ARTHROPOD PESTS OF OYSTER MUSHROOM (*PLEUROTUS OSTREATUS*) AND THEIR MANAGEMENT IN WESTERN KENYA

BY

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DECLARATION

Declaration by student

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DEDICATION

This work is dedicated to my son (Dan) and his dad (Fred) who have been my source of motivation during my study and sacrificed their interests for my academic benefit.

ABSTRACT

Oyster mushroom *Pleurotus ostreatus* is a major source of food and income in western Kenya. The production of oyster mushroom in this region has been hampered by arthropod pests that cause serious production losses in terms of quality and quantity. Development of an efficient pest control measure is based on the identification of pests infesting oyster mushroom in this region. This study aimed at identifying arthropod pests of oyster mushroom in western Kenya and developing an Integrated Pest Management (IPM) strategy to manage them. Oyster mushroom was established at on-station, old and new mushroom farm sites during short rains in 2012, dry season in 2013 and long rains in 2013. Nylon netting, decis (insecticide), potted *Tagetes Minuta* + High spawn rate, Nylon net + potted *T. minuta* + High spawn rate (IPM) as pest control measures were evaluated on their efficacy in managing these pests and enhancing oyster mushroom growth and yield. Pests were sampled per site, season and treatment for identification and data on their numbers per site, season and treatment recorded. Data on days to full spawn run, pinning, first and second flush of oyster mushroom and yield; fresh weight (gms) was also collected. The percent biological efficiency was calculated. Data collected was subjected to Analysis of Variance (ANOVA) using Genstat Version 12 and significant means separated by Tukeys test. Mites *Pygmephorus* spp. (Acaridae) and dipterans *Megaselia scalaris* (Phoridae), *Bradysia* spp. (Sciaridae), *Culicoides* spp. (Ceratopogonidae) and *Anatrichus* spp. (Chloropidae) were identified. All pests except *Bradysia* spp. were reported at the three sites and in all the seasons. The mean number of pests was highest during short rains compared to long rains which recorded the lowest mean number of these pests. Old oyster mushroom farm recorded the highest mean number of these pests with the new oyster mushroom farm recording the lowest mean number. The IPM treatment consisting of Nylon net + potted *T. minuta* + High spawn rate recorded the lowest mean number of these pests compared to Normal spawn rate. The oyster mushroom in IPM treatment took the shortest duration in terms of days to the various developmental stages of oyster mushroom while recording the highest fresh weight of 150g/kg and percent biological efficiency of 30% after the second flush. There were significant differences ($p < 0.05$) in terms of mean number of pests and growth and yield of oyster mushroom amongst the different treatments. Thus, this study recommends IPM for management of arthropod pests infesting oyster mushroom in this region. This control measure will not only control the pests but also enhance growth and yield of oyster mushroom for food and income.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
IPM	Integrated Pest Management
KAPAP	Kenya Agricultural Productivity and Agribusiness Project
KIRDI	Kenya Industrial Research and Development Institute
MDG	Millennium Development Goals
NMK	National Museums of Kenya
RCBD	Randomized Complete Block Design

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CHAPTER ONE

INTRODUCTION

1.1 Background information

Mushroom cultivation in Kenya has been gaining popularity since 1970s contributing to reduction of malnutrition, job creation among the youth, income generation from sale of surplus mushrooms, poverty and hunger eradication especially in the rural areas. Mushroom cultivation also plays a key role in helping Kenya deliver the global commitment of achieving the Millennium Development Goals on health, poverty and hunger (MDG1&3) (Gateri *et al*., 2010).

The annual mushroom production in Kenya is estimated at 500 tonnes with annual production potential of 100,000 tonnes and an estimated consumption of 650 tonnes annually (Onchonga *et al*., 2013) indicating that the demand outstrips the supply. The 500 tonnes produced annually have a farm gate value of KES. 225 million with a retail value of KES. 340 million (Onchonga *et al*., 2013). A bulk of this production is a domain of the large scale farms which constitutes 90-95 % of the total production with small scale production being concentrated in Nyanza, Western and Coastal areas.

The exotic mushrooms currently commercialized in Kenya are button *(Agaricus bisporus)* and oyster (*Pleurotus spp.*) with the former constituting the bulk of the mushrooms produced in Kenya. Button is mostly grown by large scale farmers due to the high capital requirements and sophisticated technology that it requires making this mushrooms expensive and out of reach by most Kenyans in terms of affording it (Gateri *et al*., 2010). On the other hand oyster mushroom production is preferred by the small scale farmers and is gaining popularity among these farmers. This is due to

low investment costs, adaptability of oyster mushrooms to a wide range of weather conditions, easy availability of substrate materials, simple and easy cultivation procedures, no land requirements as production is indoors and high yields compared to button (Gateri *et al*., 2009; Sanchez, 2010). These are the factors that the small-scale farmers have found to be affordable for the improvement of their livelihood through oyster mushroom production in Kenya (Kamau, 2007). Moreover fresh oyster mushrooms fetch high market prices of between Kshs 300 to Kshs 600 a kilo gram (Bert, 2011).

There has also been an increasing demand of these mushrooms by the health conscious consumers due to the nutritional and medicinal value found in oyster mushrooms. Oyster mushroom is rich in proteins (25-50%), vitamins (riboflavin, biotin, niacin, B complex, A, D, C), essential amino acids, sugars (17-47%), mycocellullose (7-38%) as well as minerals like phosphorous, potassium, sodium and calcium of about 8-12% (Noble, 2009; Omomowo *et al*., 2013; Ashraf *et al*., 2013). These goes in hand with the therapeutic benefits in oyster mushrooms which include boosting immunity, lowering blood pressure, preventing cancers and liver disintegration diseases as well as lowering cholesterol (Kanyi, 2010; Diana *et al*., 2006). Recently oyster mushroom has been reported to be a good source of dye for the textile industry where it has the potential to achieve 100% dye decolorisation potential with improvement of culture and technological conditions thus widening the ready market for oyster mushroom (Singh *et al*., 2013).

The realization of benefits of oyster mushrooms by the small scale farmers in Kenya has been hampered by a number of factors which include limited availability of quality spawn (mushroom seed), limitations in proper production and postharvest

handling skills, lack of extension services, diseases and pests (Gateri *et al.*, 2009). Among these factors pests account for serious yield losses of in terms of quality and quantity estimated at 70% worldwide (Chandra *et al*., 2007). In Kenya there is no documentation of yield losses associated with pests on oyster mushroom.

These losses are due to the high susceptibility of oyster mushrooms to pests caused by the favourable oyster mushroom cultivation conditions that favour the prevalence of these pests (Royse, 2003). These pests not only cause yield losses but they can as well lead to a total crop failure in a single outbreak. As a result farmers are usually forced to employ all possible pest control measures to prevent such losses from occurring. But the adopted pest control measures have been characterized by failure to successfully control the pests and enhance growth and yield due to lack of sufficient knowledge on the pest species infesting the oyster mushroom. Thus the pest control measures are usually not employed promptly and efficiently once infestation occurs.

Western Kenya contributes to the 65% of oyster mushroom produced in Kenya as well as constituting a majority of the small scale producers of oyster mushrooms in Kenya (Odendo *et al*., 2009). Butere is one of the regions in western Kenya that is characterized by a large number of small scale farmers in oyster mushroom production (Mapesa, pers.comm.). This has been caused by the presence of small land acreage per family that cannot support other agricultural activities for their livelihoods. The production of oyster mushroom in this region under self help groups has also favoured production of oyster mushroom in the region. Such self help groups have enabled the farmers access production materials and equipments at favourable prices, access market for the produce as well as extension services through technicians employed by the groups. Also the availability of sugarcane leaves as

substrate for oyster mushroom production has enhanced its production in the area at reduced costs.

Pest infestation causing serious yield losses has hampered the realization of the advantages associated with oyster mushroom cultivation by these farmers. Thus the farmers have been forced to adapt various pest control measures to avoid such losses. These pest control measures include chemical control whose use has been hampered by increased consumer demand on chemical free foods, limited number of pesticides approved for use in mushrooms (Staunton *et al*., 1999). This goes hand in hand with the high sensitivity of the primordial to chemical vapors especially where deformation occurs in oyster mushrooms (Royse, 2003).

Physical methods such as use of nylon nets as physical barriers against pest infestation and cultural methods such substrate sterilization, proper sanitation, good farming practices and hygiene which are all aimed at preventing the introduction of pests into the oyster mushroom cultivation units have been used. But despite the use of these pest control, the occurrence of these pests in the production unit and the losses associated with these pests is still of great concern. This might be due to lack of sufficient knowledge and information by these farmers on the pest species infesting the oyster mushroom. This has led to poor adoption and application of the pest control measures against the particular pests resulting to prevalence of these pests despite the use of the control measures. Also lack of sufficient knowledge on how to combine these pest control measures through integration has affected the control of these pests since the individual pest control measures that have been adapted by these farmers have been associated with individual shortcomings.

The aim of this study is to enhance the production of oyster mushroom in western Kenya by first identifying the arthropod pest species infesting the oyster mushroom. This identification will form a basis for the development of an Integrated Pest Management (IPM) strategy to manage the pests while reducing the quality and quantity yield losses associated with these pests. The integration of this pest control measures will allow the pest control measures used by these farmers to complement each other's shortcomings while enhancing pest control in oyster mushroom farms. This goes a long way in enhancing and even increasing the contribution of western Kenya to the 65% of oyster mushrooms produced in Kenya.

1.2 Statement of the problem

Despite the losses associated with pest infestation on oyster mushroom in western Kenya, limited information exists on the species of pests infesting oyster mushroom and their occurrence in the region. As a result of lack of this information, the pest control measures that have been adapted by farmers in this region have failed to successfully control these pests while enhancing growth and yield of oyster mushroom.

Moreover the individual pest control measures used by these farmers have been associated with individual shortcomings including affecting growth and yield of oyster mushrooms thus leading to failure of these pest control measures to manage these pests efficiently. Similarly, lack of sufficient knowledge on how to efficiently combine the pest control measures that are efficient in managing these pests has as well hindered the successful management of these pests.

1.3 Justification of the problem

There is need to establish the species of the pests infesting oyster mushroom in western Kenya as well as their occurrence. This will form the basis for the development and adoption of an efficient pest control measure that will manage these pests while enhancing growth and yield of oyster mushrooms. This is because the right pest control measure will be applied on the right pest at the right time and in the right manner.

Since individual pest control measures used in western Kenya have been associated with individual shortcomings to successfully control these pests, knowledge on how to combine these pest control measures that are compatible, safe, sustainable and ecologically sound will enhance control of these pests. This is because combination of these pest control measures in IPM strategy will exclude the pests economically and ecologically by bringing together pest control measures that are compatible, safe, sustainable and ecologically sound.

The integration of these single component control measures in an IPM strategy will enable them to complement each other's individual shortcomings. This will also provide an efficient pest control measure that will favour the growth and yield of the mushroom crop while discouraging the infestation and prevalence of these pests.

1.4 Study objectives

1.4.1 Overall objective

To enhance the production of oyster mushroom in western Kenya through the control of pests infesting oyster mushroom using Integrated Pest Management (IPM) strategies.

1.4.2 Specific objectives

- To determine the arthropod pest species associated with oyster mushroom and their seasonal occurrence.
- To evaluate the efficacy of select pest control methods in management of arthropod pests infesting oyster mushroom.

1.4.3 Research Hypotheses

- Identification of arthropod pests species infesting oyster mushroom will enhance pest management in Western Kenya
- Population dynamics of arthropod pest species infesting oyster mushroom will change in based on seasons and farm types
- The integration of pest control measures will efficiently manage arthropod pest species of oyster mushroom

CHAPTER TWO

LITERATURE REVIEW

2.1 Oyster (*Pleurotus* spp.) mushroom

Oyster mushroom is one of the popular edible mushrooms and accounts for 14% of the total world production of edible mushrooms (Chandra *et al*., 2013). Oyster mushroom belongs to the Genus *Pleurotus*; Family Pleurotaceae and are primary decomposers found almost worldwide. *Pleurotus ostreatus* is the most important commercial species in which many commercial strains are developed and cultivated. This is because *P. ostreatus* is adapted to a wild range of climatic condition and substrate materials (Kong, 2004; Royse, 2003).

In Kenya there is very little documentation on the *Pleurotus* species being cultivated with majority being recognized by cap colour; white, grey and yellow oyster mushrooms. In western Kenya *Pleurotus citrinopileatus* that has been growing in the wild in Kakamega forest is now being domesticated (Musieba *et al*., 2012) for commercial oyster mushroom production with *Pleurotus ostreatus* and *Pleurotus eryngii* being already commercialized in this region.

2.1.1 Cultivation of oyster mushroom

Oyster mushroom are grown from mycelium propagated on a base of steam-sterilized cereal grain mostly rye or millet where its mixture (mycelium and grain) form the spawn which is used as the mushroom seed (Royse, 2003). Poor spawn preparation compromises the quality of the spawn in various aspects including serving as the initial source of pest inoculum to mushrooms established from such spawn. Spawning is mainly done under sterile condition where failure to observe sterility provides room

for pest infestation especially flies which prefer laying eggs on spawned substrate (Royse, 2002).

Spawn is applied at a rate based on wet weight (w/w) of the substrate expressed as a percentage and the spawn application rate can be as low as 0.8% to as high as 10% (Custodio and Christopher, 2004; Upadhyay, 2006). It has been observed that as the spawn application rate increases, the duration of spawn run reduces due to increased inocula points within the substrate. This rapid spawn run reduces the time non-colonized substrate is exposed to insect pests such as sciarid and phorid larvae and also shortens the crop cycle reducing pest build up in mushroom production units (Royse, 2003; Upadhyay, 2006).

The substrate is usually formulated before spawning at 80% bulk substrate, 20% Nitrogen supplement and 1% pH buffer (Custodio and Christopher, 2004) and sterilized. Substrate sterilization is usually done using several methods such as autoclaving, chemical sterilization and pasteurization (Pani, 2013) but chemical and autoclave sterilization is still not feasible in Kenya. Pasteurization by either immersion in hot water at about 80^{o}C for 30 minutes, bulk steaming at 60^{o}C for 6 hours or drum steaming at 95^{o}C for 4 hours are substrate pasteurization methods used in Kenya. Sterilization of the substrate destroys the micro-organism and pests within the substrate creating optimum conditions for mushroom mycelial growth (Taurachand, 2004; Noble, 2009; Diana *et al*., 2006). Poor substrate sterilization characterized by low temperatures over a short period of time provides room for the survival of micro-organisms and pests which infest the mushroom once established on such substrate. The infestation of mushroom mites has greatly been associated by poor substrate sterilization.

The spawned substrate is usually incubated indoors to provide optimum growing conditions for the mushrooms. Oyster mushroom requires cool temperatures of 23 - 25^{o}C, high Relative Humidity of 85 - 95%, proper aeration, sanitation and darkness (Musieba *et al*., 2012). Insect pests especially sciarids and phorids have been reported to have a high adaptation to this conditions as they successfully reproduce and multiply under these conditions. The ventilation and entrances of the incubation rooms are usually covered by impervious netting material to prevent entry of insect pests especially flies whose infestation is favoured by the odour produced by actively growing mushroom mycelial during incubation.

2.2 Pests of oyster mushroom

Oyster mushrooms are highly susceptible to pest infestation due to the favourable environmental conditions of the mushrooms which not only enhances the growth of oyster mushrooms but also favour the prevalence of these pests (Keil, 2002). Pests infesting oyster mushroom have been reported to have a high adaptability to the oyster mushroom conditions.

The humidity, temperature and dark conditions observed during the production of oyster mushroom have been reported to favour prevalence of pests. Also the substrate on which the oyster mushroom is established has been reported to provide a sufficient breeding site for pests by serving as a major source of food for these pests (Cha, 2004). Several pests infest oyster mushroom but insect pests of the order Diptera have been found to be the notorious pests as their presence in oyster mushroom production units is not only a nuisance but they also phoresy other pests and diseases. The larvae of these pests have been reported to feed on the mushroom mycelium and can lead to a total crop failure in a single outbreak (Royse, 2003). In Kenya there is no

documentation of pests infesting oyster mushroom but Ogutu, (pers. Comm.) reported that insect pests (Dipterans) were serious oyster mushroom pest in western Kenya. In this region these pests are not only a nuisance in production units and cause of yield losses but also infested the spawn lowering its quality.

2.2.1 Mushroom flies

2.2.1.1 Taxonomy and identification

Dipterans of the family Sciaridae, Phoridae and Cecidae have been identified and found to be pest of economic importance in mushroom production. Sciarid flies of the genus *Lycoriella* and *Bradysia* have been considered to be the most significant pests of cultivated mushrooms due to their very low economic threshold (Shamshad *et al*., 2008; Shamshad, 2010). *Bradysia pauper* in Sri lanka (Gnaneswaran and Wijayagunasekara, 1999), *Lycoriella solani* in Pennsylvania (Royse, 2003) and *Lycoriella mali* in Zimbabwe (Chidziya *et al*., 2013) have been reported as sciarids of economic importance in oyster mushroom production but as minor pests. To date no work has been documented in Kenya on the species of sciarids infesting oyster mushrooms.

In reference to phorids, *Megaselia halterata* (Wood) and *Megaselia nigra* (Diptera; Phoridae) are the two phorid species that attack mushrooms belonging to the genus *Megaselia*. *Megaselia nigra* are minor pests and attacks the mushroom sporophores at conditions of natural day light. *M. halterata* is the second most important pest of button mushroom after sciarids (White, 1991; Jess and Bingham 2004) while *Megaselia tamiladuensis* has been reported as pest of oyster mushroom in this genus. *Heteropeza pygmaea, Mycophila speyeri* and *Mycophila barnesi* are the cecids that have been reported to infest mushrooms. *M. barnesi* is the most common of the three

species and can cause substantial loss in yield with *Mycophila* spp. reported to infest oyster mushrooms (Staunton *et al*., 1999). Despite a majority of these mushroom flies having being identified and found to infest oyster mushroom, to date no work has been documented in Kenya on the species of these mushroom flies infesting oyster mushrooms.

The identification of mushroom flies has mainly been done based on the morphological characteristics of pinned specimens with reference to older identification keys during identification (Gnaneswaran and Wijayagunasekara, 1999). This form of identification faces a number challenges especially with emergence of new sub species where such keys often offer little information for the efficient identification of such species.

Mushroom flies of the genus *Megaselia* have been characterized by taxonomical complexities aggravated by sexual dimorphism (Disney, 2008; Zuha and Omar, 2014) hence use of keys based on pinned specimen for identification is done cautiously. Despite this, sciarids have a characteristic wing venation that distinguishes them from other mushroom flies during identification characterized by a v-shaped vein at the central wing venation (Gnaneswaran and Wijayagunasekara, 1999).

Furthermore sciarids of the genus *Lycoriella* and *Bradysia* are distinguished from each other by the presence or absence of bristles on the fore tibia which is absent in *Lycoriella* and whiplash hair on the inner sides of the gonostyles which is absent in *Bradysia* (Shamshad *et al*., 2008). *Bradysia ocellaris* are further identified on the basis of a characteristic lateral yellow stripe on the abdomen (Shamshad, 2010).

The identification of these mushroom fly species in Kenya has not been fully studied although flies have been reported by oyster mushroom farmers in Western Kenya as major causes of yield loss in oyster mushroom. Thus there is need to carry out the identification of flies infesting oyster mushroom in this region for efficient control of these pests.

2.2.1.2 Biology of mushroom flies and their damage on oyster mushroom

The occurrence and prevalence of mushroom flies is greatly affected by prevailing external weather conditions leading to seasonality in their prevalence. Navarro *et al*., (2002) observed that the numbers of sciarids across the seasons in Spain were low with their numbers peaking in spring. Similar Navarro *et al*., (2002) in Spain observed high prevalence of adult phorids in spring and autumm with a low prevalence in winter and summer. This indicates that extreme external weather conditions characterized by extreme temperatures greatly affects the activity of mushroom flies.

Chidziya *et al*., (2013) observed that the development of sciarids is temperature dependant where sciarids do not develop at temperatures below 10^{o}C while high temperatures of 32.2^{o}C caused injurious effects on all the developmental stages of sciarids. But temperatures of 25^{o}C are favourable for growth of sciarids. The duration of phorid development is temperature dependant and may vary between 15 days at 24 - 27^{o}C to 50 days at 16 - 21^{o}C while increase in air temperatures especially during spawn run facilitates shorter life cycles of phorids (Biosciences institute of North Ireland, 2013).

Thus extreme external weather conditions characterized by extreme temperatures greatly affects the activity of mushroom flies. Thus the prevalence of mushroom flies

in western Kenya will be well established when their prevalence on oyster mushroom farms is observed across the seasons in this region since seasons present varying external weather conditions that impact their activity.

The development of an efficient pest control measure against mushroom flies is based on a clear understanding of their biology. This will ensure that the right control measure is applied at the right time and the right way in reference to the destructive stages of the pest. Swarms of phorid and sciarid flies are usually found outside a mushroom farm and easily fly into the mushroom growing rooms when physical barriers are not efficiently put in place. Once mated, the females are attracted to odour of cooling substrate after pasteurization and oduor from actively growing mushroom mycelium.

Physical barriers such as nylon netting and air filters have been used on entry points and ventilation to limit entry of these flies into the production units (Coles, 2002). Sealing of cracks in the production unit at the end of a cropping cycle has also been used to exclude these flies from production units. Once in the growing room the females lay eggs on substrate during cooling and spawning discriminating against substrates that have been treated by pesticides (Keil, 2002). The incorporation of insecticide in to the substrate before spawning has been adopted by some mushroom producers to discourage the females from ovipositing (Shamshad, 2010). The hatched larvae preferably feed on moist actively growing mycelium than on dense mycelia because fully grown mycelia are water repellant (dry) and studded with calcium oxalate crystals (Keil, 2002). Rapid colonization of the substrate by the mycelium through increased spawn rate has been utilized in managing these larvae. Since fully colonized substrate prevents the larvae from feeding successfully.

A high number of larvae feeding on the mycelium can hinder successful spawn run by contaminating the substrate with feacal material preventing the mycelium from colonizing such substrate (Chidziya *et al*., 2013). This is in addition to the fact that a large of the larvae reduces the amount of mycelium colonizing the substrate. This discourages the flies from laying eggs on substrate will minimize the damage associated with the larvae once eggs are hatched. Crop damage, yield and quality losses of oyster mushroom by the adult flies result from their ability to mechanically spread mushroom diseases like *Trichoderma* spp., *Verticillium fungicola* and *Pseudomonas tolaasii* (Shamshad, 2010). The adult flies are also carriers of pyemotid mites and nematodes which are also mushroom pests (Chidziya *et al*., 2013). Hence limiting and excluding the flies from the production unit will minimize the losses caused by flies on mushroom.

2.2.2 Mushroom mites

2.2.2.1Taxonomy and identification of mushroom mites

The mushroom mite Acari: Siteroptidae and Acari: Pygmephoridae are cosmopolitan nuisance pest in the cultivation of mushrooms with the family Pygmephoridae being encountered commonly in commercial mushrooms. Mites of the family Pygmephoridae are commonly referred to as Red pepper mites (RPM) or pigmy mites. *Pediculaster fletchmanni* (RPM) as reported by Kheradmand, *et al*., (2006), *Siteroptes mesembrinae* as reported by Terras and hales, (1995) and *Brennandania lambi* (RPM) as reported by Gao and Zou, (2001) are destructive mushroom mites in commercial production of white mushroom (*Agaricus bisporus*). But there is no documentation of pigmy mites infesting oyster mushrooms, Gao and Zou (2001) report reveals that *Brennandania lambi* (Acari: Pygmephoridae) do not survive in

mycelia of oyster mushroom *Pleurotus ostreatus* and *Pleurotus sajor-caju*. Past record reveals that no work has been documented in Kenya on the species of mushroom mites infesting oyster mushrooms.

Solenidia located on the legs is one of the special morphological characteristic used in the identification of pigmy mites of the genus *Pygmephorus* which consists of four sub genera. The female mites of this genus are used in their identification since the male mites are uncommon. The presence or absence of a single tarsal claw on the first pair of legs of female *Pygmephorus* spp. is the distinguishing factor among these mites (Wicht, 1970). The identification of pigmy mite in Kenya has not been fully studied although mites have been reported by oyster mushroom farmers in Western Kenya.

Mushroom flies are the carriers of mushroom mites (Navarro *et al*., 2002; Chidziya *et al*., 2013) hence the external prevailing weather conditions that affects the occurrence of these flies might also affects the occurrence of mushroom mites in mushroom farms. Thus the establishment of the occurrence of mushroom flies in Western Kenya in reference to the seasons in this region might shade light on occurrence of mushroom mites in this region in reference to seasons in this region.

2.2.2.2 Biology of mushroom mites and their damage on oyster mushrooms

The biology of mushroom mites is essential in development and adoption of an efficient pest control measure and its application at the right time and manner.

Mushroom mites usually gain entry into the compost by clinging onto sciarid and phorid flies (Navarro *et al*., 2002) when the mites attain their migratory stage after being overcrowded in the substrate. Apart from using flies to gain entry into the

compost, mites in a hypopus state adhere on moving objects such as rodents, personnel's clothes and footwear and implements to gain entry into mushroom growing rooms. In hypopus state, mites body becomes flattened covered with a thick shell to protect them from exposure to hostile conditions but when conditions become favourable hypopus sloughs as the mites take their normal appearance (Tsarev, 2003).

Under favourable conditions mushroom mites multiply rapidly with a female mushroom mite laying upto 160 eggs in 5 days at 20^{o}C which hatch into adult mites. The life cycle of pigmy mites is temperature dependant where at higher temperatures the generation time decreases. The generation time of pigmy mites is normally 4 - 5 days but at lower temperatures of 10^{o}C the generation time is about 7 days while at 20^{o}C is about 4.8 days and at 25^{o}C is about 3.9 days (Columbia ministry of Agriculture and lands, 2009).

Generally mushroom pests of the genus *Pygmephorus* do not feed on mushroom mycelium or fruiting bodies but they feed on weed moulds such as species of *Trichoderma* (Smiley, 2009; Wicht, 1970). *Trichoderma* spp. competes with mushroom mycelium for resources in the substrate. The occurrence of *Pygmephorus* spp. in mushroom substrate is usually an indicator of a poorly prepared substrate that is unfavourable for growth of mushroom mycelium thus favouring the growth of weed moulds on which this mites feed on (Tsarev, 2003; Smiley, 2009). These mites are among the key transmitters of *Trichoderma* spp. in mushrooms as they possess a pair of specialized spore-carrying structures (sporothecae) in which they transport spores of *Trichoderma* and other weed moulds to substrates as mushroom substrate offer favourable conditions for spore germination and hyphal survival (Terras and Hales, 1995).

Although mushroom mites do not cause direct damage on mushrooms, *Trichoderma* spp. causes serious fungal diseases in mushroom production as it can lead to complete crop failure. Thus understanding the biology of mushroom mite is essential in successfully control of mites and the damage they cause on mushrooms in western Kenya.

2.3 Pest control methods for pests infesting oyster mushrooms

2.3.1 Physical control methods

These are various pest control measures that are usually put in place to prevent pests from reaching sites where they can cause damage on oyster mushrooms and are measures that mainly exclude pests from mushroom production units. The exclusion of pests from mushroom growing units is highly favoured by the fact that mushroom production is done within closed doors in environmentally controlled rooms (Shamshad, 2010). The physical pest control measures that are used in managing pests of oyster mushroom include maintenance of the integrity of the production unit through filtering air entering the production unit using air filters (Geosel, 2011), observation of a regular repair program of the production unit and use of impervious netting material on doors and ventilation (Illankoon and Wijesekara, 2002).

Keeping the doors closed throughout the production period (Staunton *et al*., 1999), limiting the number of times the doors to the production unit are kept opened and destruction of heavily infested substrate bags are as well physical pest control measures. Since mushroom flies have been reported as some of the major pests infesting oyster mushroom in western Kenya, several physical control measures have been adapted by the mushroom farmers in this region to exclude these pests. These includes maintaining the integrity of the production units through regular repair

programs, use of impervious netting material on doors and ventilations and keeping doors closed throughout the production period with observation of minimum number of times that the doors are kept opened as well as destroying heavily infested substrate bags.

Despite the use of these physical pest control measures, the occurrence of these mushroom flies in the production units is still a major concern by these farmers. This is attributed to the fact that mushroom flies infest oyster mushroom during substrate conditioning and spawning thus oyster mushroom is usually infested by these pests even before introduction into the production units. In cases where the previous cropping cycle had been characterized by heavy mushroom fly infestation which hide in cracks of the production units (Columbia ministry of Agriculture and lands, 2009), new infestations may occur on subsequent crop cycles despite the existence of physical barriers to exclude the pest. This indicates that the single or individual use of these physical pest control measures cannot successfully exclude these pests.

2.3.2 Cultural control methods

These methods entail all the normal oyster mushroom production techniques that make the environment less supportive of pest prevalence while supporting the growth and yield of the oyster mushrooms (Columbia ministry of Agriculture and lands, 2009).

In oyster mushroom production substrate sterilisation is a key cultural practice that ensures the destruction of pests in the substrate that may interfere with the proper performance of mushrooms (Coles and Barber, 2002). Substrate sterilization at raised temperature for a considerable period of time ensures the destruction of pests in the

substrate. In western Kenya substrate sterilisation is mainly done using steam where substrate packed in polythene bags is placed raised above water in a metal drum for 2 hours fueled by firewood. This sterilisation technique has been characterized by the presence of substrate potions that have not been sterilized where such potions serve as sources of pest infestation once the mushroom has been established in such substrates. Also failure of the farmer to observe constant temperature within the 2 hours of pasteurization results to poorly pasteurized substrate susceptible to pest infestation.

The conditioning of the substrate under aseptic condition after sterilisation as well as spawning under aseptic condition is also a cultural control method in oyster mushroom production that prevents pests from infesting the substrate especially the flies which prefer laying eggs on such substrate (Columbia ministry of Agriculture and lands, 2009). Despite this, failure of farmers to provide aseptic condition during conditioning and spawning has been characterized by heavy mushroom fly infestation.

Shortening the cropping cycle to a maximum of three flushes (Illankoon and Wijesekara, 2002) is another cultural practice but has not been fully adapted by the farmers in western Kenya due to the desire to maximize the yields through lengthened crop cycles.

Shortened crop cycle can also be attained by increasing the spawn rate which shortens the production duration of oyster mushrooms minimizing exposure of substrate to pest infestation (Royse, 2003). 5% spawn rate is the commonly used spawn rate but as the rate decreases from 5% to 1% the duration to the various developmental stages of oyster mushroom increases while the yield reduces (Royse, 2003). An increase of the

spawn rate from 5% - 9% spawn rate has been characterized by increased yield and reduced duration to various developmental stages of oyster mushrooms (Upadhyay, 2006).

In spite of this spawn rates of 10% w/w and above have been characterized by reduced yield and increased duration to various developmental stages of oyster mushrooms as well as uneconomical (Bhatti *et al*., 2007). But 8% and 9% spawn rate of oyster mushroom (*Pleurotus ostreatus*) have been reported to have no significant differences in terms of duration to developmental stages and yield of oyster mushroom. Hence either of these two rates can be utilized to shorten the crop cycle while enhancing yield of oyster mushrooms. The use of increased spawn rate to shorten the crop cycle of oyster mushroom has not been adopted by farmers in western Kenya as a pest control measure.

Shortened crop cycles reduces number of insect pest generation and the overall fly population as it shortens the period within which the larvae are exposed to favourable conditions to successfully complete their larval stages (Coles and Barber, 2002).

Farm sanitation is as well a key cultural practice that is observed during all stages of oyster mushroom production and covers all measures necessary to prevent mushroom pests from entering, developing and spreading on the farm since most of this pests are spread by workers, rodents and equipments (Columbia ministry of Agriculture and lands, 2009). Since sanitation helps in management of oyster mushroom, farmers in western Kenya have adapted it to enhance pest control as well as growth and yield of the mushroom but failure to observe sanitation in some farms has been characterized by heavy pest infestation.

Steam heat treatment (cook-out) of growing rooms after completion of a cropping cycle is a cultural practice that forms the backbone of sanitation in mushroom farms. Steam heat treatment enhances the control of pests by eliminating pests that build up from previous crop cycle which may serve as source of infestation once a new crop is established (Staunton *et al*., 1999). Although steam heat treatment is a cultural practice in mushroom production, it has not been fully adopted by farmers in western Kenya due to financial constrains.

The regulation of humidity and temperature in oyster mushroom production units at 80 - 90% Relative Humidity and temperature of 20^{o} - 30^{o}C for successful growth of oyster mushroom through watering is a cultural practice that also enhances pest control (Coles and Barber, 2002). These wide ranges of humidity and temperature levels are not favourable for the prevalence of the pests. Regulation of the temperature and humidity levels within these ranges in oyster mushroom production units in western Kenya has not been successfully due to poor access to temperature and humidity measuring equipments as well as the high illiteracy levels among these farmers.

It is evident that cultural pest control measures revolve around manipulating the oyster mushroom cultivation conditions to favour the growth and yield of the crop while discouraging the prevalence of the pests (Coles and Barber, 2002). But failure to keenly observe these conditions has been associated with heavy pest infestation and consequently great yield losses in western Kenya since they are the very basic practices in oyster mushroom production. Hence cultural practices as pest control measures in oyster mushroom production cannot be successfully utilized for efficient pest management in oyster mushrooms when used individually.

2.3.2.1 Repellant plants

Repellant plants are plants that are majorly established as companion plants to deter pests from infesting the crop of interest and majorly viewed as a cultural pest control measure in crop production. The use of repellant plants in managing pests infesting mushrooms has not been fully documented but the use of plants botanical extracts with pesticidal properties has been used as biopesticides against some of the pests infesting mushrooms. Erler *et al*., (2009) reported the efficiency of neem products and hot-water extracts of *Origanum onites* and *Pimpinella anisum* leaf extracts in managing *Megaselia halterata.* Bussaman *et al*., (2012) reported the efficiency of *Curcuma xanthorrhiza* and *Zingiber montanum* rhizome extracts and *Ocimum sanctum* and *Melissa officinalis* leaf extracts in management of mushroom mites *Pygmephorous* spp. Marigold is one of the companion plants that their repellant properties have been used against flies infesting horticultural crops.

Mushroom flies are key pests in oyster mushroom production, thus the utilization of repellant plants as companion crop in combination with other pest control measures will enhance their management against these flies. Mexican marigold *Tagetes minuta* is an annual herb that grows widely in Africa and occurs naturally in western Kenya. Based on its availability *T. minuta* can been established as companion plants in oyster mushroom production units to enhance management of mushroom flies in this region. Taylor, (2013) reported that borneol is one of the active ingredients in *T. minuta* that has a natural insect repellant property against Dipterans. Thus, indicating the potential of *T. minuta* in managing dipterans infesting oyster mushroom. Although *T. minuta* has been used by some of the mushroom farmers in this region to manage these flies

research studies needs to be carried out on its efficiency in managing these pests especially when used in combination with other pest control measures.

2.3.3 Biological control methods

These are pest control methods that provide an oyster mushroom grower with natural tools to control pests (Rinker, 2002). These natural tools are usually natural enemies of these pests and act as either predators or parasites of major pests that attack oyster mushrooms. Biological pest control measures have not been fully exploited in controlling pests of oyster mushrooms although biological pest control measures have been used successfully in managing sciarids and phorids infesting button mushroom (Shamshad, 2010).

Howardula busseyi is entomopathogenic nematodes that occur naturally in phorid population at times within the fly where the nematode immature stages are usually parasitic in adult male and female phorid. When the female phorid lays eggs on spawned substrate, it also deposits the second stage nematode larvae where the female and male nematode larvae develop and mate in the substrate with the fertilized fourth-stage females of the nematodes entering the phorid larvae or young pupae and develop into adults the phorid body cavity but while inside they deplete and disorganize the flies' food reserves and lays eggs which hatch into larvae that enter the female phorid oviducts. Apart from depleting the flies' nutrients, the nematode reduces the phorid egg oviposition by 50 - 100% (Shamshad, 2010; Keil, 2002).

Steinernema feltiae an entomopathogenic nematode carries bacteria that are deadly to the sciarid fly larvae at their older stage and young pupal stages. Once inside the larvae, the nematode makes its way into the body cavity of the insect and releases a

bacterium that rapidly kills the insect host within 48 hours by blood poisoning as the immature nematodes feeds on the new bacterial cells and host tissues to develop into adults (Shamshad, 2010; Keil, 2002; Scheepmaker *et al.*, 1996).

Hypoaspis miles are predatory mite that feeds upon larvae of Cecids, phorids and sciarids. The mites are suitable for the management of these insect pests because they are mobile and can easily search for the prey while the mushroom growing conditions are suitable for their reproduction and can live for several weeks without food (Keil, 2002; Shamshad, 2010).

Bacillus thuringiensis subspecies *isralensis* (Bti) is a bacterium that produces both a protein crystal and a spore which when once eaten by the larva of the insect pest, the crystal degrades in the alkaline gut of the insect reducing its ability to feed leading to the death of the larvae within 48 hours due to starvation. This bacterium has been found to cause 70 - 80% control of sciarid larvae and also phorid (Shamshad, 2010).

Biological pest control measures in oyster mushroom production has not been feasible in western Kenya due to insufficient technological support and financial constrains facing the mushroom farmers in addition to the fact that production is done on small scale basis.

Despite this, the use of natural enemies in management of pests infesting oyster mushrooms has majorly been directed to insect pests. The population of natural enemies usually takes long to peak up and stabilize yet oyster mushroom has a short cropping period. Also there is very little knowledge on how to effectively apply the natural enemies since the susceptible stages of the pests majorly the larval stage are usually lodged in the substrate.

2.3.4 Chemical control methods

Preventive application is one of the pesticide application technique used against mushroom pest where it serves the same role as physical exclusion. This application technique is used as a backup if some entry points have been overlooked. Mostly this technique kills the flies before entering the growing room as pesticide is used in routine application to the substrate or in the atmosphere within the growing room (Coles, 2002). Predictive application is usually based on monitoring the fly population and application knowledge for timely application of the pesticide.

Despite of these modes of application, there are a limited number of pesticides that have been registered for use in management of pests that attack mushroom. Those used against insect pest are usually broad spectrum with activity against a wide range of insect pests (Shamshad, 2010). Most of the pesticides used are mainly those used against horticultural pests making their effect against oyster mushroom pests non beneficial to the mushroom farmers. This is in addition to the fact that the few registered pesticides are widely and repeatedly used against these pests leading to increased chances of resistance to these pesticides by the pests (Shamshad, (2010).

Pesticides applied through incorporation of the pesticide into the substrate during spawning have been characterized by inhibited mycelia growth, delayed fruiting, total yield reduction and decrease in yield numbers (Shamshad, 2010). This is because different strains and species of oyster mushrooms react differently to these chemicals. Bendiocarb which was initially effective in controlling phorids was later found to be associated with reduction in yield and mushrooms numbers when mixed into the compost while at the higher rates of 100 and 1000 µg g^{-1} adversely affected the crop

(Shamshad, 2010). Endosulfan, an organochlorine insecticide/acaricide, has been reported to strongly inhibit mycelial growth in petri dish tests.

Some insect pests such as sciarid have been reported to have developed resistance to some insecticides like abamectin, chlorfenvinphos, pirimiphos-ethyl and diazinon (Shamshad, 2010) especially when there dosages are altered to higher or lower levels.

Babar *et al*., (2012) reported that spinosad and trichlorphon gave maximum mushroom yields while effectively controlling phorid flies by reducing emergence of adult flies followed by deltamethrin then spintoram and permethrin and Malathion respective.

In western Kenya deltamethrin (Decis) has been used as an insecticide against mushroom flies and is usually applied using preventive application technique. Deltamethrin has been reported to efficiently manage the insect pests of oyster mushroom in this region but with adverse effect on growth and yield of the oyster mushroom characterized by delayed fruiting and low fresh weights of harvested mushrooms.

But generally the dependence on chemical control techniques leads to over dependence on pesticides, pesticide resistance and probable pest problems. The increasing populations of health conscious consumers and changing attitudes towards environment, less persistent and non toxic pest control methods have been given preference over chemical control methods. This has made chemical pest control methods to be used as a last resort in management of pests infesting mushrooms inspite pesticides offering a quick and cost-efficient solution to these pests (Coles, 2002). Thus the development of a pest control measure that is environmentally friendly, less persistent and non toxic pest control method in managing pests infesting

oyster mushroom in western will enhance demand of oyster mushroom among the health conscious consumers.

2.3.5 Integrated Pest Management (IPM) strategy

Integrated pest management (IPM) is a strategy in crop production that effectively manages pests using combination of pest control methods. In this strategy cultural, physical, biological and chemical pest control methods that are economical, environmentally friendly and sustainable are integrated in consideration of the health of the crop and management of the pest with minimum impacts on the environment (Columbia ministry of Agriculture and lands, 2009). IPM aims at creating conditions that are optimal for the crop but less favourable to the development of the pest. This goes hand in hand with minimizing negative impacts on workers health, providing ecologically sound pest control program, reducing the production cost, limiting dependency on pesticides and providing a positive public image that enhances marketing potential (Fleischer, 2002). IPM has been efficiently and successfully used in managing pests infesting various crops. IPM strategies have been used successfully in managing insect pests of horticultural crops such as cabbages and tomatoes (Sardarna and Sabir, 2007) as well as those infesting field crops such as cotton.

IPM strategies against pests infesting mushroom has not been fully documented although Fleischer, (2002) and Shamshad, (2010) reviewed on the efficiency of IPM strategy in managing pests infesting mushrooms. In all the three cases the researchers emphasized the importance of farm design and the use of physical barriers which entailed dust and fly filters, impervious growing rooms, correctly filtered air handlers, correct temperature and humidity control, effective disinfectant door mats and strict execution of all hygienic measures designed for the prevention of pest dispersal by

personnel. Also an effective IPM strategy not only minimizes the risk of introducing pests from outside the farm but also reduces the chances of pest spreading on the farm from infected crops to clean crops at any stage of the mushroom crop development as reported by Shamshad, (2010).

But these practices in IPM strategy can be summarized into four principals: monitoring, exclusion (physical control), sanitation (cultural control) and pest control (Shamshad, 2010). In spite of this the development of an IPM strategy for pests infesting mushrooms is based on the understanding of four principals; Identification and understanding of the biology and behavior of the pest, regular pest monitoring, a decision on when to control, choice of control measures and evaluation of the programs' efficiency through record keeping.

To date no work has been documented on IPM strategies in management of pests infesting oyster mushroom; in Kenya the use of IPM strategies in management of pests infesting oyster mushroom has not been exploited.

Therefore for the successful development of an IPM strategy in western Kenya against pests infesting oyster mushroom, there is need to establish the pest species that attack oyster mushroom through identification with reference to their composition and incidences. This will form the basis for the successful development of a safe and sustainable pest control measure. Similarly since the physical, chemical and cultural pest control measures used in western Kenya have been associated with an individual shortcoming in managing these pests with some affecting the development and yield of oyster mushroom, the integration of these pest control measures that are compatible, safe, sustainable, ecologically sound and environmentally friendly in an

IPM strategy will enhance the management of these pests while enhancing growth and yield of the mushroom.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Establishment of oyster mushroom *Pleurotus ostreatus*

3.1.1 Experimental sites

The research study was carried out at Lunza division, Butere sub-county, Kakamega County western Kenya. The area is located at an altitude of between 1240 m - 1641 m above sea level with an average annual temperature of 22.5^{o}C having the minimum temperature of between 11^{o}C - 13^{o}C and maximum temperatures of between 28^{o}C - 32^{o}C (WaSSIP report, 2011). Lunza division lies between latitudes 0^{o}25'S and longitude 34^{o}53'E.

The area receives an average annual rainfall range of 1250 - 1750mm with two rain patterns made up of the short rains between September and December and the long rains between April and July. The experiments were set up across three seasons; short rains of 2012, dry season of 2013 and long rains of 2013. In each season oyster mushroom (*Pleurotus ostreatus*) was established at three sites within Lunza, on-station and on farm. The on-station site was located at Lunza market Marama West Mushroom Growers Group offices Lunza, one on-farm at Imanga village where the farmer had never grown oyster mushroom and the other on-farm at Matibira village where the farmer had been growing oyster mushroom for the past two years.

3.1.2 Substrate preparation and spawning

Sugarcane leaves were used as the substrate and were collected locally from standing sugarcane plantation. The leaves were chopped mechanically using a chaff cutter at the On-station site and manually at the farmer's sites to 5 - 10 cm length after which they were soaked in water for 24 hrs and drained. Wheat bran 100 g and 10 g of lime

was added to 1 kg wet weight of the substrate after which the substrate was packed into clear polythene bags of 1 kg net wet weight and the necks tied using polythene strands. The substrate was sterilized with steam for 90 minutes On-station and 2 hours on-farm.

The sterilized bags were left to cool under sterile conditions for 24 hours after which the bags were spawned based on treatments under sterile conditions. At the on-station site spawning was done inside an improvised laminar air flow while at the on-farm sites spawning was done inside a clean sterilized-room with closed doors and windows. The spawn was obtained locally from the Marama West Mushroom Growers Group. The spawned bags were tied using sterile polythene strands and sterile cotton wool introduced at the neck for aeration. Labeling of the bags was then done before incubating them in clean and sterilized rooms.

3.1.3 Experimental design

The experiment was designed in a Randomized Completed Block Design (RCBD) where the study was carried out at three sites across the three seasons in the area with six treatments replicated three times (Figure 1).

REP 1	**T4**	**T3**	**T5**	**T1**	**T6**	**T2**
REP 2	**T4**	**T3**	**T2**	**T6**	**T1**	**T5**
REP 3	**T4**	**T3**	**T6**	**T5**	**T2**	**T1**

Figure 1: Experimental layout of the study per site and across the three season

The (T) denotes the treatments per replication (Rep) with each treatment per replication consisting of five substrate bags. This experimental design was similar across the three seasons per site. The treatments which were pest control methods entailed;-

Nylon netting (T1)

The sterilized substrate bags were spawned at 6% w/w spawn and each bag was covered by a three layered nylon netting material before being arranged in the incubation room (Plate 1).

Plate 1: Nylon netting on substrate bags during incubation (Source: Author, 2012)

Synthetic insecticide (T2)

Decis (deltamethrin) a synthetic insecticide was incorporated into the substrate after cooling. 0.4 ml of decis was added to 1 litre of water according to the method by Babar *et al*., (2012). Then 10 ml of this mixture according to Tsarev, (2003) was sprayed uniformly to 1 kg substrate under sterile conditions before spawning the bags at 6% w/w spawn rate. The spawned bags were then arranged into the incubation room (Plate 2).

Plate 2: Substrate bags treated with decis (Insecticide) under incubation (Source: Author, 2012)

Potted *T. minuta* + High spawn rate (T3)

The sterilized substrate bags were spawned at 8% w/w spawn rate under sterile condition and introduced into the incubation room. Four potted *T. minuta* plant were placed in the incubation and fruiting rooms. Polythene cages were used to prevent the volatiles from *T. minuta*) from spreading to the other treatments (Plate 3).

Plate 3: Substrate bags spawned at 8% w/w spawn rate under incubation inside a Polythene cage with potted *T. minuta*) (Source: Author, 2012)

IPM-Nylon netting + *T. minuta*) + High spawn rate (T4)

The sterilized substrate bags were spawned at 8% w/w spawn rate and covered with a three layered nylon netting material after which the bags were placed in the incubation room. Four potted *T. minuta*) plants were introduced in the incubation and fruiting rooms. Polythene cages were used to prevent the *T. minuta*) volatiles from spreading to the other treatments (Plate 4).

Plate 4: Substrate bags of IPM treatment under incubation inside a polythene cage (Source: Author, 2012)

High spawn rate (T5)

The sterilized substrate bags were spawned at 8% w/w spawn rate and incubated (Plate 5).

Plate 5: Substrate bags spawned at 8% w/w spawn rate under incubation (Source: Author, 2012)

Normal spawn rate (Check) (T6)

The sterilized bags were spawned at 6% w/w spawn rate, the spawn rate normally used by the oyster mushroom farmers in the area and incubated (Plate 6). This treatment served as the check.

Plate 6: Substrate bags spawned at 6% w/w under incubation.
(Source: Author, 2012)

3.1.4 Incubation, fruiting and pinning

The incubation rooms were cleaned and fumigated using bavistin before the introduction of the spawned bags. The bags were incubated in the dark at 25 $\pm$ 2^{o}C to allow for mycelial colonization of the substrate (spawn run). The end of the incubation period was marked by dense white mycelium covering the entire substrate bag. Days to full spawn run were recorded. The growing rooms were kept humid at 85 - 90% Relative Humidity with a 12 hrs light/12 hrs dark photoperiod at 23 $\pm$ 2^{o}C. The growing rooms were watered twice-thrice daily to enhance the humidity and induce fruit body formation. The temperatures during the incubation and fruiting period were recorded. The days to pin head formation after spawn run were also recorded, as well

as the days to the first and second flushes. The substrate blocks were watered after the first flush to enhance second flushing and after the second flush the substrate blocks were destroyed. After each flush, harvesting of the mushrooms was done by hand and the fresh weight of each harvest recorded.

3.2 Assessment of infestation of the mushroom by pests

The established oyster mushrooms were inspected on weekly basis for pest infestation during the incubation and fruiting stages of oyster mushrooms. Each bag was inspected visually for pest infestation and the bags that were found to be infested were isolated for sampling of the pests. The sampling of the pests was done by extraction of the pests from the bags under sterile conditions using an aspirator where only the adult stages of the insect pests were sampled. The insect pests that were also seen moving around the bags were also sampled and formed part of the pests that were sampled from that particular bag.

The pests per bag were sorted out based on their morphological characteristics like colour and size using a hand lens, counted, labeled and preserved in 70% alcohol for further sorting and identification at the National Museums of Kenya (NMK) Department of Invertebrate Zoology. The morphologically sorted pests were labeled using alphabets and their numbers were recorded weekly per treatment for every site and in all the three seasons.

3.2.1 Taxonomical identification of the pests

Since from the preliminary mushroom dipterans and mites were the pests that had been reported in western Kenya, identification keys of dipterans and mites were used during identification. The pinned flies were observed under a microscope where their

morphological characteristics of colour, leg length and segments, wing venation and the setae (antennae, position of eyes in relation to the antennae) were used in the identification of the flies. Specimen vouchers at the collection of National Museums of Kenya (NMK) Invertebrate Zoology Department were also used in identification of the flies. The photos of the various body parts were taken using a digital microscope to aid in further identification to species level. The images of some dipterans were sent to Dr. Henry Disney at Zoology Department University of Cambridge to aid in further identification to species level.

In case of mites they were prepared and mounted before identification. The mite preparation procedure was according to (Mugambi, pers. Comm.) Since the mites were of the same morphological characteristic a sample of 10 mites was taken. The mites were cleared with lactic acid for 30 minutes and treated with potassium hydroxide (KOH) for 15 minutes to remove haemetin while the remaining fatty material was cleared using xylene. The mites were washed with acidic alcohol for 5 minutes and transferred to xylene. They were then washed sequentially at different concentrations of ethanol namely 70% - 80% - 90% and finally in absolute alcohol.

To accelerate the clearing process, the abdomen of the mites were punctured and dipped sequentially in different concentrations of ethanol 70%, 80%, 90% and absolute. The mites were then stained with acidic fuslichin for 5 minutes and were dipped in clove oil for 5 minutes to clear the chemicals that are used during the clearing process. Mites were then mounted individually on glass slides using gum arabic and covered with cover slips. The mounted specimens were finally placed in an oven at 50^{o}C to allow the gum to dry for a week. Photos of the mounted mites were

taken for the images to aid in further identification. Identification key stated by Wicht, (1970) was used in identification of the mites.

3.3 Assessment of the growth and yield of the mushroom

The days to full spawn run, days to formation of pin head, days to first flush and days to the second flush per bag was recorded to assess the growth of the mushrooms per treatment. The harvested mushroom per flush per bag was weighed and the fresh weight recorded as well as the total fresh weight of the mushroom at the end of second flush. The % biological efficiency (% BE) (ratio of fresh weight of mushroom harvested/substrate dry weight, expressed in %) was calculated to assess the yield of the mushroom.

3.4 Statistical analysis

The data collected was subjected to analysis of variance (ANOVA) using GENSTAT statistical software version 12 to establish whether there were significant differences per treatment. Significant differences were tested at 5% level of significance and significant means were separated by Tukeys test. The data on number of pests that were sampled was subjected to a logarithmic transformation [Log_{10} (x+2)] where x scores were incidences of the pests while 2 was a constant. The transformed data was then subjected to analysis of variance (ANOVA).

CHAPTER FOUR

RESULTS

4.1 Pests infesting oyster mushrooms

The pests infesting oyster mushrooms in western Kenya were composed of flies and mites. *Megaselia scalaris* (Diptera: Phoridae), *Culicoides* spp. (Diptera: Ceratopogonidae), *Anatrichus* spp. (Diptera: Chloropidae), *Bradysia* spp. (Diptera: Sciaridae) and *Pygmephorus* spp. (Acari: Pygmephoridae) were the pests that were found to infest oyster mushroom farms. All these pests were recorded at the three sites across the three seasons but with exception of *Bradysia* spp. that was reported only during the short rains at the On-station site. These pests were found to infest oyster mushroom during the incubation phase and none were found to infest the fruiting phase.

4.1.1 *Megaselia scalaris* (Diptera; Phoridae)

The identification of this pest to the genus level was based on their small body size, yellow-brown body colour, characteristic apically rounded wings with two broad outer veins interiorly and weak oblique veins posteriorly (Plate, 7a), humped back and short antennae (Plate, 7b). Their legs were segmented and covered with hairs (Plate, 7c).

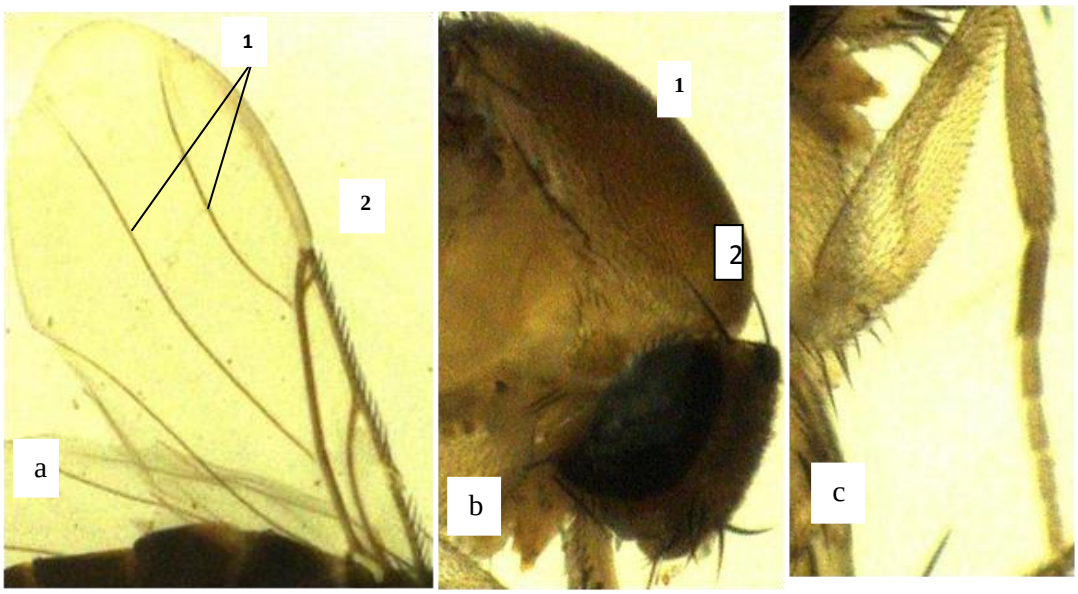

Plate 7: Morphological features of taxonomic importance of adult *Megaselia scalaris*. a- apically rounded. 1- Weak oblique veins. 2- Broad outer veins, b- 1- humped back. 2- Antennae (aristate type). c- Fore leg, segmented covered with hair.
(Source: Author, 2013)

Further identification of this pest to species level was based on the shorter but wider abdominal tergite of the females and presence of the hypopygium on the males characterized by a thick, feathered bristles at the tip of the anal tube (Disney and Copeland, pers. comm.).

In reference to the damage associated with *M. scalaris*, their larvae stages were observed in the substrate feeding on the oyster mushroom mycelium and as a result they created patches of uncolonised substrate. The adult stages of *M. scalaris* were found to be carriers of mushroom mites *Pygmephorus* spp. that were also recorded in these study. The *Pygmephorus* spp. was found stuck on legs *M. scalaris*.

4.1.2 *Culicoides* spp. (Diptera; Ceratopogonidae)

The identification of this pest was based on their small body size, slender elongated abdomen, long legs and humped back (Plate 8a). Their mouth parts were characterized by a short stout piercing mouth parts (Plate 8a) while their wings were slightly beaded along the outer vein emerging from the hind part of the humped back (Plate 8b). Their antennae were long and segmented with the female antennae being setaceous (Plate 8c) while the male antennae being plumose (Plate 8d).

The damage associated with *Culicoides* spp. on oyster mushroom was indirect as their hatched larvae were found feeding on the substrate that had not been colonized by oyster mushroom mycelia during the incubation period.

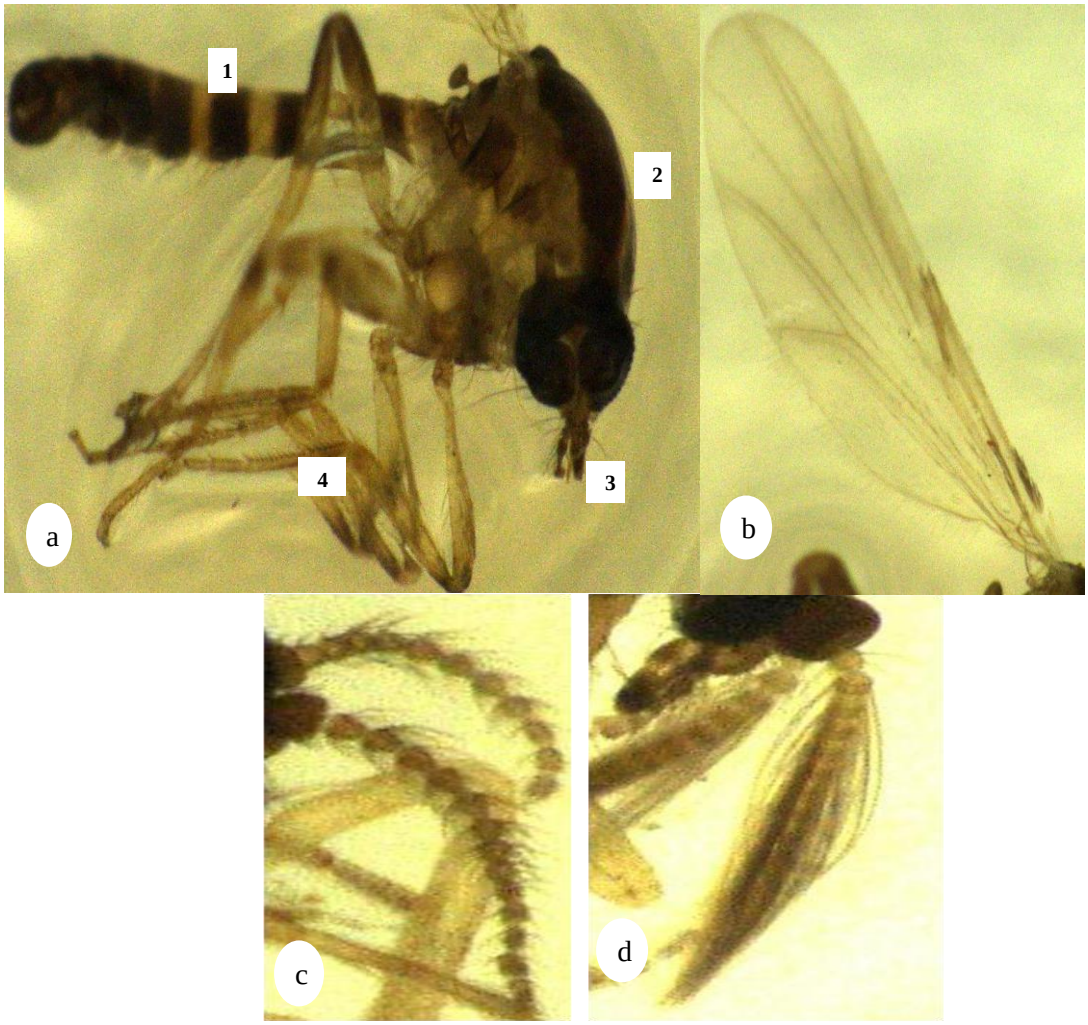

Plate 8: Features of taxonomic importance of adult *Culicoides* spp., a- 1-abdomen slender elongate, 2- humped back, 3-short piercing mouth parts, 4-long legs. b- Slightly beaded wing emerging from the humped back. c- Female antennae, (setaceous antennae), d- male antennae (plumose antennae). (Source: Author, 2013)

4.1.3 *Anatrichus* spp. (Diptera: Chloropidae)

Two species of this pest were sampled and were distinguished by the presence or absence of anal vein on the wing but both species were identified as belonging to the genus *Anatrichus* based on their small sized and dark coloured body characterized by a distinct wing venation consisting of a broken coastal (Plate 9a, b). Both species consisted of compound eyes and short thickened unsegmented antennae emerging from almost the base of the eyes.

In reference to the damage associated with *Anatrichus* spp. on oyster mushroom, their larvae were observed feeding on substrate patches that had not been colonized by oyster mushroom mycelium where this substrate patches were already been infested by larvae of *M. scalaris* and *Culicoides* spp. In addition to this, larvae of some *Anatrichus* spp. were observed feeding on oyster mushroom mycelium.

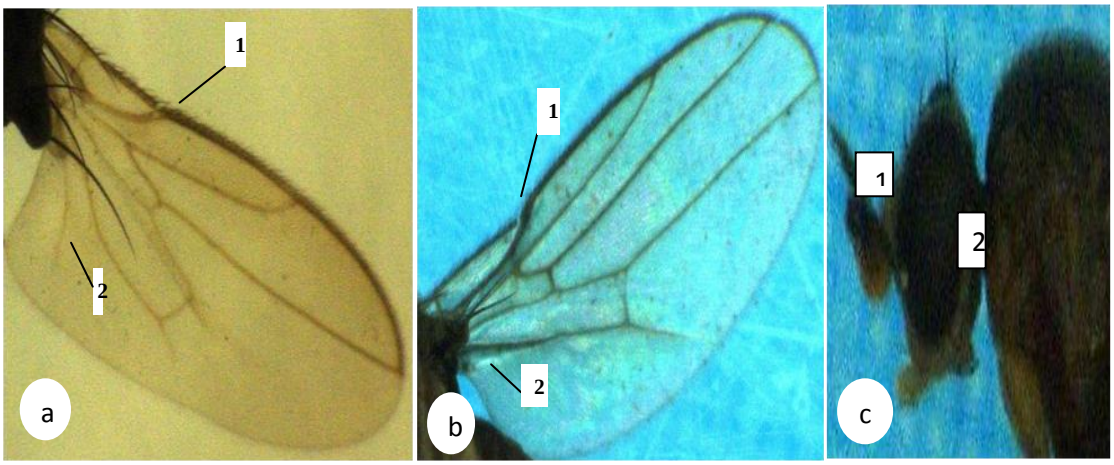

Plate 9: Morphological features of taxonomic importance of adult *Anatrichus* spp., a – species 1-wing venation, 1-coastal break, 2- anal vein. b- Species 2-wing venation, 1- coastal break, 2- anal vein absent. c -setae of *Anatrichus* spp. with thickened unsegment antennae, compound eyes. (Source: Author, 2013).

4.1.4 *Bradysia* spp. (Diptera; Sciaridae)

The features used in identification of this pest were their small sized dark coloured body with a characteristic wing venation of a distinct v-shaped venation at the center of the wing (Plate 10a). Their legs were long and segmented with compound eyes located at the base of the distinct thickened segmented antennae (Plate 10b). The abdomen was elongated and tapering (Plate 10b).

In reference to the damage associated with *Bradysia* spp., their larvae were observed to feed on oyster mushroom mycelium.

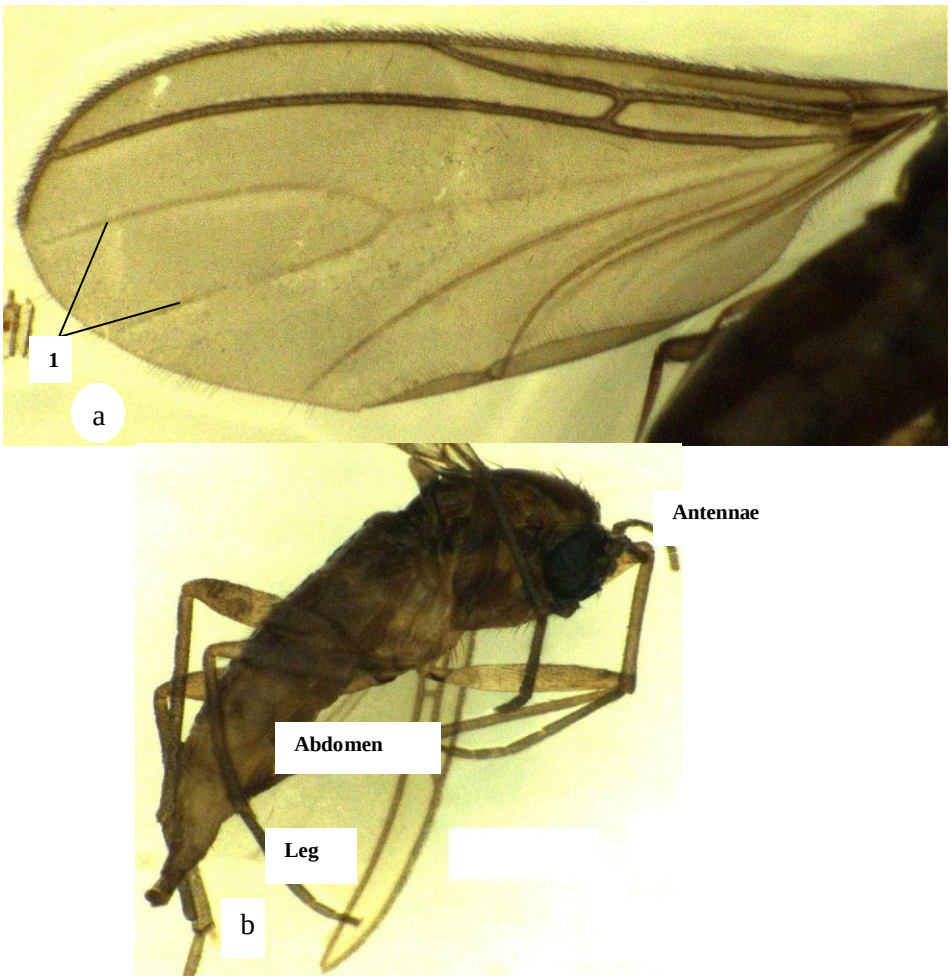

Plate 10: Morphological features of taxonomic importance of adult *Bradysia* spp. a -wing venation 1-v-shaped veins at the center, b - side view of the *Bradysia* spp., long segmented legs, short broad segment antennae, compound eyes at the base of the antennae, elongated tapering abdomen. (Source: Author, 2013)

4.1.5 *Pygmephorus* spp. (Acari; Pygmephoridae)

The identification of this pest was based on their small sized yellowish-brown coloured body characterized by flattened appearance, four pairs of segmented tarsal with clawless tarsus I, pincher mouth parts, incomplete epimeron III, anal setae and no true antennae (Plate 11)**.** *Pygmephorus* spp. was found stuck on the legs of the *M. scalaris* during sorting for identification.

Pygmephorus spp. caused indirect damage on oyster mushroom as they were observed to occur on substrate patches that had been colonized by the weed mould, (*Trichoderma* spp.). This created patches of substrate uncolonised by mushroom mycelium.

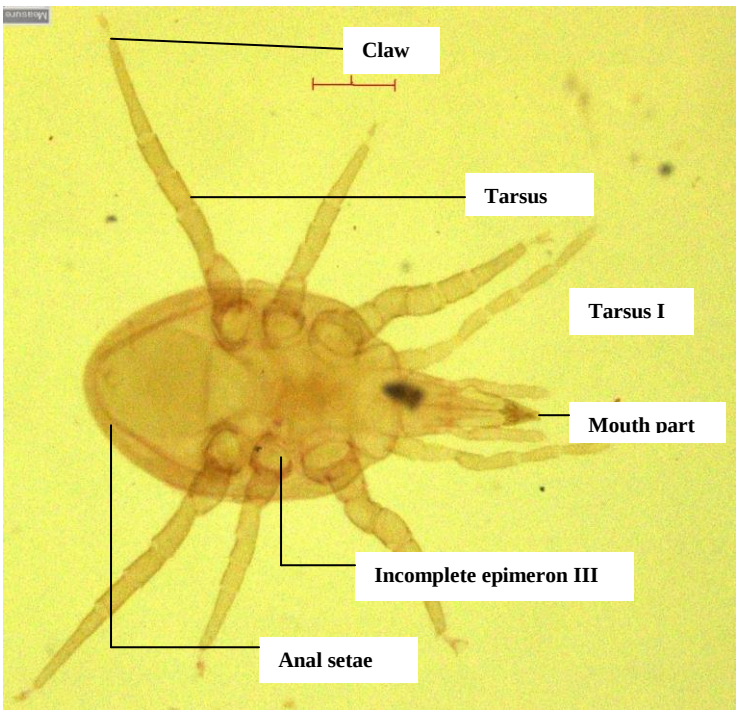

Plate 11: Morphological features of taxonomic importance of *Pygmephorus* spp. Ventral view; four segmented tarsal with clawless tarsus I, pointed mouth part, anal setae and incomplete epimeron III. (Source: Author, 2013).

4.2 Population dynamics of pests infesting oyster mushroom

A high mean number *M. scalaris, Culicoides* spp., *Anatrichus* spp. and *Pygmephorus* spp. was recorded at the old oyster mushroom farm followed by the on-station site with the new oyster mushroom farm recording the lowest mean number of these pests (Figure 2). The short rains of 2012 recorded the highest mean number of *M. scalaris* with its population decreasing into the dry season of 2013 with the long rains recording the lowest population of *M. scalaris*. The same population trend was also observed for *Pygmephorus* spp. during these three seasons.

On the other hand the population of *Anatrichus* spp. increased from the short rains of 2012 in to the dry season of 2013 before decreasing into the long rains of 2013. In reference to *Culicoides* spp., the highest mean number of *Culicoides* spp. was recorded during the long rains of 2013 followed by the short rains with the dry season of 2013 recording the lowest mean number of *Culicoides* spp. (Figure 2).

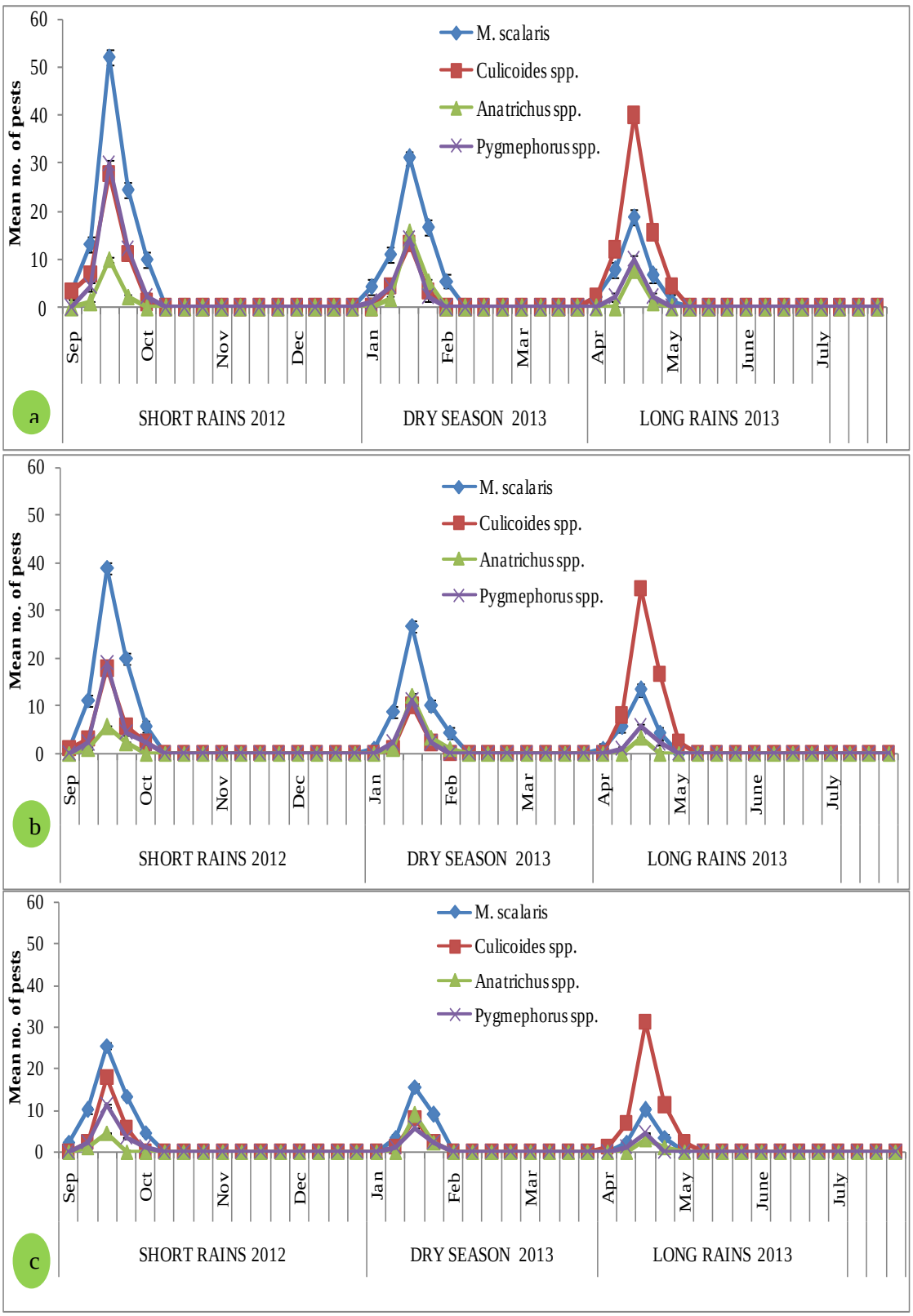

Figure 2: Mean number of pests attacking oyster mushroom during short rains 2012; dry season 2013 and long rains 2013. a- Old oyster mushroom farm, b- on-station, c- New oyster mushroom farm

4.3 Efficacy of different pest control measures in managing pests infesting oyster mushroom

The lowest number of pests was recorded in the treatment that combined Nylon netting + Potted *T. minuta* + High spawn rate (IPM) compared to Normal spawn rate (Table 1). The reduction of the mean number of pests infesting oyster mushroom in various treatments was then followed by Decis (insecticide), Potted *T. minuta* + High spawn rate, High spawn rate and Nylon net respectively compared to Normal spawn rate (Table 1).

Table 1: Mean number of pests attacking oyster mushroom in different pest control measures

TREATMENTS	Pest species			
	M. scalaris	***Culicoides* spp.**	***Anatrichus* spp.**	***Pygmephorus* spp.**
IPM	1.40^{a}	1.11^{a}	0.00^{a}	0.13^{a}
Decis (Insecticide)	6.15^{b}	1.78^{ab}	1.35^{a}	2.07^{a}
T. minuta + High spawn rate	7.33^{bc}	4.45^{bc}	1.78^{a}	3.19^{a}
High spawn rate	8.90^{cd}	5.91^{c}	3.42^{a}	4.67^{a}
Nylon net	11.73^{d}	11.22^{d}	9.44^{b}	8.11^{b}
Normal spawn rate	31.48^{e}	21.53^{e}	13.56^{c}	13.07^{c}

Means followed by the same letter in the column are not significantly different ($p < 0.05$) (Tukeys)

This table indicates that the IPM treatment that combined Nylon netting + Potted *T. minuta* + High spawn rate reduced the occurrence of these pests on oyster mushroom while Nylon netting treatment had a reduced effect on the occurrence of these pests on oyster mushroom as it was characterized by high a number of these pests infesting oyster mushroom.

The occurrence of *M. scalaris*, *Culicoides* spp. *Anatrichus* spp. and *Pygmephorus* spp. in the different treatments showed significance difference in terms of their mean numbers ($p < 0.05$) (Appendix I, II, III and IV) respectively.

4.4 Performance of oyster mushroom in different pest control measures

Oyster mushroom in the treatment that combined Nylon netting + Potted *T. minuta*) + High spawn rate (IPM) recorded the shortest duration in terms of mean number of days to full spawn run, pinning, first flush and second flush compared to Normal spawn rate (Table 2).

Table 2: Performance of oyster mushroom under different pest control measures

TREATMENTS	Mean of Days to Full spawn run	Pinning	First flush	Second flush
IPM	26.00[a]	28.14[a]	31.15[a]	35.70[a]
T. minuta + High spawn rate	29.04[b]	31.70[b]	35.30[b]	40.30[b]
Decis (Insecticide)	32.33[c]	35.33[c]	38.93[c]	44.74[c]
High spawn rate	32.85[c]	36.14[c]	40.00[c]	47.30[c]
Nylon net	40.19[d]	43.40[d]	47.70[d]	54.44[d]
Normal spawn rate	42.51[e]	46.92[e]	51.78[e]	59.43[e]

Means followed by the same letter in the column are not significantly different ($p < 0.05$) (Tukeys)

The performance of oyster mushroom in terms of days to full spawn rate, pinning, first flush and second flush was then followed by *T. minuta* + High spawn rate, Decis (Insecticide), High spawn rate and Nylon net respectively compared Normal spawn rate (Table 2) .

This indicates that the IPM treatment that combined Nylon netting + Potted *T. minuta* + High spawn rate enhanced the growth of oyster mushroom. The oyster mushrooms in this treatment was characterized by a shortened duration of oyster mushroom to the various developmental stages in terms of days to full spawn, pinning, first flush and second flush. On the other hand the Nylon netting treatment did not enhance the growth of oyster mushroom. The oyster mushrooms in this treatment was

characterized by a lengthened duration to the various developmental stages in terms of days to full spawn, pinning, first flush and second flush.

The performance of oyster mushroom in terms of days to full spawn, pinning, first flush and second flush across the treatments showed significant difference ($p < 0.05$) (Appendix V, VI, VII, VIII) respectively.

In reference to the of yield of oyster mushroom in different treatments, the mushroom in the treatment that combined Nylon netting + Potted *T. minuta* + High spawn rate (IPM) recorded the highest yield of oyster mushroom in terms fresh weight (gms) and percent biological efficiency compared to Normal spawn rate (Table 3). The performance of oyster mushroom in terms of fresh weight (gms) and percent biological efficiency in different treatments was then followed by Potted *T. minuta* + High spawn rate, High spawn rate and Nylon spawn rate compared to Normal spawn rate (Table 3). The oyster mushroom in Decis (insecticide) recorded the lowest yield of oyster mushroom in terms of fresh weight (gms) and percent biological efficiency compared to Normal spawn rate (Table 3).

Table 3: Mean fresh weight (gms) and percent Biological efficiency of oyster mushroom in different pest control measures

TREATMENTS	FRESH WEIGHT (gms)	PERCENT BIOLOGICAL EFFICIENCY
IPM	131.4[a]	26.3[a]
T. minuta + High spawn rate	117.7[b]	23.5[b]
High spawn rate	112.5[c]	22.5[c]
Nylon net	98.0[d]	19.6[d]
Decis (Insecticide)	86.3[e]	17.3[e]
Normal spawn rate	83.9[f]	16.8[f]

Means followed by the same letter in the column are not significantly different ($p < 0.05$) (Tukeys)

This indicates that the IPM treatment that combined Nylon netting + Potted *T. minuta* + High spawn rate enhanced the yield of oyster mushroom as the mushrooms in these treatment were characterized by a high yield in terms of fresh weight (gms) and percent biological efficiency. The decis treatment on the other hand affected the yield of oyster mushroom negatively. Oyster mushrooms in this treatment had low yield in terms of fresh weight (gms) and percent biological efficiency. The fresh weight (gms) and percent biological efficiency in the treatments showed significance difference ($p < 0.05$) (Appendix IX, X) respectively.

CHAPTER FIVE

DISCUSSION

5.1 Pests infesting oyster mushrooms

Pests of the order Diptera and Acari were pests that were reported on oyster mushroom farms in Western Kenya. This observation is similar to that of Gnaneswaran and Wijayagunasekara, (1999) where dipterans were among the pests that were reported on oyster mushroom farms in Sri Lanka. Despite Dipterans of the family Sciaridae and Phoridae observed in this study having also being observed by Chidziya *et al*., (2013) and Gnaneswaran and Wijayagunasekara, (1999) on oyster mushroom in Zimbabwe and Sri lanka respectively, those of the family Ceratopogonidae and Chloropidae have not been reported on oyster mushroom.

Megaselia scalaris were the most prevalent pest species of oyster mushroom (*P. ostreatus*) in western Kenya where their larvae were the destructive stages as they fed on oyster mushroom mycelium. The larvae of *M. scalaris* which have varied feeding habits are also myceliophagus and feed on mycelium of cultivated mushroom. This was also observed by Disney and Ševčík, (2011), Johal and Disney, (1994) and Disney, (2008) but the larvae were also reported to feed on the sporophores during fruiting. The absence of *M. scalaris* on the sporophores in this study is in agreement with the findings of Sanchez, (2010) that the fruiting bodies of oyster mushrooms are rarely attacked by pests.

The adult flies of *M. scalaris* were the key carriers of *Pygmephorus* spp. that were also reported to infest oyster mushroom in this study. Although *M. scalaris* has not been reported to carry *Pygmephorus* spp., Navarro *et al*., (2002) observed that

Megaselia halterata were the key carriers of *Pygmephorus (Brennandania lambi)* on button mushroom in Spain.

Sciarids have been considered a serious pest in commercial production of mushroom due to the direct damage caused by their larvae feeding on mycelium. Despite this, the number of *Bradysia* spp. a sciarid and fungus feeding dipterans in this study was very low. O'Connor and Keil, (2005) reported that mushroom variety influence developmental time, survivorship, weight and reproduction of sciarid having the hybrid strain of button mushroom (*Agaricus bisporus*) as the most favourable host for sciarids. Thus *P. ostreatus* in this study might be having a negative effect on developmental time, survivorship, weight and reproduction of *Bradysia* spp. resulting to the almost insignificant numbers of *Bradysia* spp. infesting the oyster mushrooms in western Kenya.

Since *M. scalaris* whose numbers were high in this study have been reported to be preying upon or parasitizing larvae of other fungus feeding dipterans as reported by Disney *et al*., (2013), the larvae of *Bradysia* spp. a fungus feeding Dipteran might have been preyed upon or parasitized by the *M. scalaris* larvae resulting to low incidences of *Bradysia* spp. On the other hand Gnaneswaran and Wijayagunasekara, (1999) reported that *Bradysia* spp. was a minor pest of *P. ostreatus* in Sri lanka due to their low larval density in the substrate. But Kumar, (2006) while screening *Pleurotus* spp. against sciarid larvae infestation under laboratory conditions revealed that *P. eryngii* proved to be most susceptible to sciarids followed by *P. ostreatus* and *P. sajor-caju.*

Culicoides spp. has not been reported as a pest of cultivated oyster mushroom but the occurrence of *Culicoides* spp. in oyster mushroom production units in this study could be associated with the production of octenol (1-octen-3-ol) by the mushroom. Octenol in presence of CO_2 attracts *Culicoides* spp. as observed by Cilek and Kline, (2002) and Bhasin *et al*., (2000) where a combination of octenol and CO_2 is also used in traps against *Culicoides* spp. Cotton, (2009) and Tasaki *et al*., (2013) reported that linoleic acid which is produced from breakdown of the substrate during mycelial colonization by several enzymes in mushroom is further broken down where 1-octen-3-ol is produced as one of the products. The synthesis of octenol by mycelium colonizing the substrate during the incubation phase of oyster mushroom is similar to the findings of this study as *Culicoides* spp. were sampled during the incubation phase of oyster mushrooms.

Once attracted, *Culicoides* spp. would sneak into the production units through cracks and openings where the females oviposited on the moist organically rich substrate as also reported by Hill and Macdonald, (2008). The hatched larvae fed on the substrate lowering the quality of the substrate for mycelia colonization.

Anatrichus spp. has also not been reported as a pest of cultivated mushrooms but the occurrence of *Anatrichus* spp. in oyster mushrooms farms in this study might be due to the fact that the larvae of Chloropidae family have varied feeding habits including being saprophagus where the larvae feed on the frass following damage by other insects (Nartshuk, 2011). Thus after the damage caused by *M. scalaris*, *Culicoides* spp. and *Pygmephorus* spp. on mushroom mycelium and substrate, the larvae of *Anatrichus* spp. would feed on frass of the already contaminated substrate. The larvae of *Anatrichus* spp. might have also fed on the oyster mushroom mycelium leading to

patches of uncolonised substrate. Nartshuk, (2011) reported that larvae of some Chloropidae species fed on fungi as well as mushroom mycelia thus having oyster mushroom as a fungi this pest fed on the mycelium during the incubation phase.

In this study two feeding habits; saprophagus and myceliophagus were exhibited by the *Anatrichus* spp. larvae in addition to the fact that two species of this pest were reported in this study. Nartshuk and Kurina (2014); Nartshuk, (2014) observed that Chloropidae larvae of the genus *Gaurax* were both saprophagus and mycophagus.

On the other hand Witch, (1970) reported that pests of the order Acari were pests of economic importance in commercial production of button mushroom (*Agaricus* spp.) but this study provides the first documentation of *Pygmephorus* spp. (Acari; Pygmephoridae) infesting cultivated oyster mushroom. The infestation of *Pygmephorus* spp. in this study was majorly due the occurrence of *M. scalaris* which were their key carriers. *Pygmephorus* spp. were found stuck on the legs of *M. scalaris* flies during sorting of the sampled species for identification an observation that was also made on *Megaselia halterata* in Spain (Navarro *et al*., 2002). Also Woodhall *et al*., (2009) and Chidziya *et al*., 2013 reported that *Pygmephorus* spp. cling on mushroom flies once they attained their migratory stage. This migratory stage is attained when the *Pygmephorus* spp. get overcrowded due to multiplication leading to competition for the limited resources in the substrate.

Pygmephorus spp. might have also infested the mushrooms from the raw substrates that were kept in mushroom production farms for mushroom production as also reported by Tsarev, (2003). Equally Tsarev, (2003) observed that the suckers that characterizes their body during hypopus state, a state they take under hostile

conditions allows the *Pygmephorus* spp. to be transmitted successfully by adhering on the personnel and equipments used in handling the substrate.

Pygmephorus spp. possesses a pair specialized spore-carrying structures (sporothecae) in which they transport the spores of *Trichoderma* spp. and other weed moulds (Terras and Hales, 1995). Once *Pygmephorus* spp. had been transmitted by *M. scalaris* onto the substrate, the *Trichoderma* spp. spores from *Pygmephorus* spp. germinated in the substrate providing food for *Pygmephorus* spp. since in this study *Pygmephorus* spp. were seen feeding on *Trichoderma* spp. Mycelial growth of *Trichoderma* spp. competed with oyster mushroom mycelium for nutrients in the substrate (Pecchia, 2009) discouraging the growth of oyster mushroom mycelium consequently creating patches of uncolonised substrate but colonized by *Trichoderma* spp.

In addition to this indirect damage caused by *Pygmephorus* spp. on oyster mushroom in this study, Woodhall *et al*., (2009) reported that *Pygmephorus* spp. contaminates the substrate making the substrate un-fit for mushroom mycelium to colonize the substrate when they occur in large numbers. Thus due to the availability of food *(Trichoderma* spp.), the numbers of *Pygmephorus* spp. increased hindering colonization of mushroom mycelia. Although Navarro *et al*., (2002) reported that *Pygmephorus (Brennandania lambi)* was a myceliophagus mite causing serious yield losses on button mushrooms in Spain this was not observed in this study.

The observation of all these pests only during the incubation phase of oyster mushroom in this study is in agreement with the findings of Sanchez, (2010) that

incubation phase of oyster mushroom is more susceptible to pest infestation compared to the fruiting phase.

5.2 Population dynamics of pests infesting oyster mushroom

The prevalence of *M. scalaris* is evidently seasonal where their occurrence is dependent on the prevailing weather conditions as also reported Jess and Bingham, (2004); Navarro *et al*., (2002). The temperature and humid conditions during the short rains of between September and October 2012 (Appendix XI) favoured the general development *M. scalaris* from hatching to emergence of adult flies. The temperature of 25 $\pm$ 2^{o}C and Relative Humidity >75% during this short rains favoured the prevalence of *M. scalaris* an observation that was also made by Koch, (2013) on reared *M. scalaris*. The high temperature and low rainfall resulting into high humid conditions during the dry season of January through to March 2013 and the relatively low temperatures of high rainfall and low humidity during the long rains of April to July 2013 provided relatively hostile weather conditions that did not favour the general development of *M. scalaris*.

The occurrence of *Culicoides* spp. during the long rains and short rains might be due to the damp conditions that favoured their breeding based on the findings of Hill and Macdonald (2008) while the dry conditions during the dry season discouraged their breeding leading to the low number of the *Culicoides* spp. during the dry season.

The occurrence of *Anatrichus* spp. was generally very low during the three seasons. This may be due to the fact that *M. scalaris* prey upon or parasitize larvae of other fungus feeding dipterans (Disney *et al*., 2013). Hence the larvae of *Anatrichus* spp.

that fed on mushroom mycelium might have been preyed upon or parasitized by *M. scalaris*.

In reference to the population trend *Anatrichus* spp. during the three seasons, the high temperature and low humidity during the dry season favoured the prevalence of this pest compared to the prevailing weather conditions during the short and long rains of this area. Berim, (2003) observed that temperatures of $25 \pm 2^{o}C$ favour the flight and mating of chloropids. Also Lindblad and Solbreck (2008) observed that warm sunny weather conditions favour the activity of chloropids compared to cold and cloudy weather conditions.

Although Pecchia, (2009) reported that the multiplication and life cycle of *Pygmephorus* spp. is highly dependent on temperature, the prevalence of these mites across the seasons in this study was based on the successful prevalence of *M. scalaris.* Thus the environmental conditions in this area may not be conclusively used to describe the seasonal abundance of these mites. But generally the environmental conditions that influenced the prevalence of *M. scalaris* also influenced the occurrence of *Pygmephorus* spp. since they were transmitted by *M. scalaris.*

Under favourable conditions *Pygmephorus* spp. multiplies rapidly having a female *Pygmephorus* spp. laying up to 160 eggs in 5 days that hatch as mature mites within a day (Pecchia, 2009; Wicht, 1970). Thus at that rate of multiplication *Pygmephorus* spp. would attain their migratory stage at the third week of oyster mushroom incubation period similar to the time when a majority of *M. scalaris* flies are emerging. Their number would then decline after the third week due to depletion of resources within the substrate caused by the large number of *Pygmephorus* spp.

The continued cultivation of oyster mushroom at the old mushroom farm provided sufficient breeding grounds for these pests. This agrees with the findings of Jess and Bingham, (2004) that population of mushroom pests increases rapidly in preceding generations when efficient control measures are not observed. Thus continued cultivation of oyster mushroom at the old mushroom farm provided sufficient breeding grounds for these pests compared to the new mushroom farm. The breeding sites at the old mushroom farm provided the initial sources of pest infestation in the production unit leading to a rapid population increase of these pests once the oyster mushrooms were established. This is in comparison to the new mushroom farm where the initial sources of pest infestation were absent leading to a slow build-up of these pest populations once the oyster mushrooms were established.

5.3 Efficacy of different pest control measures against pests infesting oyster mushroom and performance of the mushroom

The high infestation of these pests in the Normal spawn rate treatment might have been as a result of early infestation of these pests during substrate cooling and spawning (Hill and Macdonald, 2008). The 6% w/w spawn rate used in this treatment also contributed to the high infestation of these pests because this spawn rate provided low inoculation points of the spawn in the substrate resulting in slow substrate colonization rate (Royse, 2003; Upadhyay, 2006). This led to the presence of substrate uncolonised by the mycelium that allowed their larvae to thrive successfully. As a result of high pest infestation in this treatment, the amount of mycelium inoculum colonizing the substrate and the vigor of mycelial growth reduced leading to slow substrate colonization. Consequently this slowed spawn run lengthening the duration to full spawn run and the duration of the subsequent developmental stages of

the oyster mushrooms under this treatment (Babar *et al*., 2012). Equally feacal material and frass from damaged substrate by these pests altered the physical and chemical properties of the substrate making the substrate unfit for colonization by the oyster mushroom mycelium (Babar *et al*., 2012; Bussaman *et al*., 2012). These reduced vigor and density of the mycelia growth also led to fruiting bodies of low fresh weight (gms) and % biological efficiency.

Decis (deltamethrin) significantly reduced the infestation of these pests by inhibiting the female from ovipositing on the substrate. Babar *et al*., (2012) reported that female *Megaselia* spp. do not oviposit on chemically treated substrate. Despite low infestation of pests in this treatment, this treatment might have altered the chemical and physical properties of the substrate causing inhibited mycelial growth hence delayed fruiting of the oyster mushrooms (Shamshad, 2010). As a result the duration of oyster mushroom to full spawn run, pin head formation, first and second flush lengthened that consequently impacted on the yield leading to the low fresh weight (gms) and Percent biological efficiency of the harvested mushrooms.

Tagetes minuta contains borneol as a chemical compound found in its pungent scent that has been found to have repellant properties against insect flies (Dipterans) (Wyss, 2011; Taylor, 2013). Hence having *M. scalaris, Culicoides* spp. and *Anatrichus* spp. as Dipterans, the repellant properties of potted *T. minuta* reduced the infestation of the adult flies in the treatments that combined potted *T. minuta*

The integration of Nylon netting + potted *T. minuta* + High spawn rate in the IPM treatment significantly managed these pests as this treatment recorded low pest infestation. The 8%w/w spawn rate provided more spawn inoculation points in the

substrate for rapid colonization of the substrate and dense growth of the mycelium thus reducing the susceptibility of the mushroom to larvae infestation (Keil, 2002). The larvae of mycelia feeding dipterans do not feed on fully colonized substrate but on substrate being colonized. The fully colonized substrate is dry and studded with calcium oxalate crystals that discourage the feeding of the larvae. Borneol a chemical compound found in the pungent scent of *T. minuta* enhanced its repellant activity against the adult flies. On the other hand, nylon netting provided physical barrier against the few flies that sneaked into the production units.

As a result of the reduced pest infestation in the IPM treatment, the duration of oyster mushroom in terms of days to full spawn run, pinning, first and second flush shortened. Equally the 8% w/w spawn rate in this treatment enhanced the vigor and density of mycelia growth leading to fruiting bodies of high fresh weight (gms) and percent biological efficiency. Thus the integration of Nylon netting + potted *T. minuta* + High spawn rate in IPM provided an all round management of pests infesting oyster mushroom in western Kenya while enhancing growth and yield of oyster mushroom. Although the use of IPM strategy in management of pests infesting oyster mushroom has not been documented, IPM strategy has been reported to be efficient in management of pests infesting Horticultural crops (Sardarna and Sabir, 2007)

CHAPTER SIX

CONCLUSION, RECOMMENDATIONS AND WAY FORWARD

6.1 Conclusion

- *M. scalaris, Culicoides* spp., *Anatrichus* spp. Bradysia spp. and *Pygmephorus* spp. were pests that infested oyster mushroom in western Kenya.
- A high mean number of these pests were recorded during the short rains and at the old oyster mushroom farm as compared to dry season and long rains and at On-station and New oyster mushroom farm sites
- IPM offered the best control measure over the other control measures that were tested
- This study documents the first occurrence of *Culicoides* spp., *Anatrichus* spp. and *Pygmephorus* spp. in oyster mushroom

6.2 Recommendations and way forward

This IPM strategy is recommended to oyster mushroom farmers since it is affordable, safe, sustainable and environmentally friendly and despite controlling the arthropod pests efficiently it also enhances growth and yield of oyster mushroom.

There is need to identify other efficient physical barriers against the adult flies so as to minimize the damage associated by their hatched larvae on oyster mushroom.

There is need to identify *Culicoides* spp., *Anatrichus* spp. Bradysia spp. and *Pygmephorus* spp. to their species level

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APPENDICES

Appendix I: Anova table for *M. scalaris* infesting oyster mushrooms

Variate: *M.scalaris* Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Blocks stratum	2	0.00842	0.0042118	9.5	
Season	2	0.049	0.0244997	55.25	<.001
Sites	2	0.02566	0.0128307	28.94	<.001
Treatments	5	0.26362	0.0527246	118.9	<.001
Weeks	4	0.21642	0.0541049	122	<.001
Season.Sites	4	0.00199	0.0004964	1.12	0.346
Season.Treatments	10	0.09166	0.0091661	20.67	<.001
Sites.Treatments	10	0.01217	0.001217	2.74	0.003
Season.Weeks	8	0.02629	0.0032863	7.41	<.001
Sites.Weeks	8	0.01142	0.0014272	3.22	0.001
Treatments.Weeks	20	0.09542	0.004771	10.76	<.001
Season.Sites.Treatments	20	0.00402	0.0002008	0.45	0.981
Season.Sites.Weeks	16	0.00482	0.0003011	0.68	0.816
Season.Treatments.Weeks	40	0.04613	0.0011533	2.6	<.001
Sites.Treatments.Weeks	40	0.01328	0.0003319	0.75	0.871
Season.Sites.Treatments.Weeks	80	0.01791	0.0002239	0.51	1
Residual	538	0.23856	0.0004434		
Total	809	1.12678			

Appendix II: Anova table for *Culicoides* spp. infesting oyster mushrooms

Variate: *Culicoides* .spp Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Blocks stratum	2	0.00139	0.0007	1.92	
Season	2	0.03579	0.0179	49.49	<.001
Sites	2	0.00789	0.00394	10.91	<.001
Treatments	5	0.14736	0.02947	81.5	<.001
Weeks	4	0.18398	0.04599	127.19	<.001
Season.Sites	4	0.00148	0.00037	1.02	0.395
Season.Treatments	10	0.03952	0.00395	10.93	<.001
Sites.Treatments	10	0.00386	0.00039	1.07	0.385
Season.Weeks	8	0.03679	0.0046	12.72	<.001
Sites.Weeks	8	0.00351	0.00044	1.21	0.288
Treatments.Weeks	20	0.09088	0.00454	12.57	<.001
Season.Sites.Treatments	20	0.00355	0.00018	0.49	0.97
Season.Sites.Weeks	16	0.00321	0.0002	0.56	0.916
Season.Treatments.Weeks	40	0.02742	0.00069	1.9	<.001
Sites.Treatments.Weeks	40	0.00472	0.00012	0.33	1
Season.Sites.Treatments.Weeks	80	0.02265	0.00028	0.78	0.913
Residual	538	0.19455	0.00036		
Total	809	0.80856			

Appendix III: Anova table for *Anatrichus* spp. infesting oyster mushrooms

Variate: *Anatrichus* .spp Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Blocks stratum	2	0.00053	0.0002659	2.09	
Season	2	0.00311	0.0015534	12.19	<.001
Sites	2	0.00172	0.0008577	6.73	0.001
Treatments	5	0.02452	0.0049046	38.5	<.001
Weeks	4	0.02675	0.0066871	52.5	<.001
Season.Sites	4	0.00013	0.0000317	0.25	0.91
Season.Treatments	10	0.00714	0.0007141	5.61	<.001
Sites.Treatments	10	0.00149	0.0001489	1.17	0.309
Season.Weeks	8	0.00504	0.00063	4.95	<.001
Sites.Weeks	8	0.00311	0.000389	3.05	0.002
Treatments.Weeks	20	0.03769	0.0018844	14.79	<.001
Season.Sites.Treatments	20	0.00053	0.0000267	0.21	1
Season.Sites.Weeks	16	0.00042	0.0000262	0.21	1
Season.Treatments.Weeks	40	0.01397	0.0003493	2.74	<.001
Sites.Treatments.Weeks	40	0.00228	0.000057	0.45	0.999
Season.Sites.Treatments.Weeks	80	0.00397	0.0000496	0.39	1
Residual	538	0.06853	0.0001274		
Total	809	0.20093			

Appendix IV: Anova table for *Pygmephorus* spp. infesting oyster mushrooms

Variate: *Pygmephorus* .spp Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Blocks stratum	2	0.00011	5.3E-05	0.22	
Season	2	0.00985	0.00492	20.68	<.001
Sites	2	0.00657	0.00329	13.8	<.001
Treatments	5	0.0454	0.00908	38.14	<.001
Weeks	4	0.06353	0.01588	66.71	<.001
Season.Sites	4	0.00167	0.00042	1.75	0.137
Season.Treatments	10	0.01271	0.00127	5.34	<.001
Sites.Treatments	10	0.00388	0.00039	1.63	0.095
Season.Weeks	8	0.01158	0.00145	6.08	<.001
Sites.Weeks	8	0.00794	0.00099	4.17	<.001
Treatments.Weeks	20	0.04221	0.00211	8.86	<.001
Season.Sites.Treatments	20	0.00366	0.00018	0.77	0.753
Season.Sites.Weeks	16	0.00315	0.0002	0.83	0.656
Season.Treatments.Weeks	40	0.01562	0.00039	1.64	0.009
Sites.Treatments.Weeks	40	0.00377	9.4E-05	0.4	1
Season.Sites.Treatments.Weeks	80	0.00962	0.00012	0.5	1
Residual	538	0.12808	0.00024		
Total	809	0.36933			

Appendix V: Anova table for days to full spawn run in oyster mushrooms

Variate: Full Spawn Run Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	2.75	1.38	0.03	
Treatment	5	5490.62	1098.12	20.45	<.001
Residual	154	8270.43	53.7		
Total	161	13763.81			

Appendix VI: Anova table for days to pinning in oyster mushroom

Variate: Pin Head Formation Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	4.11	2.06	0.03	
Treatment	5	6735.69	1347.14	21.02	<.001
Residual	154	9870.7	64.1		
Total	161	16610.5			

Appendix VII: Anova table for days to first flush in oyster mushroom

Variate: First Flush Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	1.12	0.56	0.01	
Treatment	5	7985.88	1597.18	20.38	<.001
Residual	154	12066.06	78.35		
Total	161	20053.07			

Appendix VIII: Anova table for days to second flush in oyster mushrooms

Variate: Second Flush Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	2.2	1.1	0.01	
Treatment	5	10558.8	2111.8	20.53	<.001
Residual	154	15843.3	102.9		
Total	161	26404.3			

Appendix IX: Anova table for fresh weight (gms) of oyster mushroom

Variate: Fresh Weight (gms) Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0	0	0	
Treatment	5	54530.3	10906.1	11.38	<.001
Residual	154	147550.2	958.1		
Total	161	202080.6			

Appendix X: Anova table for percent biological efficiency of oyster mushrooms

Variate: %Biological Efficiency Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0	0	0	
Treatment	5	2181.21	436.24	11.38	<.001
Residual	154	5902.01	38.32		
Total	161	8083.22			

Appendix XI: Weather data for Butere sub County

Seasons	Time (Months)	Temperature (0C)		Precipitation (mm)		Evapotranspiration (mm)
		MAX	MIN	MAX	MIN	
Dry season	JANUARY	31.6	12.9	70	50	185
	FEBRUARY	31.8	13.6	112	66	169
	MARCH	32.2	14.5	164	115	192
Long rains	APRIL	29.9	15.2	280	250	151
	MAY	28.8	15.1	297	260	141
	JUNE	28.5	14.2	179	148	131
	JULY	28.3	13.5	129	115	134
	AUGUST	28.7	13.7	182	150	146
Short rains	SEPTEMBER	29.6	13.5	160	145	157
	OCTOBER	29.8	14	172	155	165
	NOVEMBER	29.8	13.7	151	100	155
	DECEMBER	30.3	13.3	86	53	174