PHYTOCHEMICAL COMPOSITION, LARVICIDAL ACTIVITY OF *Cocos nucifera* EXTRACTS ON *Busseola fusca* Fuller AND ITS EFFECTS ON GROWTH AND YIELD OF MAIZE

BY

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DECLARATION

I certify that this thesis has not been previously presented for the award of a degree in any other university or institution. The work reported herein is my original work and all sources of information have been supported by relevant references unless inadvertent omission.

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DEDICATION

I heartly dedicate this piece of work to my loving and caring mother, the late Siza Scola Masha who makes it all worthwhile, to my father the late Mr Malick B. Ndzovu and my brother Ndzovu. M.Ndzovu.

ABSTRACT

Maize forms the staple food for most Kenyans. Although, current trends show *Busseola fusca* as the major pest causing a decline of about 400,000 tonnes of maize in Kenya. Most farmers use synthetic insecticides to control *Busseola fusca*, however, chemicals have residual effects in crop products, are expensive and harmful to the environment. Plant extracts are biodegradable and safe for human use but limited extracts that have been identified. There is a need to identify more plant extracts to control insect pests. *Cocos nucifera* is known to have antimicrobial and the phytochemical compounds of this plant may have larvicidal effects on *Busseola fusca* which may improve maize yield. However, its phytochemical composition and larvicidal activity on *Busseola fusca* has not been determined. This study aimed to investigate the phytochemical composition, larvicidal activity of *Cocos nucifera* extracts on *Busseola fusca* and its effects on growth and yield of maize. The experiment was carried out in a greenhouse at Maseno University. It was laid in a Completely Randomised Design, consisting of three replicates. The treatments consisted of coconut leaf, root and husk extracts at: 75%, 50%, 25%, 0% (negative control) and Karate (Lambda-cyhalothrin). Three holes of a depth of 2.5cm each were made in each pot. Two Pannar 15 maize seed variety were sown per hole in 20 litre plastic pots. The pots were filled with acrisol soils having a pH of 4.5-5.5. Cowdung organic manure, Diammonium phosphate and Calcium Ammoniun Nitrate were used to improve soil fertility. The seedlings were thinned to 3 plants per pot. *Busseola fusca* obtained from the International Center for Insect Physiology and Ecology in Nairobi, were released to 20 days old plants. The treatments were applied weekly and observations were made at 15 day interval. The data collected was subjected to analysis of variance and means separated at $p \leq 0.05$. The results showed the presence of tannins, saponins, steroids, terpenoids, flavonoids and glycosides. Alkaloids were absent in all the extracts. Coconut leaf extract at 75% concentration significantly increased the mean number of dead *Busseola fusca*, reduced leaf damage, reduced dead hearts and reduced the number of borer holes, while increasing chlorophyll content and increasing maize yield in grams. The mortality of stem borer, chlorophyll content and yield increased, as the number of dead hearts and borer holes reduced at the early stages of maize growth with increased exposure to the extract. The mortality of stem borer may be attributed to the phytochemicals present in the coconut extracts which have insecticidal, anti- feeding and larvicidal effects on insect pests. This in turn reduced leaf damage, dead hearts and borer holes hence increased maize yields. In conclusion, coconut extracts have potent larvicidal effects against *Busseola fusca* due to the phytochemicals present in the extract. Coconut leaf extract at 75% is recommended as the most effective biopesticides against *Busseola fusca* in order to improve the maize yields by farmers. Further research on the mode of action of the phytochemicals present in *Cocos nucifera* extracts against *Busseola fusca* is recommended.

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LIST OF ABBREVIATIONS

ICIPE	-	International Center for Insect, Physiology and Ecology
LSD	-	Least Significance Difference
PAN 15	-	Pannar 15 maize seed variety
DAP	-	Diammonium Phospate
CAN	-	Calcium Ammonium Nitrate
CTA	-	Technical Centre for Agricultural and Rural Cooperation
IPM	-	Integrated Pest Management
CIMMYT	-	International Maize and Wheat Improvement Center

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CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Maize was first introduced to Africa by the Portuguese in the 16th to 18th century it has since become a staple food in Africa. Major importers of maize were Zimbabwe, Angola, Ghana, Kenya and Mozambique (Pingali, 2011). The challenges facing maize farmers in African countries include variation in climatic conditions, poor soils, pests and diseases, low-yielding seeds and post-harvest losses (CIMMYT, 2015).

Maize is Kenya's most important crop with more than 2.1 million ha of land having been occupied by maize between 2011 and 2013 out of Kenya's 5.3 million ha of all crops (CIMMYT, 2015). This implies that maize accounts for 40% of all crop area in Kenya. Data from the Ministry of Agriculture for 2011 indicated that maize accounts for more than 51% of all staple food grown in the country. The major maize producing counties in Kenya are; Trans Nzoia, Uasin Gishu, Kakamega, Nakuru, Embu, Nyeri, Kirinyaga, Taita-Taveta and Kwale.

Kenya's per capita maize consumption is estimated at 103 kg/person/year (CIMMYT, 2015), with the 2017 annual maize demand being 52.8 million bags. However, maize production has shown a deficit with the 2017 annual production being 37 million bags of maize which fell below the annual domestic demand for the year (KNBS, 2018). This necessitates a need to carry out a study to improve maize yields in Kenya to reach self sufficiency.

Stem borers are considered to be one of the most important pests of maize in the world (De Groote, 2001). In Kenya, stem borers cause a decline in maize production by an average of 13 percent or 400,000 tonnes of maize, equivalent to the normal yearly amount of maize the country imports. This damage is valued at more than USD 90 million per year for maize (De Groote *et al*., 2011). The most prominent species of stem borers in Kenya are the *Busseola fusca* Fuller, found in the cooler and higher areas and spotted stem borer *Chilo partellus* (Swinhoe), found in the warmer and lower areas (Mulaa, 1995). Estimated crop losses of 36.9 % were obtained in

Trans Nzoia district (Kenya) when *Busseola fusca* was introduced to maize under natural conditions (Mulaa, 1995). Areas around Lake Victoria, have a mixture of the two species, similar to the mid altitudes and the southwest of the moist transitional zone (De Groote, 2001).Due to the extensive yield losses caused by maize stem borer in Kenya, there is need to alternative way of reducing the incidence of the pest and inturn improve maize.

Feeding activities of the stem borers reduce the photosynthetic area of the leaves that result to poor yield (Ofor *et al*., 2009).The larvae of *Busseola fusca* after hatching, feeds on soft surface of the leaves and then enters in to the stem through whorl and feeding on pith of the stem. This leads to stunted growth of the maize plants resulting into dead hearts when attacked at their initial stages. The larvae also enter in to the stem through lower nodes by making the borer holes (Nyukuri *et al*., 2014). Damage resulting from stem borer infestation on maize plant can cause between 20-40% losses during cultivation, and 30-90% at post- harvest and storage (Madonni *et al*., 2006). Generally, the yield losses due to stem borers range from 10 to 100% (Bosque and Mereck, 1990).Maize stem borers have been reported to cause great damage and yield losses across Africa (Davies and Pedigo, 1990). Despite the effects of stem borer, information on the effect of controlling *Busseola fusca* using coconut extracts on growth of maize is unknown.

Synthetic insecticides have been used extensively in the past by farmers for the control of stem borers, however, they have not been effective in the control of stem borers and are not environmentally friendly (Clieve, 2003). Unfortunately this method is expensive, toxic to its users and the environment as well as having undesirable effects on non-target organisms. It has led to development of resistant strains that are more difficult to control (Jembere *et al*., 1995; Okonkwo and Okoye, 1996). Botanicals are considered as alternatives to insecticides because they are cheap, environmental friendly and easily adaptable by local farmers through the abundant flora diversities that exist in the tropics (Adde *et al*., 2011). There is therefore a need to come up with a better, environmentally friendly control method for *Busseola fusca*.

Botanicals have over the years become quite promising against insect pests (Anon, 1991). Neem derivatives for instance have been effective in control of stem borers (Aliniazee *et al*., 1997; Kumar and Bhatt, 1999; Ganguli and Ganguli, 1998; Bhanukiran and Panwar, 2000). In another study, four common tropical mosses, viz: *Calymperes afzelii*, *Thuidium gratum*, *Bryum*

coronatum and *Barbula lambarenensis* were tested and reduced the number of stem borer significantly in maize in Nigeria (Ande *et al*.,2010). Aqueous leaf extract of *Nicotiana* spp. L. and *Cymbopogon citratus*, root bark aqueous extract of *Securidaca longepedunculata* F and flower extract of *Chrysanthemum cinerariaefolium* showed significant mortality against *Busseola fusca* (Shiberu *et al.,* 2013). However, information on the use of coconut extracts in the management of stem borer in maize needs investigation.

Cocos nucifera belongs to the family Arecaceae commonly known as 'coconut'. It produces fruits that are unique in terms of their morphology. It is considered as one of the high value cash crops in tropical countries given the many different categories of products that can be derived from the coconut tree (Lima *et al*., 2015 and Heenataj *et al*., 2017). In the Coastal region of Kenya, the crop's potential is not being fully exploited for maximum production of coconut-based-products for both domestic and industrial applications (Kadere *et al*., 2009). The major coconut producing countries of the world such as Malaysia and Thailand show that the coconut industry is well developed, and the fruit of the coconut palm is the main source of many food products (Severio,1996). According to a study by Ofwona (1994), the Kenyan government had shown considerable effort in promoting oil seed crops in Kenya to self-sufficiency levels. Various committees and missions were formed such as the national committee on oil crops and the mission to appraise and accelerate oil seed production.

Leaf extracts of coconut and sorghum were found to contain Antiviral principle (AVP) which was effective against tomato spotted wilt virus(TSWV) which causes bud necrosis, bud blight or ring mosaic in groundnuts (Manjunatha , 2008). A study on ***c***oconut leaf bioactivity against fall armyworms and corn earworms, showed significant mortality for the insects that fed on coconut leaves, with a significant reduction on their growth rates (Dowd *et al*., 2011). Coconut husk fibre extract, and its antibiofilm activity showed that it possess antimicrobial activity against the bacteria Pseudomonas sp., Alteromonas sp. and Gallionella sp. involved in biofilm formation (Viju *et al*.,2013).However the larvicidal activity of *Cocos nucifera* extracts on *Busseola fusca* has not been evaluated.

The available data has revealed presence of flavonoids, glycosides, carbohydrates, tannins and saponins in aqueous extracts of *Cocos nucifera* root in India. However, steroids, proteins,

alkaloids, phenols and quinines were found to be absent (Sivakumar *et al*., 2011). Another study on microscopical and phytochemical characters of *Cocos nucifera* root showed presence of carbohydrates, proteins, amino acids and glycosides though alkaloids and flavonoids were absent (Rajkumar *et al*., 2012).

In addition, Oliveira *et al*. (2009) investigated the phytochemical analysis of liquid of green coconut husk fibre (LGCHF) in Nigeria. They revealed presence of catechins, condensed tannins, flavonoids and steroids. Despite the available data on qualitative chemical analysis for *Cocos nucifera* extracts, no studies have been done on the same in Kenya. Therefore, the current study on the phytochemical composition of aqueous *Cocos nucifera* extracts was carried out.

1.2 Statement of the problem

In spite of maize having a huge importance for food security and economic wellbeing of the country, its production has declined over the years (Kiptanui, 2019). Currently the yield is estimated at 1622 kg/ha, with an average production of 3.5 million tons (KNBS, 2018). As at the end of 2019, Kenya's maize production was 3800 thousand tonnes. This was a decline of 5.33% compared to 2018. This low level of production has been attributed to harsh weather conditions, traditional farming practices, pests and diseases. Stem borers are considered to be the most important maize pests in Kenya, with the most prominent species being *Busseola fusca* F which has been documented to cause serious crop damage and losses. A lot of emphasis has been placed on the use of synthetic pesticides such as Karate(lambda-cyhalothrin) to control the pest as opposed to use of biopesticides (coconut extracts) despite the numerous side effects of the synthetic pesticides which include environmental toxicity are being experienced by most smallholder farmers in Kenya. Synthetic pesticides have been associated with increasing the cost of production in the country. In early 2019, in availability of government subsidized fertilizer spiked the cost of fertilizer which was out of reach to farmers in the open market and Agro-Vets (Kiptanui, 2019). The high cost of production has led to maize shortage in the country.

Moreover, East African tall coconut variety covers most parts of the coastal region of Kenya and some parts of the western region although the phytochemical composition is unknown. Coconut leaf extract has been documented to contain antiviral properties against tomato spotted wilt virus

as well as causing a reduction in the growth rate of fall army worms and corn earworms, (Manjunatha , 2008) and (Dowd *et al*.,2011). Most of the studies have used coconut extracts to control other pests but their use to control maize stem borer is unknown.

1.3 Justification

Maize is the most important cereal crop in Kenya and is an important staple food for more than 80 percent of the population (ISAAA, 2001). According to CIMMYT (2015), maize accounts for 40% of all crop area in Kenya. The data for 2011 from the Ministry of Agriculture indicates that maize accounts for over 51% of all staple food grown in the country. Despite the importance of maize, current trends show that Kenya is struggling to achieve self-sufficiency. The 2017 annual production was 37 million bags of maize which fell below the annual domestic demand of 52.8 million bags for the year (KNBS, 2018). This has been attributed to insect pest, the most prominent being *Busseola fusca*. There is need to identify an alternative way the agricultural sector can improve maize yields for Kenya to reach self-sufficiency through control of maize stem borer using coconut extracts as biopesticides.

Farmers have been relying heavily on synthetic insecticides which are expensive, toxic to its users and the environment as well as having undesirable effects on non-target organisms, which has led to development of resistant strains that are far much difficult control (Jembere *et al*., 1995; Okonkwo and Okoye, 1996). Biopesticides have shown great potential in controlling insect pests such as maize stem borer since they are environmentally friendly, cheap and readily available to the Kenyan farmer. It is therefore necessary to carry out a study to contribute to the available knowledge on the use of biopesticides (coconut extracts) in the management of maize stem borer.

Studies on the phytochemicals present in coconut extracts have shown the potential of the active ingredients present in the extracts being associated to the antimicrobial properties of coconut. This study soughts to provide insight on the phytochemicals in coconut being the bioactive compounds to use in the control of maize stem borer.

1.4 Objectives of the Study

1.4.1 General Objective

To investigate the phytochemical composition, larvicidal activity of *Cocos nucifera* extracts on *Busseola fusca* and its effects on growth and yield of maize.

1.4.2 Specific objectives

i. To determine the phytochemical composition of *Cocos nucifera* extracts.

ii. To determine the effect of *Cocos nucifera* extracts on *Busseola fusca* mortality.

iii. To determine the effect of the control of *Busseola fusca* using *Cocos nucifera* extracts on the growth of maize plants.

iv. To determine the effect of the control of *Busseola fusca* using *Cocos nucifera* extracts on the yield of maize plants.

1.5. Hypotheses

i. There are varied phytochemical compounds in *Cocos nucifera* extracts.

ii. *Cocos nucifera* extracts have larvicidal effects on the mortality of *Busseola fusca.*

iii. *Busseola fusca* control using *Cocos nucifera* extracts has an effect on growth of maize plants.

iv. *Busseola fusca* control using *Cocos nucifera* extracts has an effect on the yield of maize

CHAPTER TWO

LITERATURE REVIEW

2.1 Maize Production

Maize is Kenya's most important crop accounting for 40% of all crop area in the country (CIMMYT, 2015). Data for 2011 from the Ministry of Agriculture in Kenya reported maize to account for more than 51% of the staple food in the country. According to KNBS (2018), Kenya's maize demand for the year 2017-2018 was 52.8 million bags. However, maize production has shown a deficit over the years with the 2017 annual production being 37 million bags of maize, which fell below the annual domestic demand for the year KNBS (2018). Approximately 12% of the maize harvested is estimated to have been lost which translates to about 4.5 million bags (Njeru, 2019). In 2019, maize production for Kenya was 3800 thousand tonnes although maize production has fluctuated substantially in the recent years (Knoema, 2019).

The projected maize production for the year 2020 is 3700 tonnes (FAO, 2020). As at October 2020, it has been documented that Kenya is likely not to meet the growing demand for maize (FAO, 2020). According to Kiptanui (2019), the decline in maize yields has been attributed to over dependence on rain fed agriculture, poor climatic conditions, pests and diseases, low yielding seeds and post-harvest losses (CIMMYT, 2015). Due to the increased fluctuation in maize production despite the growing demand for maize among Kenyans, there is a need to carry out a study to contribute towards increase g the maize yields in Kenya.

2.2Taxonomy of African maize stem borer

Preferred scientific name**; *Busseola fusca* Fuller**

Preferred common name***; African maize stalk borer***

Domain: Eukaryota

Kingdom: Metazoa

Phylum: Arthropoda

Subphylum: Uniramia

Class: Insecta

Order: Lepidoptera

Family: Noctuidae

Genus: Busseola

Species: *Busseola fusca*

Retrieved from CABI (2017).

2.3Taxonomic description of maize stem borer

According to Harris and Nwanze (1992), the following taxonomic descriptions are used for diagnosis and keys for identification of *Busseola fusca*.

2.3.1Eggs

They are creamy-white when first laid but darken just before emergence. They are round, flattened and slightly flattened. They are characterized by approximately 70 radial ridges (crenulations) on the upper surface of each egg shell. They are about one mm in diameter. They are usually laid in batches of 30 to 100 under leaf sheaths in single a long column stretching up the stem, and may slightly be compressed by pressure from the growing stem.

2.3.2 Larvae

They are often light or dark violet to pinkish white in colour, with a distinctive grey tinge. The head capsule is dark brown and the prothorax is yellowish-brown. Its spiracles (breathing holes found along the side of the body) are elongate-oval with black edges. The caterpillars have pro-legs along the abdomen . The larvae (caterpillars) lack conspicuous hairs or markings. They grow to a length of about 40 mm.

2.3.3 Pupa

Pupae are generally 25 mm in length and shiny yellow brown to dark brown in color. Males are usually smaller than females. They have a pair of plain spines located on the terminal cremaster.

2.3.4 Adults

The adult wing-span is 25-35 mm. Females are generally larger than males. Its forewings are light to dark brown with darker markings. The hind wings are white to grey-brown. The darker colouration develops further in cold and wet conditions.

2.4. Life cycle of maize stem borer

Female adult African maize stalk borer moth mate on the night of emergence and will oviposit on the subsequent 3 to 4 nights (the exact duration depends on temperature and other factors). Each female lays eggs in a row between the stem and leaf sheath in batches of 100 to 200. Each female can lay up to 1000 eggs in a lifetime. **.** Egg laying is usually concentrated on maize plants that are less than 2 months old with the leaf sheath of the youngest unfolded leaf being the most preferred part of the leaves for the females. Eggs hatch in about 7 to 10 days and the larvae move into the leaf whorls to feed on leaves for 2 to 3 days and then either move to other plants or enter inside the maize stem. There are usually 6 larval instars although 8 are possible in unfavorable conditions. When older (third instar), they tunnel into the stems where they feed on the central stem tissue for 3-5 weeks. Only one larva is found per stem, as larvae are cannibalistic.

The larvae matures in about 35 days and grows to a length about 40 mm, when conditions are favorable during the growing season, but during dry and/or cold weather the larvae enter into a resting period, pupa stage (diapause) of 6 months or more in stems, stubble and other plant residues. The pupae are generally 25 mm in length. Prior to pupating inside the stem, the larvae cut a small hold in the stem which enables the adult moth to emerge. The adult moth will emerge after a pupal period of 7-14 days from the hole that they produced before pupation. Adults mate soon after emergence. Under favourable conditions the life cycle can be completed in 7-8 weeks.

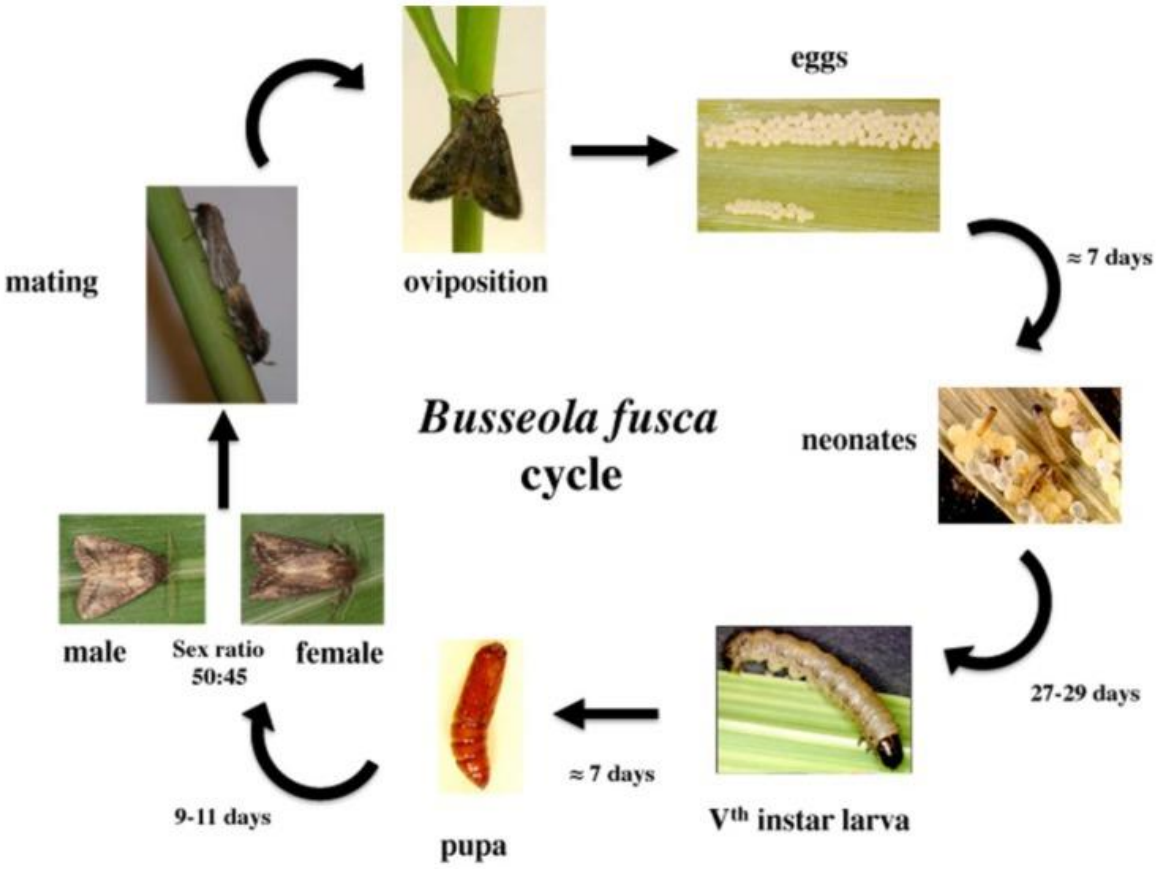

Plate 2.1. Biological cycle of *Busseola fusca* under optimal environmental conditions on artificial diet (photos on mating and oviposition from Felix (2008).

2.5 Damage of African maize stem borer (*Busseola fusca* F.) on maize plants

The larvae of *Busseola fusca* after hatching, feeds on soft surface of the leaves and then enters in to the stem through whorl and feeding on pith of the stem. This leads to stunted growth of the maize plants resulting into dead hearts when attacked at their initial stages. The larvae also enter in to the stem through lower nodes by making the borer holes (Nyukuri *et al*., 2014).

Feeding activities of the stem borers reduce the photosynthetic area of the leaves that results in poor yield (Ofor *et al*., 2009). Generally, the yield losses due to stem borers range from 10 to 100% (Bosque and Mereck, 1990). Damage resulting from stem borer infestation on maize plant

can cause between 20-40% losses during cultivation, and 30-90% at post- harvest and storage (Madonni *et al*., 2006; Robert *et al.*, 2014).

The increased damage in young maize plants is due to tenderness of leaves and stem which aged, toughened and thus became unsuitable for newly hatched larvae (Ogemah, 2003). The first generation of the larvae was thus important in terms of causing yield loss and exceeded the second generation which attacked the crop when it was already advanced in age (Muasya and Diallo, 2006). Maize stem borers have been reported to cause great damage and yield losses across Africa (Davies and Pedigo, 1990). Maize grain losses could result from; consistent feeding of the stem borer in the stem, weakness of the plants and making them more prone to lodging. This has led to a decline in grain production (Amudvi, 2009).The fore mentioned studies indicate the need to determine the larvicidal effects of coconut extracts against maize stem borer. This will contribute to a decline in maize stem borer hence increasing maize yields.

2.6 Management Strategies of Maize Stem borer

Several methods are being used to manage stem borers. The choice of the management strategy is governed by the size of the farms, age of the crop and the availability of adequate resources that can enable a farmer acquire the desired method. In Kenya, chemical, biological, cultural as well as planting of resistant maize varieties have been used. These strategies can be used to develop Integrated Pest Management (IPM) practices (Mushore, 2005).

2.6.1 Chemical control

It is the most commonly recommended method for stem borer control though its effectiveness is achieved through continuous applications. This is due to the short period that the larvae are exposed and therefore prompt and frequent applications have to be made. This can be very uneconomical to the small scale farmer (Bonhof, 2000). Screening of insecticides showed effective control of *Busseola fusca* with Carbaryl, Lamdacyhloahterin , endosulfan, and synthetic pyrethroids (Chinwada, 2002).The formulations come as granules, foliar sprays and soil applied systemics where the smallholder farmer adopted granular leaf applications because it does not require special application equipment (Ogah *et al.,* 2011; Prasad and Gupta 2012). This is despite the adverse effects of synthetic pesticides on non-target species and to the environment (Van den Berg and Nur 1998; Varela *et al*., 2003).

Stem borers are known to be physically protected from contact pesticides when they are feeding inside stems (Overholt, 1998). Moreover, the use of pesticides requires a level of know-how for its efficiency which is usually limited hence non-guaranteed success in their application (Midingoyi, 2018). Against this background, it is necessary to find a lasting and self - sustaining way of controlling the pests.

2.6.2 Cultural control Methods

It is a prophylactic (preventative) method of control concerned with the manipulation of the environment to make it unfavorable for the pest (Dent, 1991).

2.6.2.1 Manipulation of sowing dates

A study by Tekle (2016) on Distribution and Management of *Busseola fusca* indicated that early planting of maize as soon as the rains set off ,off-sets the damage caused by *Busseola fusca* ensuring high yield. Research results obtained from sowing date trials showed that early plantings suffer less from the attack by *Busseola fusca* (Azerefegne *et al*., 2001).Early infestation of stem borer has been documented to be very detrimental for maize production (Emana and Tsedeke, 1999) . It is therefore important to note that the choice of planting dates may be influenced by other factors, for example rainfall.

2.6.2.2 Intercropping

Experiments on maize/bean intercropping showed a significantly higher incidence of stalk borer and cob worms on sole maize growth compared to intercropped treatments (Tekle, 2016). Intercropping has been documented to reduce pest population on a crop by reducing the visual and olfactory stimuli which attract pests on to a crop (Hill, 1987). Intercropping is also known to affect oviposition on non - host crop plants. The larvae emerging from eggs on non - hosts die due to starvation reducing the number of larvae migrating to host plants (Ampong - Nyarko *et al.*, 1995). However, one of the problems associated with intercropping as a stem borer control measure is predicting the cropping systems which will best reduce pest abundance. This necessitates the need for an alternative way of controlling maize stalk borer.

2.6.2.3 Host plant resistance

It involves the incorporation of resistant and tolerant factors into maize cultivars has been used to control stem borers (Mushore, 2005). Various maize varieties have been evaluated for resistance

against stem borers and several lines have provided multiple resistances against oviposition and larval (Minja, 1990). Mechanisms of resistance are based on antixenosis and antibiosis.

With antibiosis, the plant resists damage by causing death to the pests or by reducing the rate of reproduction (Hill, 1987). Reduced preference for oviposition, reduced feeding due to the presence of some chemicals in the plant, reduced ability to be tunneled and plant's tolerance to leaf damage, dead heart and stem tunneling are some of the documented mechanisms of host plant resistance (Seshu - Reddy, 1998).

Production of transgenic plants *Bacillus thuringiensis* (Bt) maize hybrids have demonstrated sub-lethal effects of Bt toxins on insect performance, particularly reduced feeding and delayed larval development in insect pests (Eizaguirre *et al*., 2006). Despite the high adoption rate of Bt-maize by farmers due to ease of management, Bt-resistant *Busseola fusca* populations were reported throughout the maize production region of South Africa (Kruger, 2012).

Since the resistance mechanism is based on a single gene, insect pests are capable of developing resistance to almost any toxin if the usage of Bt maize is used for a long time (Mc Kenzie, 1996). The resistance has been documented not to be recessive as previously assumed (Campagne, 2013).Another demerit is that of the unknown effects on human health and biodiversity of beneficial insects. Hence the need to carry out a study that will provide an alternative of controlling insect pests such as maize stem borer using coconut extracts.

2.6.2.4 Utilisation of wild gramineous plants

Some of the gramineous species which have been reported to host *Chilo partellus*, *Busseola fusca* and *Sesamia calamists* in Kenya are *Hyparrenia, Panicum, Pennisetum, Setaria, Sorghum* and *Sporobolus* species. (Polaszek, 1998).Sudan grass (*Sorghum vulgare sudanese*) has been reported to attract maize stem borer females for oviposition resulting in significant yield increase in Kenya (Khan et al., 1997). Oviposition away from maize reduces larval damage, increases the efficiency of natural enemies by enabling natural enemies to colonise Sudan grass in large numbers (Khan *et al*., 1997).

2.6.2.5 Push and pull technology (PPT)

In PPT, insects are either deterred away from the main plant (push), or attracted (pull) to other areas by using stimuli that lure the insects (Yan *et al*., 2015). PPT involves intercropping maize

and *Desmodium uncinatum* in maize fields, with Napier grass planted as a border around this intercrop. *Desmodium* repels stem borer moths (push) and the surrounding Napier grass attracts them (pull) (Khan *et al*., 2001).

Studies by Khan *et al*. (1997, 2001) on the use of push-pull' system in management of stem borer reported a significantly lower maize stem borer population and damage than a maize monocrop system. Despite the success of achieved by PPT, reports by CTA, (2011) and ICIPE, (2015) show establishment of desmodium to be labour-intensive since the plot requires frequent and thorough weeding. Desmodium seeds have been reported to be expensive to acquire. Therefore, an affordable way of controlling maize stem borer such as the use of coconut extracts is necessary. This will consequently increase the maize yields in Kenya.

2.6.3. Biological control

Biological control is the use of predators, parasitoids and pathogens to maintain density of a species at a lower than would occur in their absence (DeBach and Rosen, 1991).

2.6.3.1Predators

Predators are valuable components of IPM. Ants of the genus *Lepisiota* are known to prey on stem borer eggs and pupae with *Componotus spp.* and *Pheidole spp* being the most common species (Bonhoff, 2000).

2.6.3.2Pathogens

It is the control of insects by pathogens such as entomophagous viruses, bacteria and fungi. *Bacillus thuringiensis* has been reported to significantly lower the population of stem borers in Kenya with a consequent increase in the yield (Brownbridge, 1991).However, microbial pesticides such as virus - based take long to kill insects. Fungal pesticides are difficult to produce, have a limited shelf life and are therefore useful in glasshouses where conditions are easily controlled as reported by (Mushore, 2005). Due to the fore mentioned limitations, the use of botanicals in the control of insect pests is necessary.

2.6.3.3 Parasitoids

A parasitoid is an insect whose larval stage feeds exclusively on another insect, its host, and eventually killing it (Godfray, 1994). Some parasitoids attack eggs, some attack larva, while

some attack pupae. *Trichogramma* spp parasitize on eggs of stem borers, *Cotesia* spp. are larval parasitoids while *Dentichasmiasis busseolae* are pupal parasitoids of stem borers (Sithole, 1990).

Despite the success of *Cotesia flavipes* established at the southern coastal region of Kenya through decreased stem borer density (Zhou and Overholt, 2001), host suitability studies on stem borer populations from the intended release sites need to be carried out (Ngi - Song et al., 1998). This helps in the prediction of the possibility of successful establishment of the parasitoid. It is therefore imperative to determine the larvicidal potency of botanicals such as coconut extracts against maize stem borer.

2.6.4 Use of botanicals

Neem seed extract has been documented to show pesticidal effects against maize stem borers' infestation (Wahedi *et al*. 2016). Maize plants treated with neem seed extracts recorded fewer or no dead hearts compared to the untreated control. Moss extracts of *Calymperes afzelii, Bryum coronatum, Thuidium gratum* and *Barbula lambarenensis* showed improved stem borer mortality and reduced the incidence of borer holes (Ande *et al*., 2010).

Fruit extracts of chinaberry (*Melia azedarach* L.), Endod (*Phytolacca dodecandra* L.) and pepper tree (*Schinus molle* L.) recorded a significant reduction in leaf infestation and dead heart injury by *Busseola fusca* (Fuller), and resulting in increased crop yield (Assefa and Ferdu, 1999). This shows botanicals as potent biopesticides against *Busseola fusca*.

Medicinal plant powders have been used as botanical insecticides against *Busseola fusca* under laboratory conditions (Shiberu, 2013). Ogendo *et al*., (2013) reported *Lantana camara, Tephrosia vogelii* and *Tagetes minuta* to have shown a significant reduction in stem borer incidence with a corresponding grain yield increase. This shows the potential of botanical extracts in controlling *Busseola fusca*.

Despite the success of biopesticides in controlling crop pests, some of their limitations that have been documented include; a slower rate of kill compared with conventional synthetic pesticides, shorter persistence in the environment and high susceptibility to unfavourable environmental

conditions. Because most biopesticides are not as efficacious as conventional chemical pesticides, they are not suited for use as stand-alone treatments (Chandler *et al*., 2011). A study by Sahayaraj *et al*. (2003), confirmed the dermal toxicity of neem-based insecticides (nimbicidine and vijayneem) on a useful non-target biological predator reduviid *Rhynocoris marginatus*. This necessitates the need for more research to be done on the use of botanicals against crop insect pests.

2.7. Phytochemicals and their mode of action in insects

Phytochemical compounds such as alkaloids, terpenes, polyphenols and glycosides have been documented to show larvicidal, antibacterial and antifungal activity (Odeyemi *et al*., 2008).

2.7.1 Flavonoids

Flavonoids have been documented to potential protectants through contact, oviposition deterrent, ovicidal action as well as altering moulting in insects causing death (Salunke *et al*., 2005). Flavonoids are known to play an important role in the protection of plants against plant feeding insects' and herbivores (Acheuk & Doumandji-Mitiche, 2013).The isolation of flavonoids from *Tephrosia purpuria* showed insecticidal property on *Callosobruchus maculatus* (Diwan and Saxena, 2010).

Three flavone glucosides were found to inhibit digestion in insects and also function as deterrent agents in *Nilaparvata lugens* and herbivores (Acheuk & Doumandji-Mitiche, 2013). Santos *et al*. (2016) concluded that *Tagetes erecta* and *Tagetes patula* have flavonoids that can promote its use as a natural insecticide. Flavonoids and isoflavonoids have been documented to protect the plant against insect pests by influencing their behavior, growth, and development (Simmonds, 2003; Simmonds & Stevenson, 2001). Hence flavonoids could be useful in a pest-management strategy.

2.7.2Alkaloids

Alkaloids are considered the most important group of natural substances showing insecticidal properties (Rattan, 2010). Furocoumarin and quinolone alkaloids extracted from *Ruta chalepensis* leaves showed larvicidal and antifeedant activities against the larvae *Spodoptera littoralis* (Emam, *et al*., 2009). A study by Acheuk and Doumandji-Mitiche (2013), found that

alkaloids extract of *Pergularia tomentosa* caused antifeeding and larvicidal effects. Lee (2000) documented pipernonaline and piperidine alkaloids to have mosquito larvicidal activity. Velu *et al*. (2015) concluded that alkaloids from *Arachis hypogaea* extract have larvicidal activity against chikungunya and malarial vectors. This shows alkaloids as potent phytochemicals against *Busseola fucsa.*

2.7.3 Glycosides

Glycosides are known as plant defense chemicals (Park & Coats, 2002). According to Dave and Lediwane (2012), anthraquinones isolated from Cassia species posses insecticidal activity. Glycosides extracted from *Arachis hypogaea* are known for their larvicidal activity against insect vectors (Velu *et al*., 2015).Trevisan *et al*. (2006) verified glycosides, obtained in the hydroalcoholic extract of *Kalanchoe brasiliensis* to show inhibitory effect of cholinesterase enzyme.

2.7.4 Saponins

A study conducted by Ikbai (2010) reports that saponins alter insects feeding behavior and moulting process due to their interaction with growth regulating hormones. The study further reports saponins to cause death of insects at different developmental stages. Marianna *et al* (2012), verified the insecticidal activity of saponins to be associated with their broad spectrum of action and amplitude of physiological impacts. The insecticidal activity of saponins has been attributed to their ability to primarily target the insect midgut epithelium (Geyter *et al.,* 2011). According to Ekrakene and Ogunsede (2015), saponins were better stomach and contact poison against the adult pest at increased concentration.

2.7.5 Terpenoids

Many plant compounds, majority of which are terpenoids have been known to affect insects' behaviour, growth and development, reproduction, and survival (Erdogan and Toros, 2007). A study by Rattan (2010), reported that terpenes block glucose on chemosensory receptor cells on the mouth of lepidopteran larvae. Viegas Júnior (2003) verified the repellent and insecticidal effect of sesquiterpenes to *Spodoptera littoralis* (Lepidoptera: Noctuidae). In a study by Leite *et al*. (2006) *Artemisia absinthium* leaf extract recorded 100% mortality of *Ctenocephalides canis* which is known to be rich in essential oil containing terpenes (Omer *et al*., 2007).

Studies by Martinez (2002) and Mourão *et al*. (2004), verified that triterpenes extracted from *Azadirachta indica* inhibit the feeding of insects and also affect larval development. It was also reported that triterpenes reduced the fecundity and fertility of adult insects, altered their behavior, and caused anomalies in their cells and physiology. The triterpenes also caused mortality of the insect eggs, larvae and the adults. This shows the insecticidal potency of terpenoids against *Busseola fusca.*

2.8 Coconut production

Cocos nucifera belongs to the family Arecaceae commonly known as 'coconut'. It produces fruits that are unique in terms of their morphology. It is considered as one of the high value cash crops in tropical countries, since there are many different categories of products that can be derived from the coconut tree (Lima *et al*., 2015 and Heenataj *et al*., 2017). The most recent statistics on coconut production per hectare has seen Kenya perform poorly as it is ranked position 15 out of the 17 major coconut producing countries in the world. In Kenya, the coconut sub-sector earns approximately Ksh. 3.2 billion annually- barely a quarter of its potential. This has been attributed to poor value addition strategies, lack of quality planting materials, aged orchards that are poorly managed, high pest infestations and lack of technologies for mass production of coconut planting materials and lack of trained personnel for dissemination of information (Mohamed *et al.,* 2015).

2.9 Phytochemical composition of *Cocos nucifera*

The phytochemical composition of *Cocos nucifera* endosperm tissues from Nigeria showed presence of phenols, flavonoids, alkaloids, tannins and saponins, with flavonoids being the highest followed by saponins and the least being tannins (Igwe and Ugwunnaji 2016). In the same study, the extract of *Cocos nucifera* endosperm tissue showed potent antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger* and *Penicillium notatum.* It was concluded that the activity of the extract against the organisms could be as a result of the presence of the phytochemicals, which have been reported as antimicrobial agents. Information on the phytochemical composition of *Cocos nucifera* extracts from Kenya and its larvicidal activities against *Busseola fusca* is unknown.

A study by Sivakumar *et al*. (2011), on the phytochemical screening and Anti-Bacterial activity of *Cocos nucifera* Linn root conducted in India revealed presence of flavonoids, glycosides, carbohydrates, tannins and saponins. The same study showed absence of steroids, proteins, alkaloids, phenols and quinines. Phytochemical analysis of coconut leaf extract in Brazil showed the presence of glycoside compounds, flavonoids (particularly anthocyanins), and hydrolyzable tannins, and polyphenolic compounds (Manalo *et al*., 2017). The same study postulated that the presence of the compounds could have contributed to the efficacy of coconut leaf extract against *Caenorhabditis elegans*.

2.10 Use of coconut extracts in pest control

A review of plant materials used for controlling insect pests of stored products; showed that products from *Cocos nucifera* were effective in control of pests (Dales, 1996). Coconut oil at10 ml/kg maize has been documented to cause 100% mortality in adult *Sitophilus oryzae* within 3 h and prevent reproduction and F1 emergence (Salas, 1985). Coconut oil at 10ml/kg admixed with maize and stored for 60 days caused 97% mortality in adult *Sitophilus oryzae* within 24 h and reduced F1 production by 99%. (lvbijaro and Agbaje, 1986). Although this information is available, the use coconut extracts in control of insect pest such as maize stem borer is unknown.

It has been documented that specific chemical factors in coconut leaves may also contribute to insect resistance, in addition to physical toughness (Dowd *et al*., 2011). The insect species showed reduced rates of chewed tough leaf material due to lower consumption rates making for an effective defense against many insect species. Arguably, little information has been documented on the chemical factors in coconut that contribute to insect resistance. Hence the need to determine the larvicidal effects of coconut extracts on maize stem borer.

2.11 Choice of solvent

According to a study by Abdu *et al*. (2019), when screening for phytochemicals from crude extracts from the shell powder of *Cocos nucifera* using the solvents n-hexane, ethyl acetate, dichloromethane and ethanol and water; more bioactive compounds were present in water extracts than the other solvents owing to the higher polarity of water. In the same study water extracted more steroids compared to ethanol, dichloromethane and hexane. In a study by Ghosh

et al. (2014), in qualitative phytochemical characterization of the copra extracts of *Cocos nucifera* both the aqueous extract and n-hexane extract exhibited moderate intensity of glycosides and low intensities of resins, saponins and alkaloids.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The study was carried out at Maseno University Farm in a greenhouse between February and May 2018. The experimental site at latitude 0ºI'N0º 12'S and Longitude 34º 25'E-34º47'E (Ambede *et al*., 2012). The soils were classified as acrisol deep reddish brown friable clay with pH ranging from 4.5-5.5 ,soil organic carbon and phosphorous contents are 1.8% and 4.5mg Kg^{-1}, respectively (Netondo, 1999). It is approximately 1,500m above sea level and receives an annual precipitation of 1,750mm with bimodal pattern of distribution and the mean air temperature is 28.7^0 C with a 40% relative humidity (Ambede *et al*., 2012).

3.2 Collection of Materials

3.21 Plant collection and authentication

The *Cocos nucifera* materials were collected from the East African tall coconut varieties of about 15-25m height, in Msambweni Kwale County. In Msambweni, the East African tall varieties and dwarf coconut varieties are indigenous to the area (Maurice *et al*., 2015).The authentication of the collected materials was done by the Ministry of Agriculture Kwale County.

3.2.2 Coconut Leaves

Fresh, healthy coconut leaves were collected in June in Msambweni Kwale County. Sections from the most recent fully expanded leaf (third leaf from the tip) of a 10-leaf plant were used (Oyedokun *et al*., 2011). The leaves were preserved in sterile paper bags which were sealed and transported to the Botany Laboratory at Maseno University for preparation of extract within 24hours.

3.2.3 Coconut Root

Fresh healthy samples of coconut root were collected from the tall East African coconut varieties in Msambweni. The samples were air dried under a shade for one week and twenty five root pieces, of 30cm each cut and preserved in sterile paper bags (Rajkumar *et al*., 2012).The sterile paper bags were sealed and transported to the Botany Laboratory at Maseno University for preparation of extract within 72 hours.

3.2.4 Coconut Husk

The coconut husks were obtained from coconut fruits of the tall East African coconut varieties in Msambweni, Kwale County. Four complete coconuts including the epicarp and mesocarp were collected Cyriac *et al*. (2013). The coconuts were packed in a sack and transported to the Botany Laboratory at Maseno University where the fibrous husk was removed for preparation of extract within 72 hours.

3.3 Extract Preparation

3.3.1 Coconut Leaves

The *Cocos nucifera* leaf aqueous extracts were prepared as described by Venkat *et al*. (2012), Olufemi and Oluwasegun (2015), Umar and Mwonjoria (2015) and with some modifications. Leaves of *Cocos nucifera* were washed thoroughly with distilled water to remove any dirt and debris .The leaves were air dried for 21 days and then cut into small pieces. The cut leaves were mechanically ground with a grinding machine into powder.

The powder was passed through a sieve with a mesh of 0.841mm to obtain a fine powder. One hundred gram (100 g) of the powdered leaf was poured into 1000 ml of water and left for 48hrs in a plastic bottle of 2 litre size. The mixture was shaken thoroughly, allowed to settle and decanted. The decanted solution was filtered using Whatman No 1 filter paper to obtain the crude leaf extract. The extract was stored at 4^0C for later use. The extract concentrations included 75%, 50% and 25%. The 75% concentration was prepared by diluting 75 ml crude leaf extract of *Cocos nucifera* with 25 ml of distilled water. The 50% concentration was prepared by diluting 50 ml of crude leaf extract of *Cocos nucifera* with 50 ml of distilled water and 25% concentration was prepared by diluting 25 ml of the extract with 75 ml of distilled water.

3.3.2. Coconut Roots

The root extracts were prepared as described by Sivakumar *et al*. (2011) and Umar and Mwonjoria (2015) with some modifications. The roots were cut to lengths of about 5cm and then pounded to obtain a coarse powdered root of *Cocos nucifera.* The root powder was passed through a sieve with a mesh of 0.841mm to obtain a fine powder. One hundred gram (100 g) of the root powder was poured into 1000 ml of water and left for 48hrs in a plastic bottle of 2 litre size. The mixture was shaken thoroughly, allowed to settle and decanted.

The decanted solution was filtered using Whatman No 1 filter paper to obtain the crude root extract. The extract was stored at 4^0C for later use. The extract concentrations included 75%, 50% and 25%. The 75% concentration was prepared by diluting 75 ml crude root extract of *Cocos nucifera* with 25 ml of distilled water. The 50% concentration was prepared by diluting 50 ml of crude root extract of *Cocos nucifera* with 50 ml of distilled water and 25% concentration was prepared by diluting 25 ml of the root extract with 75 ml of distilled water.

3.3.3. Coconut husks

The aqueous coconut husk extract were prepared as described by Cyriac *et al*.(2013) and Umar and Mwonjoria (2015).The fibrous husks of *Cocos nucifera* were washed with distilled water to remove dirt, cut into smaller pieces and air dried for 21 days. The dried husk was then blended using a household electric blender. The husk powder was passed through a sieve with a mesh of 0.841mm to obtain a fine powder. One hundred gram (100 g) of the husk powder was poured into 1000 ml of water and left for 48hrs in a plastic bottle of 2 litre size. The mixture was shaken thoroughly, allowed to settle and decanted.

The decanted solution was filtered using Whatman No 1 filter paper to obtain the crude husk extract. The extract was stored at 4^0C for later use. The extract concentrations included 75%, 50% and 25%. The 75% concentration was prepared by diluting 75 ml crude husk extract of *Cocos nucifera* with 25 ml of distilled water. The 50% concentration was prepared by diluting 50 ml of crude husk extract of *Cocos nucifera* with 50 ml of distilled water and 25% concentration was prepared by diluting 25 ml of the extract with 75 ml of distilled water.

3.4 Source of Maize Seeds

Clean certified seeds of PAN 15 variety were purchased from a licenced agrovet. It is known to be highly susceptible to *Busseola fusca*; free from weeds seeds and has a high germination percentage (Marcel, 2009). It takes 3½ months to reach maturity.

3.5 Planting preparations

An area was identified within a naturally illuminated greenhouse at Maseno University Farm. The conditions in the green house during the experiment were not controlled. Clearing was done in November 2017 within the greenhouse. Maize grains of PAN 15variety were sown in 45 plastic pots of 30 cm in diameter and 40 cm in height with holes at the bottom for drainage. The pots were filled with a mixture of local soil, classified as kandiudalfic Eutrodox (USDA, 1992) and organic manure at a ratio of 1:2. Seedlings were thinned to 3 plants per pot. The plants received DAP fertilizer at the rate of 2 g per plant according to Adda *et al*. (2011). CAN was applied at three weeks and six weeks interval after planting (ICIPE, 2013).

3.6 Collection and handling of *Busseola fusca*

Busseola fusca (Fuller) neonates reared on meridic diet were obtained from International Center for Physiology and Ecology (ICIPE), mass-*rearing* unit (Nairobi, Kenya). They were reared on an artificial diet as described by Onyango and Ochieng'-Odero (1994). During transportation, the vials were packed in sterilized, transparent plastic jars of 20cm by 15cm in diameter and depth respectively. The jars were covered with a mesh for aeration. The vials were cushioned with cotton to minimize collision during transportation. The diet incorporated 4 to 8-week-old sorghum powder in a nutritionally adequate diet. The larvae were reared individually in vials at ambient laboratory conditions 25–30 °C, 50–80% relative humidity, and 12hours of light to 12hours of darkness in the Botany laboratory at Maseno university for one week as documented by Onyango and Ochieng'-Odero (1994).

3.7 Experimental Design and Treatment

The experimental design was adopted from Musyimi *et al*. (2007) with some modifications. The experiment was laid out in Completely Randomised Design with three replicates. The treatments were individual potted maize plants treated with 75%, 50% and 25% concentrations of *Cocos nucifera* aqueous leaf, husk and root extract. Karate, a synthetic pyrethroid was used as the positive control and distilled water as the negative control of (0%)

A known number of *Busseola fusca* neonates were released close to the leaf whorl on 20 days old plants (ICIPE,2013). Infestation was done during morning hours between 0800 and 1100 hours in order to avoid larval mortality due to high temperature. The whorl was tapped gently before infestation to avoid the drowning of larvae in the water accumulated in plant whorl (Chouraddi and Mallapur, 2017). Immediately after infestation mosquito nets were used to enclose all the pots in order to restrict movement of the stem borers to unintended areas within the greenhouse. The potted plants were treated differently and fixed quantity of 2 liters of each concentration of the aqueous extract were prepared and partially used to treat the plants receiving the same concentration in each trial (Chouraddi and Mallapur, 2017). All the parts of the plants were copiously wetted with the extract using a local hand-held mist blower and remaining quantity of each concentration was discarded. Observations were made at 15 days interval. The maize were subjected to the treatments on a weekly basis from the time of colonization by the stem borer and this was done in the evening at 0430 hours to 0530 hours to avoid evaporation of the biopesticide due to high temperatures (Chouraddi and Mallapur, 2017).

3.8. Measurement of Parameters

3.8.1 Screening of the phytochemical composition of *Cocos nucifera* extracts

Phytochemical screening of the aqueous extracts of *Cocos nucifera* was carried out to identify presence or absence of selected chemical constituents using methods of analysis as described by Adeniyi *et al.* (2010) and Kumar *et al*. (2009) with some modifications.

3.8.1.1Test for Tannins

To 3ml of *Cocos nucifera* leaf extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicated the presence of tannins (Kumar *et al*., 2009). The procedure was repeated for husk and root extracts.

3.8.1.2 Test for Saponins

An emulsion test as described by Kumar *et al*. (2009) with some modifications was used. To 3ml of *Cocos nucifera* leaf extracts in a test tube, 5 drops of olive oil were added. The mixture was vigorously shaken. Formation of a stable emulsion indicated the presence of saponins. The procedure was repeated for husk and root extracts.

3.8.1.3 Test for Steroids

To 3ml of *Cocos nucifera* leaf extracts, 2ml chloroform was added then filtered. The filtrates were treated with 3 drops of acetic anhydride, boiled and cooled. Concentrated Sulphuric acid was added. Formation of brown ring at the junction indicated the presence of steroids (Adeniyi *et al.*,2010). The procedure was repeated for husk and root extracts.

3.8.1.4 Test for Terpenoids

To 3 ml of *Cocos nucifera* leaf extracts, 2 ml of chloroform was added, and 3ml of concentrated Sulphuric acid carefully added to form a layer (Adeniyi *et al.*,2010). A reddish brown colouration of the interface indicated the presence of terpenoids. The procedure was repeated for husk and root extracts.

3.8.1.5 Test for Flavonoids

To 3ml of *Cocos nucifera* leaf extracts, 1cm^3 of 10% sodium hydroxide solution was added. Formation of a yellow colouration, indicated the presence of flavonoids (Kumar *et al*. 2009). The procedure was repeated for husk and root extracts.

3.8.1.6 Test for Alkaloids

To 3ml of *Cocos nucifera* leaf extracts, 3ml of 1% Hcl was added heated in a hot water bath for 10minutes. Two drops of Mayer's reagent were added. A creamy precipitate indicated the presence of alkaloids in the extract (Kumar *et al*., 2009). The procedure was repeated for husk and root extracts.

3.8.1.7 Test for Glycosides

To 3ml of *Cocos nucifera* leaf extracts, 1ml of glacial acetic acid containing one drop of 0.1% ferric chloride solution was added. To this 1ml concentrated Sulphuric acid was added. A brown ring indicated presence of glycosides (Adeniyi *et al.,* 2010). The procedure was repeated for husk and root extracts.

3.8.2 Determination of Mortality of Stem Borer in maize plants

The number of dead stem borers per pot was noted and mean mortality per treatment calculated. The stems were carefully opened at the end of the experiment to get the number of dead stem borers according to Ande *et al*. (2010).

3.8.3 Determination of the effects of the control of *Busseola fusca* on growth and yield of maize.

3.8.3.1 Determination of Leaf damage in maize plants

From each pot, visual observations of leaf damage were made using the visual rating scale by (Chouraddi and Mallapur, 2017). The observations were recorded at weekly interval starting from the fourth week after introduction of the *Busseola fusca* into the pots. The mean was calculated from the three replicates according to Bediako and Thanguane (2012).

Table 3.1: Rating Scale for Assessment of Leaf Damage by *Busseola fusca*

Scale (1-9)	**Description**
1	No visible leaf feeding damage
2	Few pin holes on older leaves
3	Several shot-holes injury on a few leaves
4	Several shot-holes or small lesions injury common on several leaves
5	Elongated lesions (> 2 cm long) on a few leaves
6	Elongated lesions on several leaves
7	Several leaves with elongated lesions or tattering
8	Most leaves with elongated lesions or severe tattering
9	Plant dying as a result of foliar damage

Retrieved from Chouraddi and Mallapur (2017)

Plate 3.1: Leaf damage by *Busseola fusca*

3.8.3.2 Determination of Dead hearts in maize

The number of dead hearts was recorded through visual inspection of the potted maize plants. The total number was recorded during each inspection and the mean calculated from the three replicates according to Bediako and Thanguane (2012).

Plate 3.2: Dead heart as a result of *Busseola fusca* feeding

3.8.3.3 Determination of Borer holes in maize

The incidence of borer holes per plant was noted by direct inspection of the maize stems for borer holes. The total number of borer holes noticed was recorded per plant on each inspection. Mean stem borer holes were calculated from values recorded from the three replicates and recorded as by Ande *et al*. (2010).

3.8.3.4 Determination of chlorophyll concentration

Chlorophyll concentration was obtained by soaking the leaves in 85% acetone solution which was based on the work by Shibghatallah *et al*. (2013) and measuring its absorbance using Single Beam UV/visible Spectrophotometer at $\lambda = 663$ nm and $\lambda = 645$ nm using the equation;

$$C_{chl\text{-}a}=12.7A_{663}-2.69A_{645}$$

$$C_{chl\text{-}b}=22.9A_{645}-4.68A_{663}$$

Where Chlorophyll a concentration = $C_{chl\text{-}a}$

Chlorophyll b concentration = $C_{chl\text{-}b}$

Absorbance at λ 663 nm = A_{663}

Absorbance at λ 645 nm= A_{645}

Total chlorophyll concentration is $C_{chl\text{-}a}+ C_{chl\text{-}b}$

3.8.3.5. Determination of Maize Yield

To obtain the maize yield, the number of maize cobs in each plant was counted in all pots and recorded during harvest at the end of the experiment. The maize cobs were then shelled and dried to constant weight in an oven at 30°C to 35°C after which the yield quantity was weighed and recorded in grams (g) according to Wahedi *et al*. (2016).

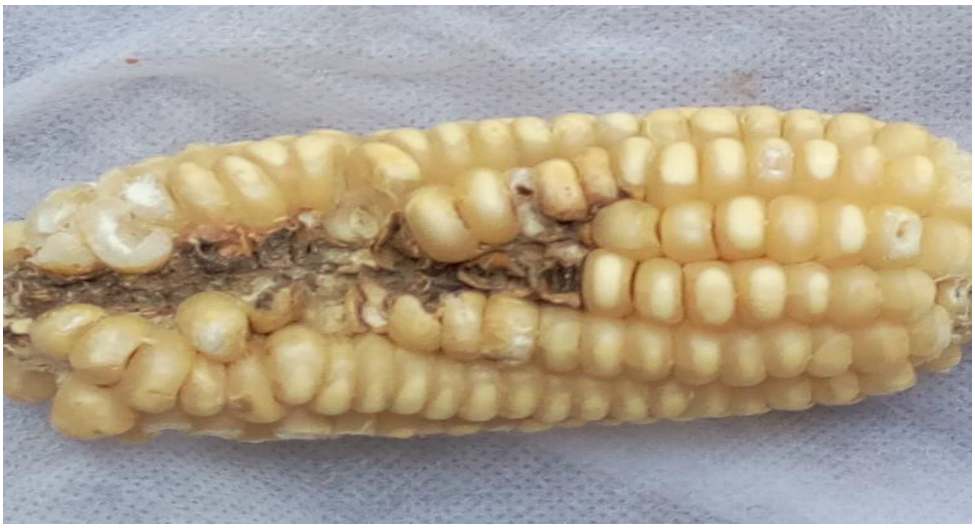

Plate 3.3: A maize cob affected by *Busseola fusca*

3.9 Data Analysis

Data obtained from the study was subjected to a univariate analysis of variance (ANOVA) to compare the effect of different concentrations of the aqueous extracts of *Cocos nucifera* on maize damage and yield. Treatment means were then separated and compared using the Least Significant Difference at 0.05 (Steel *et al*., 1992).

CHAPTER FOUR

RESULTS

4.1Phytochemical composition of *Cocos nucifera* extracts.

Qualitative chemical analysis for the phytochemical constituents of leaf, husk and root extract of East African coconut variety revealed presence of tannins, saponins, steroids, terpenoids, flavonoids and glycosides while alkaloids were absent (Table 4.1).

Table 4.1: Phytochemical constituents in *Cocos nucifera* aqueous leaf, husk and root extracts

	Presence or absence in plant part		
Phytochemical constituent	**Leaf**	**Husk**	**Root**
Tannins	+	+	+
Saponins	+	+	+
Steroids	+	+	+
Terpenoids	+	+	+
Flavonoids	+	+	+
Alkaloids	-	-	-
Glycosides	+	+	+

KEY

+: Present

-: Absent

4.2 .Mortality of Stem borer in maize plants

Generally all the extracts showed potential larvicidal activities against *Busseola fusca* for all the extract concentrations tested (Table 4.3). Thirty five and fifty days after spraying the *Cocos nucifera* extracts at the different concentrations, a significant difference was observed in the mortality of stem borer ($p \leq 0.05$; Table 4.3). However, there was no significant difference in the mortality of maize stem borer from day 65 to day 110 ($p \geq 0.05$; Table 4.3). On day 125, a significant difference in the mean number of dead *Busseola fusca* was observed compared to all the days the maize was subjected to the treatments ($p \leq 0.05$; Table 4.3).

The leaf extracts at 75% concentration showed the highest mortality effects against maize stem borer compared to the untreated control (0%), ($p \leq 0.05$,Table 4.2). The synthetic pesticide, Karate (lambda-cyhalothrin) was intermediate to the 75% leaf extract concentration (Table 4.2). The order of the larvicidal activity of the four different concentrations was 75>50>25>0%. On the other hand, the leaf extract at 50 and 25% extract concentration showed no significant difference in the mortality of maize stem borer ($p \geq 0.05$; Table 4.2).

On day 50, the 75% *Cocos nucifera* leaf extract concentration showed a significant difference in the mean number of dead *Busseola fusca* compared to the synthetic pesticide lambda-cyhalothrin ($p \leq 0.05$; Table 4.2). On the same day, the 50 and 25% leaf extract concentrations were intermediate to Karate and the untreated control (Table 4.2). The untreated control (0%) on day 50 recorded a significant difference in the mean mortality of dead maize stem borer compared to the 75% leaf extract and the synthetic pesticide lambda-cyhalothrin ($p \leq 0.05$; Table 4.2). However, no significant differences in the mortality of the *Busseola fusca* was observed in the different concentrations of the extract, lambda-cyhalothrin and the control on day sixty five after spraying ($P \geq 0.05$; Table 4.2).

Cocos nucifera husk extracts showed no significant difference in mortality of maize stem borer on day 35 across all the different concentrations as well as the synthetic pesticide ($p \geq 0.05$; Table 4.2). However, on day 50, a significant difference in the mean number of dead *Busseola fusca* was observed between Karate (lambda-cyhalothrin) and the untreated control ($p \leq 0.05$, Table 4.2). On the same day, the order of larval mortality was Karate>75>50>25>0% (Table 4.2).On

the other hand, no significant findings were observed on day sixty five across all the different husk extract concentration, the synthetic pesticide and the untreated control ($p \geq 0.05$; Table 4.2).

The *Cocos nucifera* root extract showed no significant difference in the mortality of maize stem borer on day 35 between 75% extract concentration and synthetic insecticide lambda-cyhalothrin ($p \geq 0.05$; Table 4.2).Similarly, no significant difference was observed in the mean number of dead *Busseola fusca* at 50%, 25% and the untreated control respectively.

On day 50, Karate showed a significantly higher larvicidal activity against *Busseola fusca* compared to all the different root extract concentrations ($p \leq 0.05$; Table 4.2). However, the 25% and untreated control showed no larvicidal activity against maize stem borer (Table 4.2). Generally, no larvicidal activity was observed on day 65 across all the extracts ($p \geq 0.05$; Table 4.2).

Table 4.2: The larvicidal activity of *Cocos nucifera* extracts at different concentration on mortality of *Busseola fusca* in maize plants

	Mortality of *Busseola fusca*			
	Days	**35**	**50**	**65**
Plant part	**Treatment**			
Leaf	0%	0.0000c	0.3333c	0.00a
	25%	0.3333bc	0.6667bc	0.00a
	50%	0.6667bc	1.0000bc	0.00a
	75%	1.6667a	2.333a	0.00a
	Karate	1.0000ab	1.3333b	0.00a
	LSD	0.8136	0.9395	0
Husk	0%	0.000a	0.0000c	0.00a
	25%	0.000a	0.3333bc	0.00a
	50%	0.333a	0.6667abc	0.00a
	75%	1.000a	1.0000ab	0.00a
	Karate	0.6667a	1.3333a	0.00a
	LSD	1.0504	0.8136	0
Root	0%	0.0000b	0.0000b	0.00a
	25%	0.0000b	0.0000b	0.00a
	50%	0.0000b	0.3333b	0.00a
	75%	0.6667a	0.6667ab	0.00a
	Karate	0.667a	1.3333a	0.00a
	LSD	0.6643	0.8136	0

Means with the same letter down the column are not significantly different ($p \geq 0.05$)

Table 4.3 A summary on larvicidal activity of *Cocos nucifera* extracts on mortality of *Busseola fusca* in maize plants

Variables	Mortality of *Busseola fusca*
Plant part	
Leaf	0.54a
Husk	0.35b
Root	0.22c
LSD	0.09
Extract concentration	
0%	0.05e
25%	0.19d
50%	0.35c
75%	0.73a
KARATE	0.56b
LSD	0.12
Day	
Day 35	0.47c
Day 50	0.76b
Day 65	0.00d
Day 80	0.00d
Day 95	0.00d
Day 110	0.00d
Day 125	1.40a
LSD	0.14

Means with the same letter down the column are not significantly different ($p \geq 0.05$).

4.3 Effects of the control of *Busseola fusca* on growth and yield of maize plants

4.3.1 Leaf damage in maize plants

The effect of the coconut leaf extract across all the different concentrations showed a significant reduction in leaf damage by *Busseola fusca* on day 35 and 42 ($p \leq 0.05$; Table 4.4). On day thirty five, the untreated control recorded a significantly higher leaf damage compared to all the different extract concentrations as well as karate ($p \leq 0.05$; Table 4.4). On the same day, it was observed that, the leaf extract at 75% concentration recorded a significant reduction in leaf damage ($p \leq 0.05$; Table 5).However, no significant difference was observed in leaf damage between 50% leaf extract concentration and lambda-cyhalothrin ($p \geq 0.05$;Table 4.4).

The results of the leaf extract revealed a significant reduction in leaf damage by maize stem borer on day forty two across all the treatments ($p \leq 0.05$; Table 4.4). The effect of leaf extract at 75% concentration was observed to be the best treatment in reducing leaf damage by maize stem borer. However, from the results the highest leaf damage on day forty two was detected at the untreated control (Table 4.4). The order of reduction of leaf damage across all the different extract concentration was observed to be as follows75>50>25% (Table 4.4).

The results from the effects of the husk extract exhibited a significantly reduced leaf damage across the different extract concentrations on day 35 and day 42 ($p \leq 0.05$; Table 4.4) .Although there was no statistical difference between Karate and 50% concentration on both day 35 and 42 ($p \geq 0.05$; Table 4.4). The untreated control was the least effective in reducing leaf damage by *Buseeola fusca* .

The results of the effects of the husk extract at 25%concentration and the untreated control (0%) on day forty two showed no significant difference in reducing leaf damage by *Busseola fusca* ($p \geq 0.05$; Table 4.4) . Among all the different husk extract concentration on day forty two, the 75% was recorded to be the best treatment in reducing the leaf damage by maize stem borer (Table 4.4).

The root extract at 75% concentration on day thirty five showed the highest reduction in leaf damage compared to all the treatments. However, on the same day, the untreated control (0%) and the 25% concentration showed no statistical difference ($p \geq 0.05$; Table 4.4). The effects of

the synthetic insecticide (lambda-cyhalothrin) on leaf damage were observed to be intermediate to the 50 and 75% concentration (Table 4.4). The results described in Table 4.4 revealed that the untreated control recorded the highest leaf damage followed by 25%, 50%, Karate and 75% respectively.

On day forty two, no significant difference was observed between the untreated control and the 25% root extract concentration in the reduction of maize leaf damage ($p \geq 0.05$; Table 4.4). However, from Table 4.4, the synthetic pesticide was observed to be an intermediate between the 50 and 75% root extract concentration. On the same day, the untreated control recorded the highest leaf damage while the 75% root extract recorded the least maize leaf damage (Table 4.4).

Table 4.4: A table showing Leaf damage by *Busseola fusca* in maize plants

	Leaf damage		
	Days	**35**	**42**
Plant part	**Treatment**		
Leaf	0%	8.3333a	9.0000a
	25%	6.0000b	7.3333b
	50%	3.3333c	5.0000c
	75%	1.3333d	2.0000e
	Karate	3.0000c	3.6667d
	LSD	1.4092	1.3286
Husk	0%	8.667a	9.0000a
	25%	7.333b	8.3333a
	50%	4.333c	5.6667b
	75%	2.6667d	3.0000c
	Karate	4.000c	5.3333b
	LSD	1.2428	1.4092
Root	0%	8.0000a	8.6667a
	25%	7.6667a	8.3333a
	50%	5.6667b	6.3333b
	75%	3.6667c	4.0000c
	Karate	5.3333bc	5.0000bc
	LSD	1.8789	1.9929

Means with the same letter down the column are not significantly different ($p \geq 0.05$)

4.3.2. Dead hearts in maize plants

Generally, the leaf, husk and root extracts showed a significant difference in the mean number of dead hearts in maize plants ($p \leq 0.05$; Table 4.6).On the other hand, the overall observation was that the 75% extract concentration, recorded the least number of dead hearts, followed by 50%, Karate (lambda-cyhalothrin), 25% and the untreated control (0%) respectively (Table 4.6). Fifty to one hundred and twenty five days after spraying the *Cocos nucifera* extracts, there were no statistically significant differences in the mean number of dead hearts ($p \geq 0.05$; Table 4.6).

The different concentrations of the leaf extract and lambda-cyhalothrin, exhibited no significant difference in the number of dead hearts in maize on day thirty five ($p \geq 0.05$; Table 4.5). However, the untreated control (0%), recorded the highest number of dead hearts compared to Karate (Table 4.5). The lowest number of dead hearts was observed in the 75% and 50% extract concentration (Table 4.5). Similarly, fifty and sixty five days after spraying, there were no statistically significant differences in mean number of dead hearts recorded except for the untreated control ($p \geq 0.05$; Table 4.5).

The results from the husk extracts on day thirty five, showed no significant difference in the mean number of dead hearts in the different concentrations and lambda-cyhalothrin ($p \geq 0.05$; Table 4.5). However, the untreated control recorded a significantly higher number of dead hearts compared to the different extract concentrations ($p \leq 0.05$; Table 4.5). Fifty and sixty five days after spraying, the 75% and Karate showed no significant difference in the mean number of dead hearts.

Similarly, on day thirty five, the effect of the different concentrations of the *Cocos nucifera* root extracts and Karate (lambda-cyhalothrin) showed no significant difference in the number of dead hearts ($p \geq 0.05$;Table 4.5).The untreated control(0%), showed a significantly high number of dead hearts compared to all the different extract concentrations. On the other hand, results from day fifty and sixty five showed no significant difference in the number of dead hearts in maize across all the different concentrations including the synthetic pesticide ($p \geq 0.05$; Table 4.5).However, the untreated control showed a statistically significant difference in the number of dead hearts compared to all treatments ($p \leq 0.05$;Table 4.5).

Table 4.5: Effects of different concentrations of *Cocos nucifera* extracts on dead hearts in maize plant

		Dead hearts		
	Days	**35**	**50**	**65**
Plant part	**Treatment**			
Leaf	0%	1.0000a	1.0000a	1.0000a
	25%	0.3333b	0.3333b	0.3333b
	50%	0.0000b	0.0000b	0.0000b
	75%	0.0000b	0.0000b	0.0000b
	Karate	0.3333b	0.3333b	0.3333b
	LSD	0.6643	0.6643	0.6643
Husk	0%	1.000a	1.0000a	1.0000a
	25%	0.333b	0.6667ab	0.6667ab
	50%	0.000b	0.3333bc	0.3333bc
	75%	0.000b	0.0000c	0.0000c
	Karate	0.000b	0.0000c	0.00c
	LSD	0.4697	0.6643	0.6643
Root	0%	1.0000a	1.3333a	1.3333a
	25%	0.3333a	0.6667a	0.6667a
	50%	0.3333a	0.3333a	0.3333a
	75%	0.3333a	0.3333a	0.3333a
	Karate	0.3333a	0.3333a	0.3333a
	LSD	1.2428	1.0504	1.0504

Means with the same letter down the column are not significantly different ($p \geq 0.05$)

Table 4.6: A summary of the effects of different concentrations of *Cocos nucifera* extracts on dead hearts in maize plants

Variables	Number of dead hearts
Cocos nucifera extracts	
Leaf extracts	0.33c
Husk extracts	0.38b
Root extracts	0.57a
LSD	0.04
Extract concentration	
0%	1.10a
25%	0.52b
50%	0.19c
75%	0.11d
KARATE	0.22c
LSD	0.05
Day	
Day 35	0.36b
Day 50	0.44a
Day 65	0.44a
Day 80	0.44a
Day 95	0.42a
Day 110	0.44a
Day 125	1.40a
LSD	0.06

Means with the same letter down the column are not significantly different ($p \geq 0.05$)

4.3. 3. Borer holes in maize plants

There was a significant difference in the overall mean number of borer holes from day 35 to 125 ($p \leq 0.05$; Table 4.8). A significant reduction in the mean number of borer holes was observed across all the different *Cocos nucifera* extract concentration ($p \leq 0.05$; Table 4.8). The leaf extract recorded the least number of borer holes compared to the root and husk extract (Table 4.8). The 75% extract concentration, exhibited the highest reduction in the number of borer holes and the lowest was observed in the untreated control (0%) ($p \leq 0.05$; Table 4.8).

The effects of the leaf extract at 75% recorded the highest reduction of borer holes in maize across all the days compared to lambda-cyhalothrin (Table 4.7). However, no significant difference was observed in reducing borer holes on day 35, 50, 65 and 80 across the 50 and 25% concentration as ($p \geq 0.05$; Table 4.7).The order of reduction of borer holes was 75>Karate>50>25>0% across all the treatments from day 35 to 125 ($p \leq 0.05$; Table 4.7). There was a significant increase in the number of borer holes from day 35 to day 125 (Table 4.7).

The effects of the husk extract at 75% on day thirty five were observed to significantly reduce the number of borer holes compared to all the different extract concentrations. No significant difference was observed between the 50 and 25% extract concentration ($p \geq 0.05$; Table 4.7). The untreated control (0%) recorded a significantly high number of borer holes in maize compared to all the treatments from day 35 to day 125 ($p \leq 0.05$; Table 4.7). The different treatments reduced the number of borer holes from 75>Karate>50>25% respectively (Table 4.7).

On day 35, the 75% root extract showed a significant reduction in the number of borer holes in maize compared to Karate (lambda-cyhalothrin) and the 50% extract concentration ($p \leq 0.05$; Table 4.7). On the same day, no statistical difference was observed between the 25% concentration and the untreated control ($p \geq 0.05$; Table 4.7). However, the untreated control exhibited a significantly high number of borer holes from day 35 to 125 (Table 4.7). On the other hand, the least number of borer holes throughout the growing period was observed at 75%, Karate, 50% 25% and untreated control respectively (Table 4.7).

Table 4.7: Effects of different concentrations of *Cocos nucifera* extracts on borer holes in maize plants

		Borer holes						
	Days	**35**	**50**	**65**	**80**	**95**	**110**	**125**
Plant part	**Treatment**							
	0%	5.067a	15.767a	20.333a	23.767a	17.333b	19.700b	49.300a
	25%	3.967a	4.700b	9.000b	11.200b	36.433a	44.000a	18.667b
Leaf	50%	4.300a	4.700b	6.500b	7.567b	12.800b	14.933bc	16.967b
	75%	1.300b	2.400b	3.633b	4.533b	5.767b	7.267c	10.200b
	Karate	2.633ab	3.300b	5.200b	6.767b	7.300b	9.000bc	11.600b
	LSD	2.453	9.8272	10.398	12.066	11.787	11.657	10.167
	0%	5.5333a	15.867a	24.933a	32.733a	55.333a	58.333a	63.000a
	25%	4.200ab	6.967ab	11.000b	16.733ab	22.967b	25.467b	28.167b
Husk	50%	3.8667ab	5.100b	10.533b	12.967b	19.00bc	20.667b	22.333bc
	75%	1.4233c	2.700b	3.833b	8.067b	8.333c	10.967c	13.600c
	Karate	3.1667bc	5.300b	6.867b	10.600b	12.677bc	16.333bc	19.333bc
	LSD	1.8456	9.3404	13.674	18.157	10.783	9.2399	9.0847
	0%	7.4000a	9.533a	19.200a	23.13a	29.600a	36.533a	40.133a
Root	25%	6.5333a	8.267ab	12.167ab	15.8ba	18.567ab	21.833b	24.433b
	50%	4.7333b	6.667bc	9.433b	12.1bc	17.033b	19.867b	22.833b
	75%	0.7333d	3.433d	5.567b	7.27c	11.567b	14.833b	17.200b
	Karate	2.3333c	5.167cd	6.600b	12.13bc	15.347b	18.167b	21.267b
	LSD	1.3178	2.2557	7.0483	8.21	11.158	9.7522	9.5996

Means with the same letter down the column are not significantly different ($p \geq 0.05$).

Table 4.8: A summary of the effects of different concentrations of *Cocos nucifera* extracts on the number of borer holes in maize plants

Variables	Number of borer holes
Cocos nucifera extracts	
Leaf extracts	12.23c
Husk extracts	16.83a
Root extracts	14.50b
LSD	0.98
Extract concentration	
0%	27.26a
25%	16.72b
50%	12.14c
75%	6.687e
KARATE	9.58d
LSD	1.26
Day	
Day 35	3.81g
Day 50	6.66f
Day 65	10.32e
Day 80	13.69d
Day 95	19.34c
Day 110	22.53b
Day 125	25.27a
LSD	1.49

Means with the same letters along the column are not significantly different

4.3.4 Chlorophyll concentration in maize plants

The effects of the leaf extract at 75% showed the highest chlorophyll concentration in the maize plants from day 35 to day 50 hence the most effective compared to all the treatments (Table 4.9). The results showed a significant difference in the amount of chlorophyll concentration in maize across all the treatments in both days ($p \leq 0.05$; Table 4.9).The least amount of chlorophyll concentration in the maize plants was observed in the untreated control (0%) (Table 4.9). The order of chlorophyll retention was 75>Karate>50>25>0% (Table 4.9). Generally, from day 35 to day 50 the results showed a decline in maize chlorophyll concentration in all the treatments (Table 4.9).

The husk extract demonstrated a significant difference in the reducing the maize chlorophyll concentration on day 35 from 75%, Karate, 50%, 25% and 0% (untreated control) respectively ($p \leq 0.05$; Table 4.9). A decline in the maize chlorophyll concentration was observed between day 35 and 50 (Table 4.9).Thirty five and ninety days after spraying, the highest chlorophyll retention was observed in the 75% husk extract concentration. The least amount of chlorophyll in the maize plants was recorded in the untreated control (Table 4.9).

The root extract showed a statistically significant difference in the amount of chlorophyll retained in the maize plants on day 35 and 50 ($p \leq 0.05$; Table 4.9). The 75% root extract concentration demonstrated the highest maize chlorophyll retention followed by Karate, 50%, 25% and untreated control (0%) respectively in both days (Table 4.9).

Generally, maize plants treated with the leaf extract at 75% concentration showed the highest chlorophyll retention compared to the husk and root extract at the same concentration hence the most effective extract (Table 4.9).

Table 4.9: The effects of the different *Cocos nucifera* extract concentration on total chlorophyll concentration after 35 and 50 days since initiation of the experiment

	Chlorophyll content		
	Days	**35**	**50**
Plant part	**Treatment**		
Leaf	0%	39.3667e	29.2667e
	25%	48.5633d	39.0400d
	50%	57.8700c	45.1900c
	75%	77.2300a	65.0200a
	Karate	68.7633b	56.1900b
	LSD	1.4069	1.185
Husk	0%	24.1533e	18.6000e
	25%	31.6933d	25.1767d
	50%	41.7567c	33.1133c
	75%	62.9133a	52.0000a
	Karate	54.9300b	43.1000b
	LSD	1.5172	1.2317
Root	0%	19.3133e	11.9467e
	25%	26.0967d	18.8833d
	50%	37.4100c	26.5400c
	75%	61.1600a	51.3267a
	Karate	49.28b	39.3767b
	LSD	1.1113	1.3755

Means with the same letter down the column are not significantly different ($p \geq 0.05$)

4.3.5 Maize yield

The 75% leaf extract concentration showed a significant difference in increasing maize yield compared to the Karate, 50%, 25% and the untreated control ($p \leq 0.05$; Table 4.10). Generally, the highest yields were observed at 75% leaf extract concentration (Table 4.10). The lowest yields recorded were at 0% (untreated control) (Table 4.11). The order of the maize yields obtained in all the treatments was 75>Karate>50>25>0% (untreated control) (Table 4.10).

The husk extract showed a significant difference in the maize yield obtained across all the treatments ($p \leq 0.05$;Table 4.10).However, the maize yields obtained from the untreated control were intermediate to the yields obtained from the 50 and 25% extract concentration (Table 4.10). The order of the yields in all the treatments was 75>Karate>50>25>0% husk extract concentration.

The root extract registered the lowest maize yields across all the treatments in comparison to the husk and leaf extract at different concentrations (Table 4.11).The effects of the root extract showed a significant difference between the 75%, Karate (lambda-cyhalothrin) and the untreated control ($p \leq 0.05$; Table 4.10).However, there was no significant difference in the quantity of maize yield obtained between the 50 and 75% extract concentration ($p \geq 0.05$; Table 4.10).

Table 4.10: The effects of the different *Cocos nucifera* extract concentration on Maize Yield after 125 days since initiation of the experiment

	Maize Yield (in grams)	
	Day	**125**
Plant part	**Treatment**	
Leaf	0%	54.300d
	25%	73.667cd
	50%	92.900c
	75%	182.833a
	Karate	132.600b
	LSD	20.861
Husk	0%	66.43 cd
	25%	58.87d
	50%	84.30c
	75%	175.23a
	Karate	120.43b
	LSD	22.593
Root	0%	54.167d
	25%	73.767c
	50%	85.000c
	75%	153.100a
	Karate	105.667b
	LSD	19.185

Means with the same letter down the column are not significantly different ($p \geq 0.05$)

Table 4.11: A Summary of the effect of different concentrations of *Cocos nucifera* extracts on maize yield

Variables	Quantity of yield (in grams)
Cocos nucifera extracts	
Leaf extracts	107.26a
Husk extracts	101.05ab
Root extracts	94.34b
LSD	9.84
Extract concentration	
75%	170.39a
50%	87.40c
25%	68.77d
0%	58.30d
KARATE	119.57b
LSD	12.70

Means with the same letter down the column not significantly different ($p \geq 0.05$)

CHAPTER FIVE

DISCUSSION

5.1Phytochemical composition of *Cocos nucifera* extracts

The phytochemical screening of the leaf, husk and root extract of *Cocos nucifera* confirmed the presence of tannins, saponins, steroids, terpenoids, flavonoids and glycosides. Alkaloids were found to be absent. These results are in agreement with those of (Sivakumar *et al*., 2011). The larvicidal potency of the *Cocos nucifera* against *Busseola fusca* extracts could be attributed to the presence of bioactive compounds. Plant extracts for pest control has been documented to have several advantages in terms of preventing the development of insecticide resistance due to the presence of several bio-active compounds, their low persistence in the environment and their generally low cost of use, particularly for smallholder farmers with limited income (Angioni *et al*., 2005; Caboni *et al*., 2006; Isman, 2008).

Tannins were found to be present in all the extracts and they are commonly known to produce higher concentration levels of semiquinone radicals (from tannin oxidation).The elevated levels of the radicals have been associated with increased oxidative stress in the mid gut tissues and decreased larval performance in insects (Barbehenn and Peter, 2011).

Saponins were also found to be present in all coconut extracts and they are known to have anti feeding activity against a number of insects (Barbosa *et al*.,1990).They have also been reported to show anti feeding activities against insect larvae such as those of *Spilosoma obliqued* larvae (Jain and Tripathi, 1999). Saponins have also been documented to cause upto 90% cumulative mortality of *Spodoptera littoralis* at the larval stage. The presence of saponins in all the *Cocos nucifera* shows the larvicidal potential of the extract against *Busseola fusca*. Several researches have shown saponins as growth regulators of many insect species. The effect of saponins is generally characterized by disturbance of developmental stages and moulting failure (Chaieb, 2010).

The presence of steroids in the extracts of *Cocos nucifera* indicates that the extracts have larvicidal potency against *Busseola fusca* and these results are in agreement with those of Rice, (1984) who reported Toxic effects to insects have been linked to presence of terpenoids, steroids, flavonoids, tannins and phenols. Alkaloids, indoles, steroids and tannins were present in most of the plant extracts with larvicidal activities (Amaninder and Shiva, 2018).

The presence of terpenoids in the coconut extracts can support their strong use in *Busseola fusca* control due to their pesticidal properties in Neem, which are linked to their ability to inhibit insect growth. The results are in accordance with the observation by Elumalai *et al*. (2015) who reported 100% mortality of the larvae of three species of mosquitoes when exposed to methanol extracts of *Leucas aspera,* which was attributed to the presence of major compounds such as terpenoids, steroids, tannins, flavonoids and glycosides.

The presence of flavonoids in the extracts of *Cocos nucifera* indicates that the extracts have larvicidal potency against *Busseola fusca*. Flavonoids have been reported to inhibit digestion in insects and function as deterrent agents (Acheuk & Doumandji-Mitiche, 2013). Flavonoids have larvicidal activity against chikungunya and malarial vectors (Velu *et al*., 2015). Based on the fore mentioned results from different research studies, the presence of flavonoids in coconut extracts supports their larvicidal potential against maize stem borer.

Alkaloids were not identified from the plant extracts although they are commonly known to have larvicidal and antifeedant activities against insect pest and larvae (Emam *et al*., 2009, Acheuk and Doumandji-Mitiche, 2013, Lee, 2000, Velu *et al*., 2015).These results are in agreement with those of Sivakumar *et al*., (2011), who observed absence of alkaloids in the aqueous extract of *Cocos nucifera* root in India

The presence of glycosides in the extracts of *Cocos nucifera* can support their potential larvicidal properties against *Busseola fusca* .This is because glycosides have been documented to possess larvicidal activities against larvae of the camel tick (Al-Rajhy *et al*., 2003). They also exhibit insect growth inhibitory activity against the cotton pest insect. Glycosides also possess insect anti feeding on gypsy moths (Bowers and Puttick, 1989). Based on the above mentioned research

studies, the presence of glycosides in the extract may have affected the activity of *Busseola fusca.*

The effectiveness of most botanicals against insect pests has been attributed to the presence of phytochemicals such as saponins, alkaloids, tannins as reported by Mizubuti *et al*. (2007) hence the larvicidal activities of *Cocos nucifera* leaf, husk and root extract against *Busseola fusca* can be attributed to the phytochemicals present.

5.2 .Mortality of Stem borer

Cocos nucifera leaf extract at 75% was the most effective in causing mortality of *Busseola fusca* compared to the husk and root extract. These findings differs with reports by Lima *et al*.(2015) who worked with butanolic coconut husk extract with showed 81.30% larvicidal activity. This difference could be as a result of the different solvents used during the extraction process and the concentrations used since various botanical concentrations give different results. The method of extraction and the solvent system used dictates the quality of extracts obtained (Odhiambo *et al*., 2009; Javaid and Rehman, 2011; Mahlo *et al*., 2013 and Bandor *et al*., 2013). Dabur *et al*. (2007) reported water extracts to be more effective than organic extracts. In the current study, the *Cocos nucifera* leaf, husk and root extract respectively showed a significant difference ($p \leq 0.05$; Table 4.3) in the mortality of *Busseola fusca* .The results also recorded a significant decline in the mortality of maize stem borer from the 75% extract concentration to the untreated control ($p \leq 0.05$; Table 4.3). This shows the potency of the different *Cocos nucifera* extracts, at different concentrations in reducing *Busseola fusca* larvae. These findings are in agreement with those of Shiberu, (2013) who reported that botanicals of *Chrysanthemum cinerariaefolium* recorded 75% mortality of *Busseola fusca* and the efficacy of the botanicals declined with a decrease in extract concentration level. However, the results of this study showed the 75% leaf extract concentration to cause the highest mortality of maize stem borer. These results differ with some of the observations made by Shiberu, (2013) who reported that the synthetic pesticide recorded the highest mortality of stem borer among all the treatments. This difference can be attributed to the use of aqueous leaf extract of Nicotiana spp. and *Cymbopogon citratus*; root bark aqueous extract of *Securidaca longepedunculata* F., and flower extract of *Chrysanthemum*

cinerariaefolium which may have different bioactive compounds that cause mortality of stem borer compared to the *Cocos nucifera* aqueous extracts.

The leaf extract at 75% concentration showed the highest reduction in the stem borer loads compared to the synthetic insecticide Karate. Ogendo *et al*. (2013) findings on the use of Lantana, Tephrosia and synthetic insecticide treatments, contradict with the findings of this study where the synthetic insecticide caused the highest mortality of stem borer, compared to the biopesticides. This could be attributed to the fact that he used exploratory rates of application of individual botanical pesticides to generate response curves; while in this study the plants were each treated with a fixed quantity of 2litres of each concentration of the aqueous extract. Leaf extracts at high concentrations have also been reported to contain the highest concentration of phytoconstituents (Azad and Sarker, 2017). Phytochemicals such as saponins, flavonoids, tanins and glycosides have been reported to have higher larvicidal activities against insect pests when in high concentrations (Rice, 1984 and Amaninder and Shiva, 2018).Hence the 75% leaf extract concentration may have a higher concentration of the phytochemicals, which are known to have larvicidal potential against insect pest thus causing the highest mortality of *Busseola fusca*.

The negative control (0%) caused no mortality of *Busseola fusca* compared to the leaf, husk and root extract at the different concentrations. These observations concur with those of Ogendo *et al*. (2013) who reported reduced the stem borer loads by 53, 59 and 47%, respectively by Lantana and Tephrosia treatments compared to the untreated control.

There was a significant difference in the mortality of *Busseola fusca* ($p \leq 0.05$) in day 35 as well as day 50 in the leaf, husk and root extract. There was a significant increase in the mortality of *Busseola fusca* from day 35 to day 50.These findings are in agreement with those of Calatayud *et al*. (2014) who reported that *Busseola fusca* neonates migrate to the whorl where they feed on young and tender leaves deep inside the whorl. It was also reported that the larvae can remain in the whorl especially for older plants for 6-8 weeks .From the above mentioned findings, by day 35, *Busseola fusca* was in its second instar hence any dead larvae could be seen in the whorl. By day 50, the *Busseola fusca* larvae have reached the third instar and any larvae that died between day 35 and 50 could still be observed and recorded. No significant increase in mortality of maize stem borer was observed from day 65 to day 125 ($p \geq 0.05$) in the leaf, husk and root extract.

These results are also in agreement with the Calatayud *et al*., (2014) who reported that at the third instar the larvae migrates and penetrates into the stem. Hence from day 65-125 no further increase in the mortality of *Busseola fusca* was observed the larvae had penetrated into the stem.

5.3 Effects of the control of *Busseola fusca* on growth and yield of maize

5.3.1 Leaf damage

The leaf extract at 75% concentration indicated a significant difference in the reduction of maize leaf damage ($p \leq 0.05$; Table 4.4). The *Cocos nucifera* leaf extract at 75% concentration caused the highest reduction in maize leaf damage compared to the husk and root extract. This is in agreement with reports by Dash *et al.* (2017) who reported that aqueous extracts had maximum number of phytoconstituents particularly on the vegetative parts like leaves and stems. Hence the 75% leaf extract concentration may have had the highest number of phytochemicals. According to the findings of this study, the presence of tannins, saponins, steroids, terpenoids, flavonoids, and glycosides has been associated with the larvicidal activity of the extract. Therefore the high mortality of maize stem borer caused by the 75% leaf extract may be attributed to the high number of phytochemicals present in the extract which are associated with larvicidal activities against insect pests. High mortality of maize stem borer by the 75% leaf extract in this study inturn reduced maize leaf damage on maize plants by *Busseola fusca* .This concurs to previous studies which have shown tannins to have decreased larval performance in insects (Barbehenn and Peter, 2011); saponins have shown anti feeding activity against a number of insects (Barbosa *et al*.,1990); steroids, terpenoids, flavonoids and glycosides have shown larvicidal activities (Rice, 1984 and Amaninder and Shiva, 2018).These findings differ from those of Calatayud *et al*.(2014) who reported that young *Busseola fusca* larvae do not consume any leaf tissue outside the whorls of plants. This difference could be attributed to the fact that his reports were based on field experiments while in this study the maize used as grown in a naturally illuminated green house.

The highest leaf damage was observed at 0% (negative control) extract concentration across all the extracts. These findings are in agreement with those of Ogendo *et al*. (2013) who reported the highest damage of *Busseola fusca* on different plant parts was with the untreated control.

The leaf, husk and root extract at all showed a significant difference in the reduction of leaf damage at day 42. This is because by day 42, the *Busseola fusca* has reached the 3rd instar where the larvae migrates to the lower parts of the plant where they penetrate into the stem (Calatayud *et al*.,2014).

5.3.2Dead hearts

The 75%, 50%, 25% and Karate showed no significant difference in the number of dead hearts ($p \geq 0.05$) across all the days when the maize plants were subjected to the leaf extract. These results can be explained by the reports by Groote,(2001) who observed that, under severe infestation, the larvae, either in the leaf whorl or in the stem, can cuts through the meristematic tissues; the central leaves dry up to produce the 'dead heart' symptom, resulting in the death of the plant. In this study since only three plants were grown per pot and the experiment had three replicates the number of dead hearts showed a slight variation at 75%, 50%, 25% and Karate except for the 0%.

The 0% concentration recorded the highest number of dead hearts across all the extracts. These results are in agreement with those of Ogendo *et al*. (2013) who reported the highest damage of *Busseola fusca* on different plant parts with the untreated control.

There was no significant difference ($p \geq 0.05$) in the number of dead hearts from day 65 to 125 at 75%, 50%, 25%, 0% and Karate. These findings are in agreement with the report by Groote, (2001) who noted that once a dead heart has been formed it results in the death of the plant. This confirms the fact that the dead hearts formed from day 35 would either increase in day 50 after which the number remained constant.

Maize stem borer is known to be an internal feeder although the 3rd and 4th instars are known to be the most destructive stages on the leaf surface. This could be the reason why after the potted maize plants were exposed to the *Cocos nucifera* extracts the number of dead hearts remained constant after the 50th day to the end of the greenhouse trials. This observation could also be because the different larval stages of maize stem borer normally develop successfully inside the maize stem and dead hearts usually occur at the initial stages of growth. These findings are in accordance to those of Nyakuri *et al*. (2014) and Shiberu, (2013) who reported that the larvae of *Busseola fusca* feeds on soft surface of the leaves, then enters into the stem through the leaf

whorl leading to stunted growth of the maize plants and dead hearts when attacked at their initial stages.

Cocos nucifera leaf extract was the best in reducing the number of the dead hearts compared to the husk and root extract. This could be due to the presence of a high concentration of flavonoids in the leaf extract which have been found to have insecticidal, antiovipositional, antifeedant and inhibitory effects. These effects could have reduced the population of maize stem borer in the maize plants and inturn reducing the number of dead hearts .These observations concur with those of Pavela and Herda (2007) who reported that the oil from seeds of an Indian plant *Pongamia pinnata* which contained 5-6% flavonoids which were suspected to be responsible for the insecticidal, antiovipositional, antifeedant and inhibitory effects of pongam oil.

5.3.3Borer holes

The leaf extract at 75% concentration, exhibited the highest reduction in the number of borer holes. This could be attributed to the presence of high concentrations of flavonoids, terpenoids , saponins and glycosides which are feeding inhibitors to most lepidopteran larvae where *Busseola fusca* belongs. This is in agreement with reports by Dash *et al.* (2017) who reported that aqueous extracts had maximum number of phytoconstituents particularly on the vegetative parts like leaves and stems. These findings concur with earlier studies by Schoonhoven, (1982) and Koul, (2008) who reported presence of deterrent receptors in lepidopteran larvae which responded to flavonoids, glycosides and terpenoids in botanical extracts which inhibited food intake in herbivorous insects. The observations made in this study are also supported by those of Golawska, (2007) who reported saponins to lower the ability of herbivorous insects to ingest phloem and xylem sap of plants.

There was a significant difference ($p \leq 0.05$) in the number of borer holes between day 35 and day 50 across all the extracts. These findings are in agreement with those of Groote, (2001) who observed that after hatching of *Busseola fusca* the first instars move into the leaf whorls where they feed and develop on the bases of the leaves, causing lesions(borer holes). From day 65 to 125 the difference in the number of borer holes across all the extracts is minimal except for the 0% concentration. These findings are in agreement with those by Groote, (2001) who reported that by the late third or early-fourth instars stem borers bore into the stem, feeding on tissues and

making tunnels hence no further borer holes are expected. This difference could be because Groote, (2001) findings are based on field experiments as well as natural infestation by *Busseola fusca*. Whereas this study was done in a naturally lit green house with artificial infestation of the stem borers. Hence the possibility of the stem borers leaving the whorl to feed on the leaves could have contributed to the slight increase in the number of borer holes in the subsequent days.

5.3.4 Chlorophyll content

The leaf extract at 75% concentration showed a significant difference ($p \leq 0.05$) in the reduction of the amount of chlorophyll content. The chlorophyll content determines the level of photosynthesis and productivity of maize (Egli and Rucker, 2012).According to Calatayud *et al*. (2014), the neonates of *Busseola fusca* migrate first to the whorl and feed on the young and tender tissues of the whorl. This inturn reduces the chlorophyll content of the maize plant until the *Busseola fusca* reach the 3rd instar when they migrate to the lower parts of the plant to penetrate into the stem (Calatayud *et al*., 2014).

The 75% extract leaf extract concentration has larvicidal potential against *Busseola fusca* due to the phytochemicals present in the extract (Rice, 1984 and Amaninder and Shiva, 2018). The fore mentioned studies confirm that 75% leaf extract concentration shows the least reduction in chlorophyll content due to its potential in causing mortality of *Busseola fusca* that feeds on the leaves reducing the chlorophyll content.

There was a significant difference ($p \leq 0.05$) in the reduction of the chlorophyll in the maize in the leaf, husk and root extract at day 35 and 50.These findings are in agreement with Hltaywayo *et al*. (2016), who reported a decline in chlorophyll content of maize from the initial stages of growth towards maturity.

High leaf chlorophyll content has been reported to show positive correlation with pest incidence (Sadat and Chakraborty, 2017). This differs with the results of this study where the 75% leaf extract was observed to have the highest chlorophyll content while at the same time causing the highest mortality of *Busseola fusca*

5.3.5 Maize yield

The 75% leaf, husk and root showed a significant difference ($p \leq 0.05$) in increasing maize yield compared to the Karate, 50%, 25% and 0%. These findings are in agreement with those by Ogendo *et al*. (2013) where Lantana and Tephrosia treatments, sprayed at 48, 54 and 35% showed improved grain yields compared to untreated control and also produced higher maize grain yields compared to the synthetic insecticide, Karate.

The leaf extract showed a significant difference ($p \leq 0.05$) in increasing maize yield. This could be due to the fact that, the extracts were found to contain phytochemicals such as flavonoids which influence growth and development of insect pest and by so doing they cause mortality of the pests while glycosides have larvicidal activities as well as anti-feeding effects against insect pests, (Hikal *et al*., 2017).

In this study the leaf extract at 75% has been reported to be the most effective in causing mortality of stem borer, reducing leaf damage, reducing dead hearts and borer holes as well as chlorophyll content. These results are in agreement with those by Ajala and Saxena (1994) who reported that damage parameters such as foliar damage and dead hearts had an influence on grain, after artificial infestation of three-week-old maize plants with maize stem borer. The reduction in the number of ears harvested due to larval infestation was found to be the primary cause of grain yield loss (Groote, 2001).

These findings are in agreement with those of Loko *et al*. (2017) and Jose and Sajatha, (2017) pointed that phyto-constituents such as tannins, saponins, flavonoids, terpenoids and steroids may be responsible for mortality of insect larvae since they are anti-feedants hence inhibit the larval feeding behavior.

CHAPTER SIX

CONCLUSION, RECOMMENDATIONS AND SUGGESTIONS FOR FURTHER RESEARCH

6.1 Conclusion

(i) *Cocos nucifera* leaf, husk and root extracts from tall East African coconut varieties contain phytochemicals such as tannins, saponnins, steroids, terpenoids, flavonoids and glycosides except alkaloids.

(ii) *Cocos nucifera* leaf, husk and root extract all-cause mortality of *Busseola fusca*. The leaf extract at 75% concentration proved to cause the highest reduction of *Busseola fusca* population. This reflects that the *Cocos nucifera* extracts are endowed with potent anti-feeding and larvicidal properties which may be attributed to the phytochemicals present in the extract.

(iii) The control of *Busseola fusca* using *Cocos nucifera* leaf, root and husk extracts at varied concentrations proved to reduce the number of dead hearts, reduce the number of borer holes, reduce leaf damage and improve chlorophyll concentration in maize plants. The 75% leaf extract concentration was the most effective.

(iv) From this study it can be concluded that by controlling *Busseola fusca* the quantity of yield obtained from potted maize plants in the greenhouse generally increased .The control of the pest using the 75% leaf extract produced the highest quantity of maize yields.

6.2. Recommendations

(i) *Cocos nucifera* aqueous extracts from the tall East African coconut varieties in Msambweni especially the 75% leaf extract can serve as a good bio-resouce for generating botanical pesticides against *Busseola fusca*.

(ii) The fact that *Cocos nucifera* tall East African varieties are found both at the coastal and western region of Kenya, it makes the plant readily available to access.The extracts obtained are deemed to be environmentally friendly hence should be used as alternatives to the conventional insecticides for the control of *Busseola fusca*

6.3 Suggestions for further research

(i) There is a need to carry out further research on the mode of action of the phytochemicals present in *Cocos nucifera* extracts against *Busseola fusca*.

(ii)Further research investigations need to be carried out to confirm the concentrations of the phytochemicals present in *Cocos nucifera* extracts and their contribution in causing mortality of *Busseola fusca* .

(iii)Based on the results of this study, further research can be carried out on the larvicidal activity of *Cocos nucifera* extracts against other growth parameters besides those mentioned in this study.

(iv)Further research is needed on effect of *Cocos nucifera* extracts (dwarf East African variety) against *Busseola fusca* and their effect on growth and yield of maize.

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APPENDICES

Appendix 1: A stand of Pannar 15 maize variety in the green house.

Appendix 2: ANOVA tables

Appendix 2.1: ANOVA table on the effects of *Cocos nucifera* extracts on mortality of *Busseola fusca* in maize plants

Source	DF	Sum of squares	Mean squares	F value	Pr >F
Model	218	167.6507937	0.7690403	7.28	<.0001
Error	96	10.1460317	0.1056878		
Corrected Error	314	177.7968254			

Appendix 2.2: ANOVA table on the effects of *Cocos nucifera* extracts on dead hearts in maize plants

Source	DF	Sum of squares	Mean squares	F value	Pr >F
Model	218	89.28253968	0.40955293	21.13	<.0001
Error	96	1.86031746	0.01937831		
Corrected Error	314	91.14285714			

Appendix 2.3: ANOVA table on the effects of *Cocos nucifera* extracts on maize yield

Source	DF	Sum of squares	Mean squares	F value	Pr >F
Model	218	52165.15891	239.28972	18.98	<.0001
Error	96	1210.53742	12.60976		
Corrected Error	314	53375.69633			

Appendix 2.4: ANOVA table on the effects of *Cocos nucifera* extracts on the number of borer holes in maize plants

Source	DF	Sum of squares	Mean squares	F value	Pr >F
Model	28	78642.91022	2808.67537	17.38	<.0001
Error	16	2585.48889	161.59306		
Corrected Error	44	81228.39911			