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Development of bio-pesticide for management of spodoptera frugiperda (j. e. smith) and other lepidoptera pests of maize in Tanzania

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DEVELOPMENT OF BIO-PESTICIDE FOR MANAGEMENT OF SPODOPTERA FRUGIPERDA (J. E. Smith) AND OTHER LEPIDOPTERA PESTS OF MAIZE IN TANZANIA

Winisia Esau Makirita

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

This study was conducted to evaluate the infestation levels and develop management option for a Lepidoptera namely fall armyworm (*Spodoptera frugiperda* J. E. Smith). The survey was carried out in three regions of northern Tanzania namely; Kilimanjaro, Arusha and Manyara regions, and bioassay tests were conducted at the Hunan University of Technology and Nelson Mandela African Institution of Science and Technology. Six villages per region were surveyed, and a total of 210 maize growers were interviewed in all regions during maize growing season in 2018. A scale of 0 (no damage) to 9 (100 % damage) was used to assess severity of *S. frugiperda* in the study area. Performance of bio-based formulation against *G. mellonella* and *S. frugiperda* were measure by the percentage of insect mortality recorded 2 days and 9 days post-treatment for entomopathogenic nematodes and plant extracts treatments respectively. Data collected were analyzed using GenStat software 16th edition and SPSS version 21. Results indicated that *S. frugiperda* incidence and severity level on maize were 66.59 % and 5.422; 52.96 % and 4.756; 52.64 % and 3.989 for Arusha, Kilimanjaro and Manyara regions respectively. The commonly applied pest management options by farmers in the study area were synthetic pesticides (86 %) and non-synthetic methods (14 %). Laboratory experiment showed that, formulations from *Tephrosia vogelii* and *Dolichous kilimandscharius* caused *S. frugiperda* larvae mortality of up to 70 % and 60 % respectively. Bio-based formulations from entomopathogenic nematodes (40 IJ/ml) caused *G. mellonella* larvae mortality of up to 100 %. On the *S. frugiperda*, the same nematodes concentration caused high mortality 48 h after treatment indicating that it can be used against *S. frugiperda*. Bio-formulation of nematodes in UV protecting ingredients caused higher larvae mortality (20 %) than the aqueous formulation (0 %) under direct sunlight for 6 h, indicating that nanoparticles protected the nematodes against UV radiation. Of the two biopesticide formulations, entomopathogenic nematodes had high performance, and thus, this study recommends the use of entomopathogenic nematodes for the management of *S. frugiperda* and other Lepidoptera. However, further study on their performance in different agricultural systems is needed.

DECLARATION

I, Winisia E. Makirita do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this thesis is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

Winisia Esau Makirita	**Date**
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The above declaration is confirmed

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Name and signature of the supervisor 1	
Dr. Ernest R. Mbega	**Date**
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Prof. Xiaolong Li	**Date**
Name and signature of the supervisor 3	

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CERTIFICATION

The undersigned certify that they have read the dissertation titled **"Development of bio-pesticide for management of *Spodoptera frugiperda* (J. E. Smith) and other Lepidoptera pests of maize in Tanzania"** and approve for submission to the NM-AIST senate for award consideration of the PhD degree in Life Sciences of the Nelson Mandela African Institution of Science and Technology.

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Dr. Ernest R. Mbega **Date**

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Prof. Xiaolong Li **Date**

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DEDICATION

This work is dedicated to my parents Esau and Florance Makirita for their love and inspiration in my academic life.

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LIST OF ABBREVIATION AND SYMBOLS

NPs	Nanoparticles
DMSO	Dimethyl sulphoxide
TLEM	*Tephrosia vogelii* leaves methanol extract
TLEE	*Tephrosia vogelii* leaves ethyl acetate extract
TLEC	*Tephrosia vogelii* leaves chloroform extract
DORM	*Dolichos kilimandscharius* root methanol extract
DORE	*Dolichos kilimandscharius* root ethyl acetate extract
DORC	*Dolichos kilimandscharius* root chloroform extract
IPM	Integrated Pest Management
ºC	Degree centigrade
%	Percentage
UV	Ultraviolent radiation
Fe_3O_4	Iron (II, III) oxide
ZnO	Zinc oxide
TiO_2	Titanium (IV) oxide
mg/L	Milligram per litre
ppm	Parts per million
IJ/ml	Infective juveniles per millilitre
µl	Microlitre
h	Hour
RH	Relative humidity
nm	nanometre
HUT	Hunan University of Technology
NM-AIST	Nelson Mandela African Institution of Science and Technology

CHAPTER ONE

INTRODUCTION

1.1 Background

Lepidopteran including fall armyworm (*Spodoptera frugiperda* J. E. Smith) are currently considered to be the most injurious pests of economic importance in Africa (Sisay, 2018). They are known to threaten food and income security to the majority of farmers in the African continent. Lepidoptera pests are mostly found in all regions of the continent depending on the environmental conditions. They feed on plants, stored grains and fabric (Kondidie, 2011). Numerous Lepidoptera pests are indigenous to Africa with more than 21 species. *Busseola fusca* (Fuller) (indigenous) and *Chilo partellus* (invasive) are among the predominant pests that can significantly cause yield loss from 0-100 % in different regions and seasons (Sylvain, Manyangarirwa, Tuarira & Onesime, 2015). In the past decade *C. partellus* and *C. sacchariphagus* invaded the continent, and caused injuries that were more significant than that caused by the indigenous species. *Chilo partellus* was estimated to cause (annual) yield losses ranging from 15 % to 100 % (Sylvain, Manyangarirwa, Tuarira & Onesime, 2015). While farmers are struggling on how to get rid of these pests, the invasive *S. frugiperda* has now worsened the situation. Unlike other Lepidopteran, *S. frugiperda* is a polyphagous pest that feeds on a broad host range of cultivated crops and non-cultivated-crops worldwide (Abrahams *et al*., 2017; Souza, Carvalho, Moura, Couto & Maia, 2013).

Recently the *S. frugiperda* was reported to colonize almost all of Sub Saharan African countries, threatening the national and individual incomes, food and nutrition security (Day *et al*., 2017; Prasanna, Huesing, Eddy & Peschke, 2018). It is reported to cause massive damage on maize fields, although other crops such as rice and sorghum are at risk (Abrahams *et al*., 2017). The *S. frugiperda* is estimated to cause maize loss of more than 41 % in some African countries such as Ghana and Zambia (Abrahams *et al*., 2017). A preliminary investigation indicated that, *S. frugiperda* cause economic loss of about US$ 2.5 to 6.2 billion per annual for just 12 major maize growing countries in Africa (Hailu, Niassy, Zeyaur, Ochatum & Subramanian, 2018). In Tanzania *S. frugiperda* is expected to reduce maize production by 57 % (Abrahams *et al*., 2017). Thus, the combined effects of previously known Lepidoptera and the new invasive *S. frugiperda* could result in complete crop failure and substantial economic loss which will, in turn lead to income, food and nutritional insecurity if not contained.

Management of Lepidoptera pests has been through synthetic pesticides, however the biology and behaviour of caterpillars the larvae stage of the pest have constrained the approach (Abrahams *et al*., 2017). Apart from the effect associated with synthetic pesticides, their accessibility to the majority of smallholder farmers is limited (Abrahams *et al*., 2017). Many of the cheapest and most widely used synthetic pesticides in Africa fall into the mode-of-action classes to which Lepidopterans have developed resistance (Belay, Huckaba & Foster 2012; Carvalho *et al*., 2013; Nyirenda *et al*., 2011). Synthetic pesticides have been reported to have magnification effect to non-targeted organisms, and they also tend to accumulate in the environment (Viana & Prates, 2003; Wilson & Mushobozi, 2009), and due to resource-scarce nature of the majority of farmers, they often are unwilling or unable to buy the appropriate safety equipment during application of synthetic pesticides putting their health's at risk.

An alternative to synthetic pesticides would, therefore, provide a sustained control strategy. Such alternatives that are environmentally friendly include biological-based control strategy and use of resistant crop varieties (Stokstad, 2017). Genetically modified and resistant crops through considered as an alternative approach, they are reported to be attacked by *S. frugiperda* in the Western hemisphere including Brazil (Farias *et al*., 2014; Horikoshi *et al*., 2016; Omoto *et al*., 2016). Other suggested managerial option has been the application of predators, parasitoids and entomopathogens against the pest (Ríos-Díez & Saldamando-Benjumea, 2011; Rios-Velasco, Gallegos-Morales, Berlanga-Reyes, Cambero-Campos & Romo-Chacón, 2012; Tavares *et al*., 2010). In some countries such as America, parasitoid option has been practiced for several crop pests (Beserra & Parra, 2003; Souza *et al*., 2013), but is limited in Africa. Biopesticides are effective and environmentally friendly for pest management; their performance is against a broad range of pests and in most case, they are compatible with other management options (Gul, Saeed & Khan, 2014). Despite their potentiality, the application of biopesticide formulations for management of Lepidoptera including *S. frugiperda* is limited in Africa.

Therefore, this study aimed at identifying and developing bio-control approaches that could be used to manage Lepidopterans including *S. frugiperda* in maize crop by smallholder farmers in Tanzania.

1.2 Problem statement and justification

Lepidoptera comprises some of the devastating pests hampering crop production and economic development throughout the African continent (Dejen, Getu, Azerefegne & Ayalew; Sylvain *et al*., 2015; Abrahams *et al*., 2017). Majority of maize growers particularly in Tanzania use synthetic insecticides to control lepidopteran pests. However, the use of synthetic pesticides has been associated with negative consequences such as development of resistance of the pests, health effects to users and destroying biological active microbes and parasitoids (Abrahams *et al*., 2017). The most vulnerable stage for control in Lepidoptera is the caterpillar, which are often inaccessible to pesticides due to their tendency of hiding in the whorls and reproductive parts of the host plant, limiting the effects of spraying (Abrahams *et al*., 2017). Information on application of other control options like bio-based formulation especially in Tanzania is limited. In this study, therefore extracts from *D. kilimandscharius* and *T. vogelii* were tested for their ability to manage Lepidoptera pests. In addition, the study also tested effect of entomopathogenic nematode formulations against selected Lepidopterans targeting particularly the fall armyworm (*Spodoptera frugiperda*).

1.3 Rationale of the study

In this study, therefore extracts from *D. kilimandscharius* and *T. vogelii* were tested for their ability to manage Lepidopterans as they contain insecticidal properties. In addition, the study also tested efficacy of entomopathogenic nematode formulations against selected Lepidopterans targeting particularly the fall armyworm (*Spodoptera frugiperda*). Entomopathogenic nematodes have been successful in managing soil-dwelling insects (Grewal, Nardo & Aguillera, 2001). This study was proposed to establish the infestation level and management practices of *S. frugiperda* on maize fields in northern Tanzania, which will serve as the baseline for development of effective pest management strategy. Such developed control measure will provide alternative pest management solution to farmers and other stakeholders with benefit to reduce the level of pesticides contamination in the environment and agricultural produce while improving health, food security and the income of smallholder maize production farmers in Tanzania.

1.4 Research objectives

1.4.1 General objective

To develop a bio-based formulation from active plant compound and entomopathogenic nematodes for application by farmers to reduce yield losses caused by Lepidopterans in Tanzania.

1.4.2 Specific objectives

(i) To determine infestation level of *Spodoptera frugiperda* (Lepidopteran) on maize fields and management practices by smallholder farmers in the Northern zone of Tanzania.

(ii) To evaluate insecticidal activity of plants extracts and entomopathogenic nematodes against *Spodoptera frugiperda* and *Galleria mellonella*.

(iii) To formulate and evaluate efficacy of the entomopathogenic nematode formulation against *Galleria mellonella*.

(iv) To formulate and evaluate efficacy of entomopathogenic nematode formulation for the management of *Spodoptera frugiperda*.

1.5 Research questions

(i) What is the infestation level of the *Spodoptera frugiperda* on maize fields and the farmer management practices in northern Tanzania?

(ii) What is the effect of different biopesticide formulation against *Spodoptera frugiperda* and *Galleria mellonella* as representation member of the order Lepidoptera?

(iii) Is it possible to formulate biopesticides for application against *Galleria mellonella* and other Lepidoptera pests?

(iv) What is the efficacy of the biopesticide formulation against *Spodoptera frugiperda?*

1.6 Significance of the study

This study has established the infestation level and management practices of *S. frugiperda* on maize fields in northern Tanzania, which serves as the baseline for development of effective pest management strategy. In this study, a bio-control strategy that can be used for management of Lepidoptera pests including *S. frugiperda* has been developed. Such

developed control measure will provide alternative pest management solution to farmers and other stakeholders with benefit to reduce the level of pesticides contamination in the environment and agricultural produce while improving health, food security and the income of smallholder maize production farmers in Tanzania.

1.7 Delineation of the study

The bioformulation developed from this study were not fully investigated; entomopathogenic nematodes formulation was not investigated under different agroecological regions and plants-based formulation (from (*D. kilimandscharius* and *T. vogelii*) was not characterized to identify chemical compounds containing insecticidal properties due to resource limitations such as shortage of funds and time.

CHAPTER TWO

LITERATURE REVIEW

2.1 Lepidoptera pests

Lepidoptera is the second largest order of insect, comprises of more than 157 424 identified species of butterfly and moth (Wahlberg, Wheat & Peña, 2013). This group of insects undergoes a complete metamorphosis, have wings and many feeds on plant materials with some being carnivorous (Kfir, Overholt, Khan & Polaszek, 2002). Lepidoptera species are widely distributed in different regions of Africa, depending on the host availability and climate conditions of a particular region (Kfir *et al*., 2002). Some of important Lepidoptera have restricted distribution, whereas others are found throughout the sub Saharan African (Kfir *et al*., 2002). Lepidoptera can be very specific to host type and others nonspecific, for instance, the *Sesamia cretica*, *Spodoptera litura*, *Busseola fusca*, *Spodoptera exempta*, *Sesamia calamistis*, *Sesamia nonagrioides botanephaga*, *Eldana saccharina*, *Maliarpha separatella*, *Chilo partellus*, *Chilo aleniellus*, *Chilo sacchariphagus*, *Chilo zacconius*, *Chilo diffusilineus* and *Scirpophaga* spp. are known to be more injurious pest of cultivated grass than broadleaf (Kfir *et al*., 2002; Sylvain *et al*., 2015; Sisay, 2018). Species such as *C. partellus* and *C. sacchariphagus* invaded Africa from Asia and India; and became a severe pest in southern and eastern African countries (Kfir *et al*., 2002; Dejen *et al*., 2014). Recently, new species of Noctuidae, namely *S. frugiperda* has invaded the continent with rapid distribution and has been reported to cause massive crop damage in more than 40 African countries. Several studies have been carried out, and investigations suggest the pest to be a more serious pest than the previously known Lepidopteran owing to its polyphagous and reproduction behaviour (Sisay, 2018). Owing to the economic importance of the *S. frugiperda*, its spread and distribution, biology, behaviour, effect of climatic condition on its biology, host range, effective management approaches such as bio-pesticides were urgently needed, and this was the basis for this study.

2.1.1 Spread and distribution of *Spodoptera frugiperda* in Africa

Spodoptera frugiperda invaded Africa in 2016, and it's migratory and dispersal ability has allowed it to drift and spread quickly to new geographical areas (Goergen, Kumar, Sankung, Togola & Tamo, 2016; Johnson, 1987; Kumela *et al*., 2018). It is unclear on how *S. frugiperda* come to Africa (Prasanna *et al*., 2018), but transportation, wind and international trades (Nagoshi & Meagher, 2008) can be associated with this invasion (Abrahams *et al*.,

2017). Recently, *S. frugiperda* has been confirmed present in Western, Central, South and East Africa (Goergen *et al*., 2016; Abrahams *et al*., 2017; Kumela *et al*., 2018). The pest spread is astounding as it has taken only two years to colonise more than 44 African countries, indicating that only one month is enough for the pest to invade the new geographical region. Its migratory ability has been thought to be its primary strategy to escape unfavourable conditions (Johnson, 1987; Luginbill, 1928). Warm weather seems to favour its survival, and this can be linked with the current invasion of *S. frugiperda* in Africa (Nagoshi, Murúa, Hay-Roe, Juárez & Willink, 2012). Prolonged drought has most likely facilitated the invasion of *S. frugiperda* in Africa (Prasanna e*t al*., 2018). Due to that, it is possible that the pest has found a new habitat in Africa. The colonization is worsened by the moth biology, host range and flying ability which speeds the dispersal rate and this could be an indicator that, the whole of Africa will be colonized in the near future. This colonization carries a potential possibility for severe domination and crops damage, if not controlled.

2.1.2 Biology, survival and multiplication of the *Spodoptera frugiperda*

Spodoptera frugiperda undergoes complete metamorphosis with multiple generations in a year (Hardke, 2011). Its generation can go up to 10, in good climatic condition (Fatoretto, Michel, Silva, Filho & Silva, 2017; Luginbill, 1928). All stages of *S. frugiperda* are affected by temperature; however, the optimal range for its development is about 28 °C (Hogg, Pitre & Anderson, 1982; Luginbill, 1928). At normal temperature life cycle is completed in less than 20 days, while it can go up to 90 days at low temperature (Jeger *et al*., 2017). Female are highly prolific producing a thousand eggs in one generation (Fatoretto *et al*., 2017; Jeger *et al*., 2017; Johnson, 1987).

Spodoptera frugiperda survival and multiplication is mostly affected by host availability. The *S. frugiperda* locates host plants for feeding, mating and oviposition which are aided by the compounds (e.g. hexanol, hexenyl acetate, limonene and linalool) emitted by the host plants (Johnson, 1987; Carroll, Schmelz, Meagher & Teal, 2006; Degen, Bakalovic, Bergvinson & Turlings, 2012). Once at the host canopy, female moth emits sex phero-hormones such as (Z)-9- tetradecen-1-yl acetate (Z9-14: Ac), (Z)-7- dodecen-1-acetate (Z7-12:Ac), (Z)-9-dodecen-1-yl acetate (Z9-12:Ac), (Z)-11- hexadecen-1-yl acetate (Z11-16:Ac) and (E)-7-dodecen-1-yl acetate (E7-12:OAc) (Sparks, 1980; Tumlinson, Mitchell, Teal, Heath & Mengelkoch, 1986; Ward, Mitchell, Sparks, Serrate & Villarroel, 1980) to attract adult males for mating (Sparks, 1979). In this case, only competitive adult males will mate which

determines the oviposition rates. Hundreds to thousand eggs are laid at the underside of the leaf in clusters (Johnson, 1987; Luginbill, 1928) and at high population; eggs can be laid anywhere (Sparks, 1979; Pantoja-lopez, 1985). The larva emergence survival depends on the predation and parasitism chance among other factors. In the process of *S. frugiperda* and host interaction, host plant emits volatile organic compound (VOC) such as terpenoids and linalool which serves as an indirect defense of the host plant but it also aide's predator and parasitoid to locate herbivorous prey and host (Degen *et al*., 2012). There are about 36 VOC previously isolated from maize infected with neonate larva (Degen *et al*., 2012), in which Linalool and 4, 8-dimethyl-1, 3, 7-nonatriene are reported to attract more *S. frugiperda* to the host plant (Carroll, Schmelz, Meagher & Teal, 2006).

In the absence of host plant induced defense (VOC) and natural enemies' eggs hatches and larva develops on the host (Sparks, 1979). Larva growth and survival rate increase with increase in host availability. However, in absence of favourite host, this pest can switch to other available host plants. The survival and multiplication of *S. frugiperda* is influenced by the availability of the appropriate host plants. Extensive studies on the alternative host plants to this devastating pest are necessary to explore more on their survival and multiplication potential.

2.1.3 Climatic conditions influencing the biology of *S. frugiperda*

Climatic conditions influence insect population dynamics and abundances (Murúa, Molina-Ochoa & Coviella, 2006). Changes in any of climatic factors may positively or negatively affect insect distribution, survival, life cycle, development time and behaviour (Hogg, Pitre & Anderson, 1982). The *S. frugiperda* outbreaks are associated with varying climatic condition that the pest migrates to new geographical regions to locate favourable climatic condition, among other factors (Ramirez-Cabral, Kumar & Shabani, 2017; Sparks, 1979). In the tropics, *S. frugiperda* survives year-round, although its population tends to fluctuate with seasonal shift (Luginbill, 1928). Temperature is one of essential climatic factors that affect *S. frugiperda* distribution and survival (Hogg *et al*., 1982). For instance; in temperate regions, *S. frugiperda* cannot tolerate extended extreme cold temperature and does not survive winter (Luginbill, 1928; Sparks, 1979). The *S. frugiperda* life cycle is also reported to be shorter in summer due to high development rate as compared with the fall (Mitchell *et al*., 1991). Other studies have also reported the effect of temperature on *S. frugiperda* growth stages; that eggs and pupae can at least tolerate cold temperature (Ramirez-Cabral *et al*., 2017), but no pupae or larva survival below 13 ºC (Perkins, 1979) and no eclosion reported at 10 ºC (Simmons,

1993). In addition, *S. frugiperda* may attain maximum growth rate and survival rate under the optimal temperature ranging from 20-30 ºC (Barfield & Jones, 1979; Luginbill, 1928) and its life cycle is reported to be completed in 30 days under optimal temperature of 28 ºC (Jeger *et al*., 2017; Luginbill, 1928). According to Barfield and Ashley (1987), 30 ºC is the maximum temperature for *S. frugiperda* growth and survival. Also, there is no survival reported at 40 ºC temperature (Simmons, 1993). Moreover, the increase in temperature within optimal range increases survival rate and shorten development time of the *S. frugiperda* (Silvain & Ti-A-Hing, 1985; Ashley & Barfield, 1987; Simmons, 1993). Furthermore, laboratory studies reported reduced development rate, fecundity and deformed emerged adult moth when temperature is above 30 ºC (Ali, Luttrell & Schneider, 1990; Barfield & Ashley, 1987; Simmons, 1993).

Besides the effect of temperature, rainfall is another climatic factor that influences *S. frugiperda* population density, either direct or indirect. For instance, in *S. frugiperda* native regions the period of mild cold rainfall is known to promote insect abundance by creating favourable propagating conditions (Luginbill, 1928). The higher number of larva and moths are reported when rainfall is plentiful as compared with dry season (Sparks, 1979). While heavy rainfall is known to reduce population density of the early instars, the late instars or adult stages are not affected (Andrews, 1988). Also soil moisture influences the emergency but in excess dryness emergence delays (Vickery, 1929). In dry season, pest population is low, and population peak is also delayed (Ramirez-Cabral *et al*., 2017). The dry season also poses indirect effect to the pest by inhibiting host growth, and there will be no pest survival when the host plant is dead. In Africa where the pest is new, information on the *S. frugiperda* biology and behaviour in relation to the African changing climatic conditions is limited. Thus, for effective management of this pest understanding its biology and behavior in relation to climatic conditions in Africa is crucial.

2.1.4 Host range

Spodoptera frugiperda feeds on about 80 plant species (Barros, Torres & Bueno, 2010; Tavares *et al*., 2010; Cock, Beseh, Buddie, Cafá & Crozier, 2017), including cash and food crops that farmer depends on (Luginbill, 1928; Prasanna *et al*., 2018). The pest feeding is shocking as it also feeds on non-cultivated plant species, including weeds and grasses, providing alternation chance (Johnson, 1987). In America where the *S. frugiperda* is native, it was identified to feed on cultivated crops such as Maize (Lima *et al*., 2010; Nagoshi *et al*.,

2012); Rice (Pantoja-lopez, 1985); Cotton (Clark *et al*., 2007; Gonçalves de Jesus *et al*., 2014); Sorghum (Harris-Shultz, Ni, Wang, Knoll & Anderson, 2015; Juárez *et al*., 2012); Potato (Tavares *et al*., 2010); Sugarcane (Hall, Meagher, Nagoshi & Irey, 2005); Beans (Barros *et al*., 2010); Tomato, Clove, Tobacco and Bell pepper (Barlow & Kuhar, 2009); and Cucurbits (Jeger *et al*., 2017).

In Africa, the rate of *S. frugiperda* spread and crop damage is astonishing because pest matches within plant species throughout the year and due to numerous host ranges, the pest can have a new choice of host preference (Abrahams *et al*., 2017; Prasanna *et al*., 2018). This is alarming to the food security and economy of Africans given that the consumed crops in *S. frugiperda* native land are also cultivated in Africa and the pest has got no limit in its host range, its survival chance in Africa is assured.

2.1.5 *Spodoptera frugiperda* infestation stage

Spodoptera frugiperda consists of four life stage; egg, larva, pupae and adult (Sparks, 1979). Larval is the most damaging stage consuming leaf mass and reduces photosynthetic leaf area (Buntin, 1986). It is reported to affect all maize growth stages (Pannuti, Baldin, Hunt, & Paula-Moraes , 2016), but the damage is severe when maize is less than 6 leaf stage (Cruz & Turpin 1983; Wiseman & Widstrom, 1984; Ghidlul & Drake, 1989). Once hatched, the young larvae disperse over several maize plants and start feeding on the ear of the maize leaf (Luginbill, 1928). The young larva instars (1-3feeds on the upper portion of the plant canopy and can only consume less than 2 % while the older instars (4-6) feed on the stalk and protected parts of the plant and can consume up to 77 % (Buntin, 1986; Luginbill, 1928; Stokstand, 2017; Sparks, 1979). Moreover, the *S. frugiperda* larva can cause direct damage to the developing maize grain, though during vegetative-stage grains can tolerate moderate to substantial levels of defoliation before significant yield loss occurs (Buntin, 1986). According to Sparks (1979), an average of ca. 14 000 sq. mm is used per caterpillar and in its early instars, numerous larval attacks one plant but for the late instars only one larva per plant. This indicates that only few larvae are enough to cause massive damage in a given farm. Thus, strategies aimed at reducing larva feeding and foliar damage are vital to rescue current situation in crop production. Efforts were needed to develop effective integrated control measures that target the destructive stage of *S. frugiperda* because single approach may not be useful as the larvae mining into the plant whorls may be challenging to eliminate.

2.2 Yield and economic loss due to *S. frugiperda* in sub Saharan Africa

Insect pests, including *S. frugiperda* are the main factor for reduced crop productivity worldwide (Midega, Pittchar, Pickett, Hailu & Khan, 2018; Oliveira, Auad, Mendes & Frizzas, 2014; Sparks, 1979). The *S. frugiperda* reduces crops yield and sometimes can lead to total crop loss (Belay *et al*., 2012; Midega *et al*., 2018). The damage level depends on the maize variety grown, planting season and geographical region (Midega *et al*., 2018). In Africa, the pest has potential to reduce maize yield by more than 41 % annually (Day *et al*., 2017). Estimations in 12 maize producing countries show that maize yield will be reduced from 38 971 000 to 22 866 000 tones which is 41 % loss (Day *et al*., 2017). This huge maize loss is estimated to cause economic loss of US$ 6.19 billion, due to crop damage and production cost (Prasanna *et al*., 2018). Also, several seed-producing sectors have reported damage caused by *S. frugiperda* which can affect seeds availability and economic viability of the seed sectors (Prasanna *et al*., 2018). It is also proclaimed to affect penetration of agricultural products from infected regions on international markets, fearing risk of introducing the invasive pest to uninfected regions such as Europe and Asia (Day *et al*., 2017; Prasanna *et al*., 2018). For example, Day *et al*. (2017) reported contaminated roses exported from Africa in 2017 were intercepted in Europe and this accelerated the need to place the conditions for exports.

In this study, maize production and economic loss due to invasive *S. frugiperda* have been estimated in 12 Sub Saharan counties based on FAO and Day *et al*. (2017) (Table 1). Many of the reported countries in Africa had their average maize productions increasing significantly between 2012 and 2016 (Table 1). During these years, productions varied among countries, and this was attributed to several production factors, including insect pest and drought (Midega *et al*., 2016; Prasanna *et al*., 2018). However, since 2016 when *S. frugiperda* was first reported to cause damage in many African countries, the loss is expected to increase. There has been attempt to gather data from many African countries on the damage caused by *S. frugiperda* without success. This is because the *S. frugiperda* is still new to many African countries, with national and international control programmes focusing on more known pests than the *S. frugiperda*.

Day *et al*. (2017), however, reported that maize production loss due to *S. frugiperda* ranged from 40 to 45 %. Such losses are enormous and the quantities are sufficient to cause food insecurity in many countries, leave alone where the *S. frugiperda* causes yield or total loss

(Belay *et al*., 2012; Midega *et al*., 2018). Based on the mean production values as shown in Table 1, and taking into consideration the loss at 41-45 % due to *S. frugiperda* as reported by Day *et al*. (2017) in Africa, it would mean many of the sub Saharan countries will suffer a significant loss in maize production. For instance, Burundi with a mean production of 16 705 000 tons per year may suffer a loss of 6 682 000 tons, which corresponds to 26.6 USD millions. Mozambique with a mean production of 151 993 000 tons may suffer a loss of 60 797 000 tons, which corresponds to 237.11 USD millions. Tanzania with a mean production of 579 523 000 tons may suffer a loss of 231 809 000 tons, which corresponds to 904.06 USD millions and for Zimbabwe with a mean production of 90 118 000 tons may suffer a loss of 36 047 000 tons, which corresponds to 140.58 USD millions. This is a considerable loss, which needs stern measures against *S. frugiperda* control. More efforts are therefore needed during these early years of *S. frugiperda* inversion to be able to rescue majority of the countries from food insecurity.

The calculations of the economic losses (Table 1) are based on the price (310 USD per ton) data extracted from FAO statistics for Zimbabwe for the year 2015, as most of the countries do not have the price available on the FAO website, and economic reports are not readily available. Actual data for many of the countries, from the production to losses are not readily available, leading to estimations that may be skewed sometimes for some countries. Some of these countries will be estimated to have more loss than the actual while others might have significant losses than reported. Thus, governments and all agricultural stakeholders need to work hard to gather data that would enable actual loss and estimations or projections to reflect reality. Also rescue individual income and national GDP and finally continent economy.

Table 1: Estimated quantities of maize production for selected Sub Saharan countries and the mean loss after the *Spodoptera frugiperda* invasion

Country/Region	Average production for 5 years from 2012- 2016 (Thousand Tons)	Average loss (Thousand Tons)	Economic loss (USD Millions)
Burundi	167.05	66.82	26.06
Ghana	1777.99	711.19	277.37
Kenya	3603.95	1441.58	562.22
Malawi	3276.49	1310.60	511.13
Mozambique	1519.93	607.97	237.11
Nigeria	9630.52	3852.21	1502.36
Rwanda	513.67	205.47	80.13
South Africa	11182.95	4473.18	1744.54
Uganda	2711.10	1084.44	422.93
Tanzania	5795.23	2318.09	904.06
Zambia	2845.49	1138.19	443.90
Zimbabwe	901.18	360.47	140.58
Total	43925.55	17570.21	6852.39

Source: *FAO Maize production statistics for the year 2012 to 2016. The price is based on the available data for Zimbabwe in 2015 of USD 390/ ton.*

2.3 Management of *Spodoptera frugiperda* in Africa

2.3.1 Synthetic chemical pesticides

Pest management is mainly by the use of chemical pesticides (Day *et al*., 2017; Jeger *et al*., 2017). Chemical pesticides such as carbaryl, trichlorfon, methyl parathion, permethrin, chlorpyrifos, spinosad, lufenuron and methomyl have been widely used to control insect pests including *S. frugiperda* (Al-Sarar, Hall & Downer, 2006; Carvalho *et al*., 2013; Ferreira, 2015; Pitre, 1986). Farmers have been applying insecticides without prior knowledge of the pest behaviour and biology (Kfir *et al*., 2002; Campos, Ferreira, Costa, Junior & Lasmar, 2014; Ferreira, 2015; Midega *et al*., 2018), which forces farmer to conduct frequent spraying and rotation of chemicals to increase efficiency (Pitre, 1986). Application of these chemicals against Lepidoptera (moth) including *S. frugiperda* has been reported with little success due to the insect biology and behaviour, as well as insect resistance (Tavares *et al*., 2010; Yu, Nguyen & Abo-Elghar, 2003). The hiding behavior in

host plants and nocturnal behaviour of the adult mouth have intricates its use (Campos *et al*., 2014; Cock *et al*., 2017; Ferreira, 2015). As a consequence, farmers are stranded and conduct multiple applications of chemical pesticides without following the recommended dose which may be associated with resistance development by the pest toward several classes of organophosphate, pyrethroids and carbamate (Al-Sarar *et al*., 2006; Fatoretto *et al*., 2017; Moura, Carvalho, Pereira, & Rocha, 2006; Pantoja-lopez, 1985), which has further complicated the management process. In Africa, regardless of the application of synthetic pesticides in managing *S. frugiperda* their efficacy is still unknown (Abrahams *et al*., 2017). The approach is environmentally and economically unfriendly to majority of smallholder farmers and in most cases, its availability and accessibility is limited (Day *et al*., 2017; Jeger *et al*., 2017). It is also known to affect non-targeted organisms including parasitoid of this pest which may further worsen the situation (Harris-shultz *et al*., 2015; Souza *et al*., 2013). Similarly, the choice of synthetic pesticide to use depends on farmer's purchasing power and knowledge (Midega *et al*., 2018). Thus, all of these challenges have opened an opportunity for development of other alternative approaches including botanicals, biological control, host resistant varieties and cultural methods for managing *S. frugiperda*.

2.3.2 Bioactive compounds from plants

Plant active compounds have been used for so long in managing a broad range of insect pests (Isman, 2006; Rattan, 2010). Several compounds such as alkaloids and terpenes from plants are known to poses insecticidal properties (Abdelgaleil, Abbassy, Belal & Rasoul, 2008; Gershenzon & Dudareva, 2007; Wink, 2000). Numerous plants have been evaluated against *S. frugiperda* in America (Isman & Grieneisen, 2014; Moreira *et al*., 2007; Tavares *et al*., 2011). Plants extracts evaluated against *S. frugiperda* provided promising results, and they can serve as good candidates in formulation of botanical pesticides. For instance, *Azadirachtin indica, Tagetes erecta* L. and *Ricinus communis* (Salinas-Sánchez, Aldana-Llanos, Valdés-Estrada, Gutiérrez-Ochoa, Valladares-Cisneros & Rodríguez-Flores, 2012; Rossi, Santos, Carvalho, Alves &. Pereira, 2012; Tavares *et al*., 2010). *Euphorbia pulcherrima*, *Trichilia pallida*, *Piper tuberculatum*, *Myrciaria cauliflora*, *Parthenium argentatum* (Alves *et al*., 2014; Céspedes, Martinez-Vázquez, Calderón, Salazar & Aranda, 2001; Risco *et al*., 2012) were reported to hinder insect moulting, development and reduce *S. frugiperda* population (Viviane *et al*., 2017). Promising results of plant such as *Myrtillocactus geometrizans*, *Cedrela dugessi*, *Annona mucosa Jacquin*, *Jatropha*

gossypifolia, *Cedrela salvadorensis*, *Passiflora alata* Dryander and *Porteresia coarctata* Takeoka against *S. frugiperda* have also been reported (Ansante *et al*., 2015; Céspedes *et al*., 2005; Ramos-López, Pérez, Rodríguez-Hernández, Guevara-Fefer, & Zavala-Sanchez, 2010; Ulrichs, Mewis, Adhikary, Bhattacharyya & Goswami, 2008).

In Africa, there exists dives and good source of insecticidal plants with active compound against various pests. For instance, plants such as *Lantana camara* L., *Piper guineense, Azadirachta indica Tephrosia vogelii*, *Tagetes minuta* L, *Melia azedarach*, *Tanacetum cinerariifolium*, *Jatropha curcas*, *Allium sativum*, *Allium cepa* and *Cymbopogon citrates* provided good result against stem borer (Mugisha-Kamatenesi *et al*., 2008; Ogendo *et al*., 2013; Kamanula *et al*., 2010). In this case plants used in management of native insect pest can be considered in managing fall armyworm. Despite, the high diversity of insecticidal plants as potential source of active compound against *S. frugiperda* only few plants have been screened for their potential. Since its invasion, smallholder famers are trying to apply locally available plants extracts in their fields, but performance depends on the type and concentration of secondary compound extracted from the used plants. For instance, *T. vogelii* and *Azadirachta indica* have been the commonly used plant by smallholder famers. This study has identified some insecticidal plants used by local community for management of *S. frugiperda* in Tanzania and these plants were screened against *S. frugiperda*.

2.3.3 Cultural approach

Cultural practices are the most manageable and available approach for pest management to smallholder farmers in Africa (Midega, Bruce, Pickett & Khan, 2015). In *S. frugiperda* native areas, cultural practices have been reported to lessen the damage that can be caused by *S. frugiperda* invasion (Pantoja-lopez, 1985). Practices such as crop rotation, changing planting season and planting of early maturing variety have been most cited (Pantoja-lopez, 1985). Also, other practices employed for the management of maize borer are cited as an alternative way to reduce the burden associated with *S. frugiperda* (Jeger *et al*., 2017; Midega *et al*., 2015; Pantoja-lopez, 1985). Since *S. frugiperda* is new in Africa, cultural practices for its management are still limited. Farmers have been trying to use available techniques such as hand picking, applying chill pepper, ash and adding soil on plant whorl to rescue the situation (Kumela *et al*., 2018). Numerous agricultural organizations are working to find appropriate measure which will be feasible to smallholder farmers. Push-pull technology developed to manage stem borer in Africa, was tested against *S. frugiperda* and results were highly

positive (Midega *et al*., 2018). The technology involves intercropping of cereals and legumes with insecticidal properties with grass. The approach is economical and environmentally friendly, and it can be easily adopted by smallholder farmers. Furthermore, different management option including crashing the egg masses, crop rotation, early planting to avoid periods of heavy infestation and planting early maturing varieties have been proposed by agricultural organizations (FAO, ICIPE and CABE) in suppressing *S. frugiperda* population (Abrahams *et al*., 2017). Unfortunate, none of the methods is documented for use by farmer to manage the invasive Lepidoptera (*S. frugiperda*) in Tanzania. This study has identified some cultural practices applied by smallholder farmers for management of *S. frugiperda*, although their application may be hindered by the pest behavior.

2.3.4 Use of resistant and genetically modified crops

Plants defensive mechanisms are important component in integrated pest management (Abel, Wilson, Wiseman, White & Davis, 2000; Gordy, Leonard, Blouin, Davis & Stout, 2015). However, insect attacks of various crops indicate low resistance ability of plants varieties. Also, providing line of resistance needs evidence of indigenous varieties being less attacked and securing traits in one variety might be difficult. Thus, seed producers opted for genetically modified rather than bred varieties and due to several insect attacks genetically modified crops have been used in pest management (Carvalho, Ruas, Ferreira, Moreira & Ruas, 2004). *Spodoptera frugiperda* causes massive damages in cotton, rice and maize in some countries including Brazil and the use of genetically modified crops has been proven to reduce damage (Abel *et al*., 2000; Gonçalves *et al*., 2014; Nuessly *et al*., 2007). The most available crop resistant hybrids are made with genes from *Bacillus thuringiensis* (Bt) (Pantoja-lopez, 1985). *Bacillus thuringiensis* endotoxin was first offered as a commercialized hybrid in 1996 and main crops being maize, cotton and soybean (Acharya, 2017; Perry *et al*., 2016; Yang *et al*., 2016). Since commercialization, Bt crops have been the most grown crops in US and Brazil (Horikoshi *et al*., 2016; Williams, 2011). Most Bt crops in the market contain various gene that is effective against several targeted pests. For instance, Cry1F, Cry1Ab, Cry1Ac, Cry1A.105, Cry2Ab2 are protein specific for above ground Lepidoptera (Acharya, 2017). Pest fatal was reported after consuming maize resistant hybrids (Abel *et al*., 2000; Aguirre *et al*., 2016). However, several Lepidoptera species have reduced susceptibility to Bt modified corns (Horikoshi *et al*., 2016; Okumura *et al*., 2013). The field evolved resistance has been reported on Cry1Ac endotoxin modified cotton and Bt corn expressing Cry1F and Cry1Ab gene (Dangal & Huang, 2015; Farias *et al*., 2014; Huang *et al*., 2014;

Storer *et al*., 2010). So far, *S. frugiperda* is the only Lepidoptera pest among the target pests that have developed field resistance to Cry1F gene in multiple locations (Dangal & Huang, 2015). Thus, several Lepidoptera pests are resistant to the first line of Bt gene including Cry1Ab Cry1F, and Cry1Ac (Harris-shultz *et al*., 2015; Horikoshi *et al*., 2016; Johnson, 1987; Omoto *et al*., 2016). Before 2010, Bt corn expressed only single toxin for the targeted pest species known as Bt corn first generation (1996-2010). Owing to resistance challenges, a study conducted by Zhao *et al*. (2003) suggested the combination of more than two genes as gene pyramid strategy that will delay the evolution of *S. frugiperda*. Thus, Bt technology was shifted to pyramid strategy with more than one Bt protein targeting specific pest (Yang *et al*., 2013). Bt corn expressing multiple toxins was commercialized in 2010 as second generation pyramided Bt product (Acharya, 2017). Since Bt crops events are selective against specific pests, thus *S. frugiperda* was major targeted pest of Bt corn event MON 89034 (Acharya, 2017). In Africa, the adoption of genetically modified crops depends on the policy and regulations of a particular country, but also the fear of public health has slowed down the adoption process (Abidoye & Mabaya, 2014; Mabaya, 2015). Currently, some African countries are using genetically modified crops, and trials are carried on by researchers to assess its economic and health risks (Bennett, Morse & Ismael, 2006; Horna, Zambrano, Falck-Zepeda, Sengooba & Kyotalimye, 2013; Tarjem, 2017). Since the pest is new in Africa, several evaluation trials need to be established to measure the effectiveness of genetically modified crops.

2.3.5 Application of microbes

Use of microorganisms as bio-pesticide has been viewed as a new and promising alternative means of pest control (Usta, 2013). Microbial pesticides that are eco-friendly, and bio-persistent are preferred to kill insects at various stages of its life cycle (Gul *et al*., 2014). Some have contact mode of action and others penetrate through natural openings, feed on the insect tissue and ultimately kill the insect (Gul *et al*., 2014). In many countries, pest management is likely to shift from chemical formulation to biological formulations including fungi, bacteria, virus and protozoan (nematodes). Recent studies are increasingly exploring the wider properties of microorganisms, which suggest new opportunities for their use in biological control systems (Han, Jin, Kim & Lee, 2014; Thomazoni, Formentini & Alves, 2014; Zibaee, Bandani & Sendi, 2013). Currently, several microbial formulations are commercially available and account for about 1.3 % of all pesticides in the market (Ramanujam, Rangeshwaran, Sivakmar, Mohan & Yandigeri, 2014). The available

formulation includes those of virus, fungi, bacteria and protozoan (Copping & Menn, 2000; Gul *et al*., 2014; Kachhawa, 2017), which are used for pest management in America and Europe with limited information on their use in Africa. One of the best and successful microbial formulation include that of *Bacillus thuringiensis* and *Metarhizium anisopliae* that have been reported to reduce *S. frugiperda* population on maize and rice fields in America and Europe. Nucleo polyhedron viruses is another formulation recommended to control *S. frugiperda* (Cisneros *et al*., 2002; Polanczyk, Silva & Fiuza, 2000; Rios-Velasco, *et al*., 2012; Sousa *et al*., 2016). Furthermore, *Beauveria bassiana*, *Metarhizium* spp and *Neoaplectana carpocapsae* Weiser are other pathogens that can be applied for the management of Lepidopterans including *S. frugiperda* (Hardke *et al*., 2011). Also, the naturally occurring entomopathogens and parasitic nematodes were reported to control armyworms (Grewal *et al*., 2001). The use of microbial control of pest is growing globally as a sustainable and cost-effective management approach. Numerous microbial formulations have a synergic effect with other biological methods in pest management (Sahayaraj, Namasivayam & Rathi, 2011). So, their application has gained more recognition in developed countries such as America and Europe (Ramanujam *et al*., 2014; Thomazoni *et al*., 2014). Unfortunately, only few biopesticides have been registered for pest management in Africa and none for *S. frugiperda*. Recently, numerous strains have been evaluated with positive outcomes against *S. frugiperda* (Prasanna *et al*., 2018), although countless experiments are laboratory based which limit their use by smallholder famers.

In this case, there is an opportunity to utilize microbes as biological control, independently or in combination with other biological methods. Unfortunately, none of the microbial methods has been reported in Sub-Saharan Africa for management of *S. frugiperda*. Therefore, this has opened window for integrating microbial formulation with other biological control methods to evaluate its efficacy in the management of *S. frugiperda*. Thus, the current study has developed nematodes production method to ensure its availability to smallholder farmers for management of *S. frugiperda*.

Entomopathogenic nematode industry has gradually grown up and is promoted to be an alternative to synthetic chemicals in eliminating insect pest in an environmentally friendly way (Makirita *et al*., 2019). The entomopathogenic nematode is a new type of biological pesticide, which has high virulence and a wide range of insecticides. It can also actively find the host and be easy to cultivate artificially. Nematodes have been successful in managing soil-dwelling insects, and several formulations have been developed to control foliage

feeding pests (Makirita *et al*., 2019). Their formulations have been developed from simple to advanced formulation to maximize their efficacy (Hussein & Abdel-Aty, 2012) including; infected cadavers, aqueous suspension, synthetic sponges, vermiculite formulation, wettable powder formulation, clay formulation, pellet formulation, gel formulation, water-dispersible granular formulation and activated charcoal formulation (Grewal *et al*., 2001; Guo *et al*., 2017; Hussein & Abdel-Aty, 2012). Despite their efficacy and safety, they are currently used in developed countries such as America with no information on their use and performance in Africa including Tanzania. Owing to the potentiality of the entomopathogenic microbes in pest management there is a need to invest in developing stable nematodes-based formulation for controlling native and invasive pest in Tanzania for sustainable crop production.

Nematodes of the genus Steinernema, have been reported to have great potential for the management of a broad range of insect pest. The genus has more than 90 species identified worldwide (Labaude & Griffin, 2018), and the number is increasing from year to year (Kary *et al*., 2009). Nematodes of this genus are most likely to be found in all habitat supporting vegetation (Spiridonov *et al*., 2004), and they have been isolated in different parts of the world, except Antarctica (Nikdel & Niknam, 2015). Recently, more than eight species of the genus have been reported to parasitize wide range of economically important insect pest of class Coleoptera (Kajuga *et al*., 2018); Diptera (Edmunds *et al*., 2017); Hemiptera (Berkvens *et al*., 2014); Isoptera (Wagutu *et al*., 2017) and Lepidoptera. These include; *Steinernema carpocapsae*, *S. glaseri*, *S. weiseri*, *S. websteri*, *S. longicaudum*, *S. downesi*, *S. feltiae*, *S. kraussei*, *S. abbasi*, *S. yirgalemense*, S. riobrave, *S. karii*, *S. jeffereyense* and *S. affine*. Species in this genus have different infectivity abilities, range and type of host, depending on their searching, scavenging strategy and host availability in a given geographical regime (Nadler *et al*., 2006). Species of the genus live in a symbiotic association with specific entomopathogenic bacteria of the genus Xenorhabdus (Burnell & Stock, 2000; Ehlers, 2001). *Steinernema* spp. enters an insect body through natural openings with the bacteria inside the gastrointestinal tract and recycles inside the host (Kenneya & Eleftherianos, 2016; Labaude & Griffin, 2018; Shapiro-Ilan & Gaugler, 2002), and together they accelerate pest mortality in 2 days. Moreover, majority of Steinernema are known to be compatible with other field inputs (Ansari, Shah & Butt

2008; Molina-Ochoa, Lezama-Gutierrez, Hamm, Wiseman & Lopez-Edwards, 1999; Rovesti & Deseo, 1990; Shapiro-ilan *et al*., 2012). The combination of *Steinernema carpocapsae* and resistant silk has effectively controlled *S. frugiperda* (Molina-ochoa *et al*., 1999).

Compatibility of Steinernema formulations with other biological formulation such as entomopathogenic *M. anisopliae* has also been reported (Ansari *et al*., 2008; Niekerk & Malan, 2014). Furthermore, *Steinernema* spp are reported to be tolerant to some agrochemicals in short exposure (Rovesti & Deseo, 1990), thus, agrochemicals and nematodes product can be simultaneously applied (Negrisoli *et al*., 2010), to increase efficacy in managing notorious pest in Tanzania and African at large. Therefore, the exploration of native entomopathogenic nematodes and developing a stable biopesticide formulation from worldwide available strain of nematodes to fit agricultural regime of Africa and Tanzania is important.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Location of the study

The study was done in the Northern Tanzania, particularly in Arusha, Kilimanjaro and Manyara regions in 2018. The regions are characterized by a bi-modal type of rainfall (short and long rains). Long rains occur from March to May and shorter rains occurs from October to November. The regions through which this study was conducted receive maximum rain of 105.8 mm (4.66-105.8 mm) in Kilimanjaro and 36.39 mm (0.29-36.39 mm) for Arusha and Manyara regions. Temperature across regions were similar with range of 13°C -30 °C. Thus, for survey six villages per region were surveyed, and a total of 210 maize growers were interviewed in all regions. The choice for location was based on maize production records and report on the damage by Lepidopterans, especially the fall armyworm. Laboratory bioassay was done at the Nelson Mandela African Institution of Science and Technology (NM-AIST) and Pest management Centre Tengeru, Arusha Tanzania and Hunan University of Technology, China.

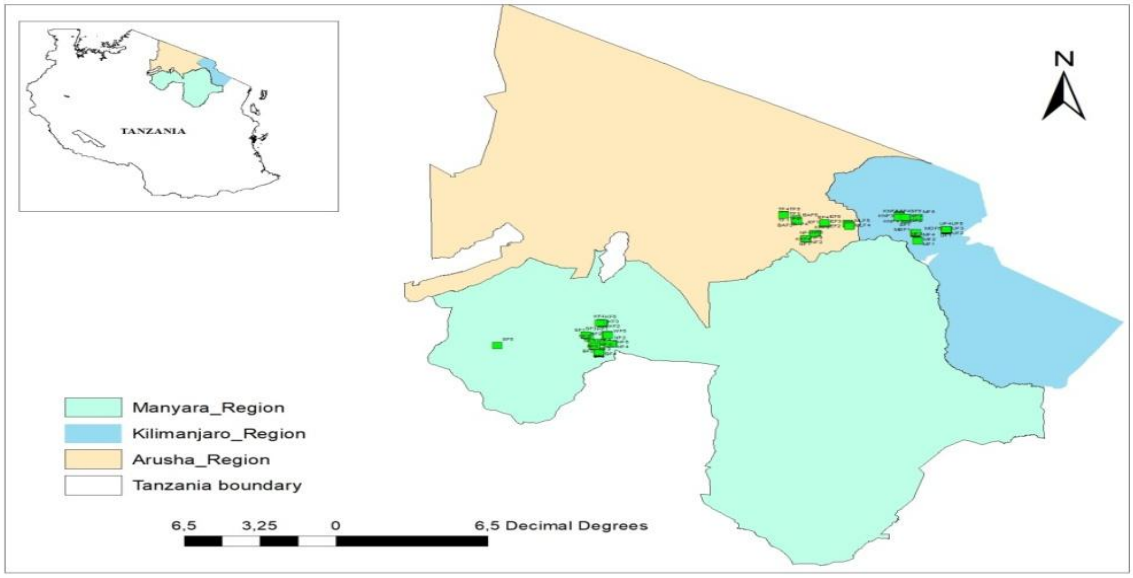


Figure 1: Map of the surveyed areas of Tanzania

3.2 Plant extracts used in this study

Root tuber of Veld lupin (*Dolichos kilimandscharicus*) (10 kg) was collected from Moshi, Kilimanjaro and aerial plant parts of fish poison bean (*Tephrosia vogelii*) leaves (5 kg) were collected from Tengeru in Arumeru District, Arusha region.

3.3 Lepidoptera insects used

The larvae of *S. frugiperda* were collected from infected maize plants at Maji ya Chai in Arumeru District, Arusha region in November 2017. The larvae were reared and maintained on maize plants, which were free of insecticides (Plate 1). After 15 ± 2 days, when the majority of the larvae reached the 4^{th} instars, they were collected and reared inside cages until adult emergence and fed with 1% honey in cotton wool. Groups of 20 to 30 adult moths were confined in cages covered with fine polyester mesh outside and inside with white paper where they oviposited the eggs. All *S. frugiperda* stages were reared at temperature 26 ± 1°C and humidity 65 ± 5 %. Eggs were collected every day and placed in plastic cups 12×8.5 cm (diameter and height) until hatched.

Galleria mellonella (Linnaeus) (Lepidoptera: Pyralidae) larvae were bought from the living culture of Ke Yun (Jiyuan Baiyun Industrial Co., Ltd. Henan province, China).

3.4 Microorganism used for the study

Entomopathogenic nematodes used in the present study were isolated from soil in Hunan University and maintained in sponge at 4°C. Nematodes were cultured frequently to maintain viability. *Xenorhabdus nematophila* bacterium was isolated from the nematodes infected *Galleria mellonella*. The same specie of the *Steinernema* have been discovered in Rwanda (Yan *et al*., 2016), while in Tanzania several other species have been discovered (Mwaitulo, Haukeland, Sæthre, Laudisoit & Maerere, 2011) which indicate the potential distribution of the said specie in Tanzania.

3.5 Metal oxides used

All metal oxides (ZnO-NPs, TiO_2-NPs and Fe_3O_4-NPs) were purchased from Aladdin Biochemical Technology Co., China. The nanoparticles sizes provided by the producers were 50 ± 10 nm, 40 and 25 nm, 35 and 20 nm for ZnO-NPs, TiO_2-NPs and Fe_3O_4-NPs respectively (Fig. 2).

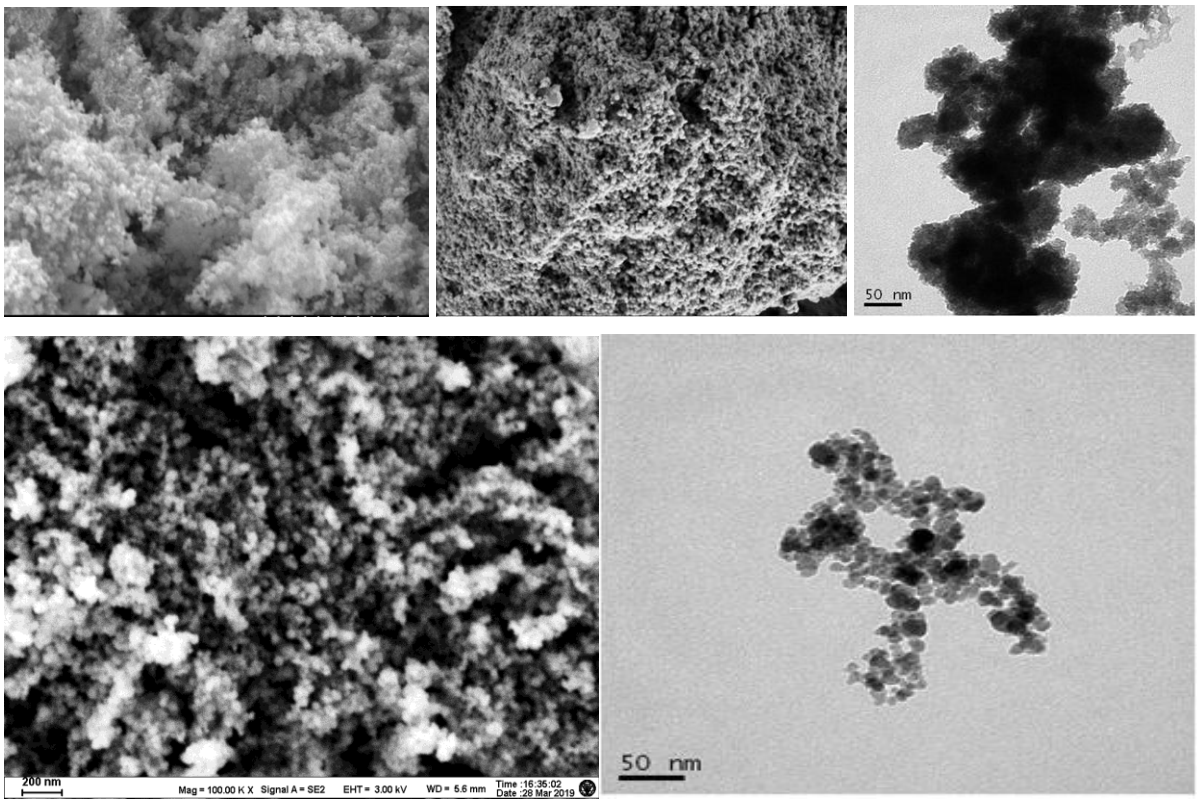


Figure 2: Scanning electron microscope (SEM) and transmission electron microscope (TEM) images of three metal oxide nanoparticles (NPs) prepared in deionized water. A) ZnO NPs, B) TiO_2 NPs, C) FeO_4 NPs

3.6 Field survey

To establish damage levels of *S. frugiperda* in maize field of the three regions of northern Tanzania, data were collected from purposefully selected 18 villages of the Kilimanjaro, Arusha and Manyara regions based on reports on *S. frugiperda* occurrences as reported by the District Extension Officers. In each village, five (5) fields were randomly selected and, in each farm, a zigzag style was used to select a 3 m x 3 m plot in triplicate. The incidence score was measured by the number of infected plants per plot divided by the total number of plants per plot times 100 %. The severity score was established in a 0-9 scale as described by Wiseman & Widstrom, (1984) with some modifications as follows; 0, no visible damage, 1-4, minimum visible damage, 5-7, moderate damage and 8-9 high damage.

In establishing the actual practice of smallholder farmers in managing fall armyworm information on farmer's management practices was obtained through interviews of different stakeholders including farmers, extension officers and Village executive officers. Smallholder farmers interviewed were those who were growing maize and old enough (minimum 18 years old) to provide information on the pest in their areas. Questioners were

designed to obtain information on the key aspects of *S. frugiperda* knowledge, maize production, maize varieties grown, *S. frugiperda* management practices, challenges, and recommendation. Global Positioning System (GPS) coordinates points for villages covered in the survey were recorded using a GPS tool.

3.7 Assessing efficacy of botanical plants against *S. frugiperda*

3.7.1 Preparation and extraction of botanical extracts

Root tuber of *D. kilimandscharicus* and aerial plant parts of *T. vogelii* leaves collected from Arusha region were washed with distilled water and dried under shade. Sample of 500 g from each plant was pulverized to obtain small particles of about 3- 11 mm. The ground particles were soaked in 1000 ml of different solvents based on their polarity to insure maximum extraction of both polar and non-polar compounds. The ground particles were first soaked in chloroform for 48 h and the respective extracts were filtered using filter paper (Whatman No 1). The obtained filtrated sample was collected in a round bottom flask, and filtrates were further sequentially soaked in ethyl acetate and methanol for 48 h. Solvents from all collected filtrates were evaporated in a vacuum using rotary evaporator under low pressure and reduced temperature. The resulting extracts 100, 70, 50 g for *D. kilimandscharicus* root methanolic, ethyl-acetate and chloroform extracts and 115, 86, 78.5 g for *T. vogelii* leaves methanolic, ethyl-acetate and chloroform extracts) were stored in closed glass vials at -4 °C and used for biological assay. Stock solution for further experiments was prepared by dissolving 100 mg in 10 ml of sterile water containing 1 % dimethyl sulphoxide (DMSO)

3.7.2 Testing plant extracts against *S. frugiperda* larvae

Newly hatched caterpillars (larva) of *S. frugiperda* were tested against two concentrations (5 % and 10 %) of *D. kilimandscharicus* and *T. vogelii* extracts obtained from the stock solution. Bioassay activity was conducted as described by Silva *et al.* (2015) with modifications. A portion of maize leaves (2 cm x 4 cm) were sprayed with a solution corresponding to each concentration on both sides and dried on paper towels for ten minutes before being placed in a petri dish (Plate 2). Then, larvae were introduced into each petri dish containing treated maize leaf and ten larvae were used for each concentration in five (5) replicates. Leaf treated with dimethyl sulphoxide served as a control. The petri dish containing treated leaf and larva were transferred to an environmentally controlled growth chamber at a temperature 26 $^{\circ}$C ± 2 $^{\circ}$C and 68 ± 2 % RH for assessment of insecticidal activity. A number of dead larvae in both treated and a control group were observed for 9 days. Mortality percentage was calculated as;

Percentage mortality = (the number of dead larval after treatment /number of larval before treatment) * 100. New fresh leaf was placed regularly on the experimental containers every after 48 h, to replace the consumed one.

3.8 Assessing the infectivity of the entomopathogenic nematodes

In investigating the effective insecticidal concentration to kill *G. mellonella*, glass Petri dishes with a 9 cm diameter containing a piece of filter paper were used. Initially, 3 L of water was added in the Erlenmeyer sharked and filtered to remove the nematodes from sponge, which were used as a carrier. The extracted nematodes were formulated into a different concentration of nematodes suspension. One (1) mL of the respective nematode's suspension was added to each Petri dish, and Petri dishes with 1 mL of distilled water alone were used as a control. The dishes were sealed with Parafilm® and placed in the climate-controlled tissue culture room at 25±1°C. This experiment had six treatments including: (a) 10 IJ/ml nematodes suspension; (b) 20 IJ/ml nematodes suspension; (c) 30 IJ/ml nematodes suspension; (d) 40 IJ/ml nematodes suspension; (e) 50 IJ/ml nematodes suspension; (f) control (water only). Each treatment was repeated six times, and mortality of *G. mellonella* was evaluated after 48 h of spraying nematodes suspension. Mortality percentage was calculated as; Percentage mortality = (the number of dead larval after treatment /number of larval before treatment) * 100. During observation, dead *G. mellonella* were dissected and observed under a microscope to confirm if their mortality was caused by entomopathogenic nematodes. Before each evaluation, *G. mellonella* were washed in distilled water to remove the nematodes that were stuck to their body, increasing the reliability of evaluating the presence of the nematodes inside the host.

3.9 Bio-based formulation for application against lepidoptera pests

3.9.1 Preparation of the bacteria and nematodes inoculums

Nematodes were cultured on nematodes growth medium in the presence of the symbiotic bacteria (*Xenorhabdus nematophila*) at 25 °C for two weeks. The fresh cultured nematodes were suspended in sterile water to form nematodes suspension. The nematodes suspension was used as inoculum for the test of NPs effects, efficacy against insect pests and nematodes-based formulation. The bacteria culture was inoculated in Nutrient broth and incubated at 25 °C and 150 rpm for 48 h. The fresh grown bacterial in the liquid medium was used as inoculum for further inoculations.

3.9.2 Testing effect of nanoparticles on the growth of nematode symbiotic bacterium (*Xenorhabdus nematophila*)

All metal oxides concentrations were prepared in nutrient broth (NB) medium in a final volume of 10 mL. Initially, 100 µL of the *X. nematophila* stock suspension was used for inoculating the NB medium, and the growth of bacteria was monitored with and without NPs at concentrations of 0.5–100 mg/L. Samples containing bacteria only were plated as positive controls. The mixtures were incubated on a shaker at 25 °C for 48 h, and the inhibition of cell growth was determined by the turbidities of the cell cultures. Aliquots were taken every after 8 h up to 48 h for measurement of the optical density at 600 nm.

3.9.3 Testing effect of metal oxides on the survival and pathogenic properties of EPNs

The evaluation of EPNs survival rates in various metal oxides nanoparticle formulations were carried out under laboratory condition at a temperature of 25 ± 1°C. Nanoparticles were suspended in deionized water in a concentration of 0.5, 2, 5 and 10 ppm, and probe sonicated to form homogeneous suspensions. Invasive Juvenile stage larvae (IJs) of the EPNs were introduced into the colloidal suspension containing respective concentrations of the three nanoparticles. Larvae kept in deionized water were used as a control group. The test was replicated three times, and nematodes mortality was estimated after 5 days of exposure. After 5 days of treatment, nematodes were washed and re-suspended in deionized water. The process however, did not allow complete removal of nanoparticles from the sample. The nematodes that survived contacts with nanoparticles at various concentrations were used for pathogenicity evaluation.

For pathogenic evaluation, one milliliter of the nematode's suspension of each concentration of NPs obtained from the previous experiment was added separately to the 10 last instars of the *G. mellonella* in a petri dish (diameter of 9 cm) lined with filter paper. The control group consisted of one milliliter of untreated nematodes larvae (IJs). The nematode concentration used was 500 IJs/ml ensured under the microscope. All treatments were replicated three times. After 48 h, insect mortality was recorded in terms of percentages and three dead insect larvae from each treatment were transferred to other petri dishes and incubated further for 48 h. The insects were later sectioned to check whether their mortality was caused by the entomopathogenic nematodes.

3.9.4 Evaluating pathogenic properties of nematodes formulation in UV Protecting Ingredients

(i) Testing survival rate of EPNs exposed under direct UV in UV protecting ingredients

The survival rate of the EPNs in three UV protecting ingredients was evaluated after UV exposure (380 nm) in a laboratory condition at 25±2 °C. The selections of the three NPs were based on low toxicity effect on the nematodes, and previous reports on enhanced efficacy of the EPNs. One milliliter of NPs suspension (0.5 %) and water containing approximately 1000 IJs were applied to each respective petri dish. Nematodes in an aqueous treatment were included as a control group. Non-treated control of water without nematodes was also used. There were three replicates of each treatment including a control group. Replicates of both treatments were exposed to UV at different time points (1, 2, 4 and 6 h). After exposure, the IJs survival rate was assessed by the active nematode movement and/or movement in response to the external stimulus.

(ii) Testing pathogenicity property of EPNs formulation direct exposed under sunlight

The nematodes protection provided by NPs in different concentration was tested in the direct sunlight exposure. About 1000 IJs in a 1 ml suspension of NPs and IJs in deionized water (control treatment) were applied to the Petri dishes. Treatments with water or/and NPs only was also used. All treatments were replicated three times. Ten *G. mellonella* larvae were introduced into each petri dish and exposed to sunlight at three-time point (5, 30 and 60 min). All tests were carried out at a temperature of 23 to 30 °C, RH 64–72 %. After the exposure time, each treatment dish was brought into the laboratory and maintained at 25 °C. *G. mellonella* larval mortality was assessed after 48 h. The tests were repeated three times.

3. 10 Assessing efficacy of nematode based formulation against *S. frugiperda*

This information is protected for patenting reasons

3. 11 Data analysis

Data collected were analyzed using GenStat software and SPSS version 21. Data on *S. frugiperda* incidence and severity were subjected to Student-Newmann-Keuls test and least significant difference (LSD) test at 5 % probability level was applied to compare the significant treatment means. Various variables were subjected to basic descriptive statistics and multiple responses to obtain the frequency of responses.

Data on insecticidal activity of entomopathogenic nematodes including mean percentage mortality of insect were plotted against the logarithms of concentrations using the Fig. P computer program (Biosoft Inc, USA). The LC_{50} and regression coefficient (R^2) were calculated from the regression equations obtained from the graphs**.** Whereas the data obtained for the insecticidal activity of plant extracts against insect were submitted to a variance analysis and Duncan's test in GenStat software was used to compare means between treatments.

Data on the performance of entomopathogenic formulation with UV protecting ingredients against insect were subjected to ANOVA and Student-Newmann-Keuls at 5 % significance level of probability was used to compare means between treatments.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 *Spodoptera frugiperda* infestation and management practices on maize fields of smallholder farmers in Northern Tanzania

(i) *Spodoptera frugiperda* incidence and severity

Survey results showed that all maize fields covered in the survey were infested by the *S. frugiperda* in all three regions of northern Tanzania with varying infestation levels among regions (Table 2) and Plate 3. Of the surveyed areas Arusha appeared to have significant ($p < 0.001$) higher level of incidence scores (66.59 %), as compared to Kilimanjaro (52.96 %) and Manyara (52.64 %) (Table 2). Based on the survey data, the severity score was significant different ($p \leq 0.05$) between regions which ranged from low (1-4) to moderate (5-7) damage as scored following the (Wiseman *et al*., 1984) visual rating scale.

By village, the infestation results among villages show that Malula (79.55 %) had the highest infestation level followed by Timbolo (76.55 %) and Kikwe (76.10 %) with the least infestation recorded in Embasen (35.57 %) (Table 3). However, the severity of *S. frugiperda* damage was low throughout the fourteen villages except for Malula, Timbolo, Kikwe and Mtakuja where it was moderate.

Plate 1: Colony of *S. frugiperda* reared in glass cages at 25-28 °C and 67-80 % RH. The insects were supplied with honey for adults and maize leaves to feed larvae. Larvae that emerged were collected for bioassay.

Plate 2: Testing plant extracts efficacy against *S. frugiperda* larvae

Table 2: Mean incidence and severity of *Spodoptera frugiperda* in Northern Tanzania

District	Incidence (%)	Severity level
Arusha	66.59 [a]	5.422 [a]
Kilimanjaro	52.96 [b]	4.756 [b]
Manyara	52.64 [b]	3.989 [c]
LSD (p=0.05)	6.12	0.578
P value	<.001	<.001

Means with the same letter(s) down the column are not significantly different (p=0.05, Student-Newman-Keuls test)

Plate 3: *Spodoptera frugiperda* larvae damage on leaves and corns of maize in the study area during the 2018 growing season

Table 3: Mean Incidence and mean severity of the *S. frugiperda* in the surveyed villages of the three regions

Village Name	Region	Coordinates	Mean Incidence %	Mean severity
Malula	Arusha	S 3021’56 E 3700’32	79.55 a	7.267 a
Timbolo	Arusha	S 3017’40E 36040’46	76.55 a	6.600 ab
Kikwe	Arusha	S 3026’2E 36050’34	76.10 a	6.667 ab
Mtakuja	Kilimanjaro	S 3029’10 E 37021’39	72.39 ab	6.733 ab
Nduruma	Arusha	S 3028’36 E 36047’27	69.46 abc	5.067 cd
Mabogini	Kilimanjaro	S 3025’56 E 37020’57	62.94 abcd	5.667 bc
Bangata	Manyara	S 3019’58 E 36044’36	62.30 abcd	4.600 cde
Signo	Manyara	S 4011’57 E 35040’10	59.86 abcd	4.533 cde
Halla	Manyara	S 4016’5 E 35048’13	54.71 bcde	3.867 cdef
Mabungo	Kilimanjaro	S 3024’48 E 37030’14	52.25 cde	4.867 cde
Nakwa	Manyara	S 4017’8 E 35042’39	51.92 cde	4.200 cde
Wangwaray	Manyara	S4011’43 E35046’52	51.58 cde	3.067 ef
Bonga	Manyara	S 4019’17 E 35044’27	51.28 cde	4.533 cde
Uchira	Kilimanjaro	S 3024’35 E 37030’21	49.66 cde	4.600 cde
Kiongozi	Manyara	S 406’45 E 35045’28	46.48 de	3.733 def
Kindi	Kilimanjaro	S 3017’52 E 37015’57	41.81 de	3.533 def
Sambaray	Kilimanjaro	S 3018’59E 37017’60	38.70 e	3.133 ef
Embasen	Arusha	S 3021’39 E 36053’17	35.57 e	2.333 f
Mean		-	57.4	4.722
LSD (p =0.05)		-	12.99	1.1355
P value		-	< 0.001	< 0.001

Means with the same letter(s) down the column are not significantly different (p = 0.05, Student-Newman-Keuls test)

(ii) *Spodoptera frugiperda* management practices by smallholder farmers in the study area

In the present study, two main types of management practices were reported by farmers including; synthetic chemicals and non-synthetic chemical methods applied by 86 % and 11.2 % of the respondents respectively. However, only 2.8 % of the respondents reported having done nothing against the pest. Sixteen (16) different brands of insecticides were reported to be used by smallholder farmers in the study area as shown in Table 4. Chemical pesticides like Duduba 450 EC (Cypermethrin 150 g/L + Chlorpyrifos 300 g/L) was the mostly (23.7

%) used across regions, followed by Duduall 450 EC (Cypermethrin 100 g/l +Chlorpyrifos 350 g/L) (10.5 %) and Supercron 500 EC (Emamectin Benzoate 21.5 g/L) (9.7 %). Other types of chemical pesticides, their applications were restricted to specific regions or villages due to their availability. Despite the intense use of pesticides, smallholder farmers have reported; ineffectiveness of the insecticides (40.9 %), high cost of insecticides (38 %), limited *S. frugiperda* management knowledge (11.6 %), limited knowledge on *S. frugiperda* biology and behavior (5.3 %) and limited technical *S. frugiperda* expertise (4.2 %) as the main constraints for effective management in the study area.

On the other hand, nonchemical methods were also used in the study area to manage *S. frugiperda* and application of these methods was reported by 11.2 % of the respondents. Whereby these methods were applied in the field followed by the application of synthetic chemicals or applied simultaneously. Non-synthetic chemical methods applied in the study area are listed below (Table 5).

Table 4: Synthetic chemicals commonly applied by farmers for the management of *Spodoptera frugiperda* in the study area

Trade Name	**Active ingredient**	**Percentage (%)**	**Frequency**
Duduall 450EC	Cypermethrin 150 g/L+Chlorpyrifos 300 g/L	10.5	22
Duduba 450 EC	Cypermethrin 100 g/l +Chlorpyrifos 350 g/l	23.7	49
Spidex 2.15EC	Emamectin Benzoate 21.5 g/L	4.7	9
Laraforce 25EC	Lambdacyhalothrin 25 g/L	1.9	4
Belt 480SC	Flubendiamide 480 g/L	6.5	14
Selecron 720EC	Profenofos 720 g/l	3.6	8
Boneforce	-	2.2	5
Supercron 500EC	Profenofos 500 g/l	9.7	20
Karate 5EC/5SC	Lambda cyhalothrin 50 g/l	3.7	8
Dudumectin11.2 %EC	Emamectin 4.8 %+Acetamiprid 6.4 %	4.1	9
Profecron 720EC	Profenofos 720 g/l	4.8	10
Prosper 720EC	Cypermethrin120 g/L +Profenofos 600 g/L	2.8	6
Libarate	Emamectin Benzoate 40 g/L+ Indoxacarb 160 g/L	6.1	13
Snow super 20 %EC	Abamectin 10 % + Emamectin Benzoate 10 %	4.2	9
Ninja 5EC	Lambdacyhalothrin 50 g/l	4.2	9
Multi-Alfplus150 EC	Emamectin Benzoate 50 g/l +Alphacypermethrin 100 g/l	1.9	4
Soap	-	5.4	11
Total		100	210

Table 5: Non-synthetic chemical methods applied by famers for management of *Spodoptera frugiperda* in the study area

Category	Name	Percentage (%)	Frequency
Cultural	Ash	27.7	58
	soil/sand	21.7	45
Biological	-	0	0
Botanical	*Tephrosia vogelii*	10.8	23
	Azadiracta indica	10.8	23
	Zingiber officinale	10.8	23
	Solanum incanum	4.9	10
	Capsicum annuum	13.3	28
Resistant variety	-	0	0
Total		100	210

4.1.2 Insecticidal activity of different bio-based control agents against *Spodoptera frugiperda* and *Galleria mellonella*

(i) Effect of plant extracts on *Spodoptera frugiperda*

This study found that an increase in exposure time increases larval mortality of *S. frugiperda* (Table 6). The effect of plant extracts on larvae mortality was concentration-dependent. High insect mortality was observed in the highest concentrations of the methanol, ethyl acetate and chloroform extracts of all botanicals. Two days after exposure, the effect of plant extracts on *S. frugiperda* larvae mortality ranged from 6.67 % to 36.67 % which was significantly ($p= 0.027$) higher than the mortality observed under the control group (0.33 %). After two days of exposure, *T. vogelii* leaves methanol extract (TLEM) caused the highest insect mortality (36.67 %), followed by *D. kilimandscharius* root chloroform extract (DORC) (33.33 %), *T. vogelii* leaves ethyl acetate extract (TLEE) (30 %) and *T. vogelii* leaves chloroform extract (TLEC) (30 %). In the ninth day of exposure, insect mortality caused by plant extracts ranged from 36.67 % to 70 % which was also significantly ($p <.001$), higher than the mortality observed in the control group (0.67 %). The highest mortality was observed in *T. vogelii* leaves methanol extract (TLEM) (70 %), followed by *D. kilimandscharius* root chloroform extract (DORC) (60 %), *D. kilimandscharius* root ethyl-acetate extract (DORE) (60 %) and *T. vogelii* leaves chloroform extract (TLEC) (56.67 %).

Table 6: Mean percent mortality of *Spodoptera frugiperda* larvae 2, 9 days after treatment with plant extracts in the laboratory experiments

Type of extracts	Concentration (%)	Percent mortality of the larva after treatments	
		2 days	9 days
TLEM	5	26.67 ^{bc}	56.67 ^{cd}
TLEM	10	36.67 ^{c}	70 ^{d}
TLEE	5	26.67 ^{bc}	50 ^{bc}
TLEE	10	30 ^{c}	53.33 ^{bcd}
TLEC	5	26.67 ^{bc}	53.33 ^{bcd}
TLEC	10	30 ^{c}	56.67 ^{cd}
DORM	5	6.67 ^{ab}	36.67 ^{b}
DORM	10	23.33 ^{bc}	53.33 ^{bcd}
DORE	5	20 ^{abc}	46.67 ^{bc}
DORE	10	26.67 ^{bc}	60 ^{cd}
DORC	5	30 ^{c}	50 ^{bc}
DORC	10	33.33 ^{c}	60 ^{cd}
Untreated	Water	0.33 ^{a}	0.67 ^{a}
P-value		0.027	<.001

Means within a column followed by the same latter are not significantly different $p < 0.05$ (Duncan's test).

TLEM- *Tephrosia vogelii* leaves methanol extract, TLEE- *Tephrosia vogelii* leaves ethyl acetate extract, TLEC-*Tephrosia vogelii* leaves chloroform extract, DORM- *Dolichos kilimandscharius* root methanol extract, DORE- *Dolichos kilimandscharius* root ethyl acetate extract, DORC-*Dolichos kilimandscharius* root chloroform extract

(ii) Infectivity of entomopathogenic nematodes against selected Lepidoptera pest

Results indicate that infectivity of the entomopathogenic nematodes against insect pest is concentration-dependent. That is, mortality of *G. mellonella* larvae increased with increased concentration of the nematode's suspension. The mortality of *G. mellonella* was 100 %, when the concentration of nematodes was higher than 40 IJ/ml, although other treatments differed from the control (Fig. 3). Statistics has shown that $R^2 = 0.9192$ and the lowest concentration that kill half insect population (LC_{50}) is 19.49 IJ/ml computed from $y = 128.53x - 115.38$.

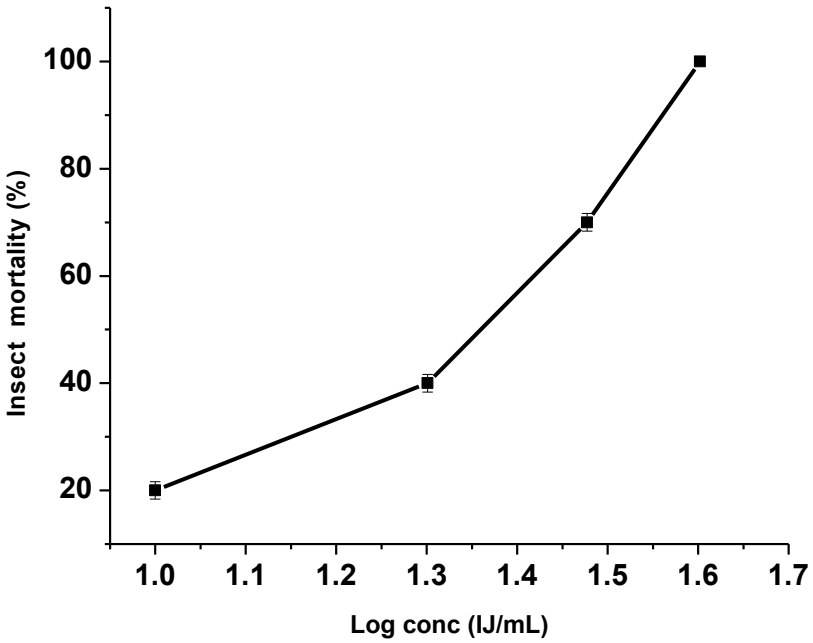


Figure 3: Mortality of *Galleria mellonella* after exposure to different concentrations of nematode suspension in the laboratory (25±5 ºC, RH of 70±10 %)

4.1.3 Formulation of the entomopathogenic nematodes for application against Lepidoptera pests

(i) Effect of nanoparticles on nematode symbiotic bacterium

Results indicated that, the toxicity of the three NPs were less toxic to the nematode symbiotic bacterial exhibiting growth inhibition in a concentration-dependent manner (Figs. 4–6). The observed inhibition had an order of Fe_3O_4 < TiO_2 <ZnO NPs, although the measurable inhibition was observed at the highest concentrations of 50 mg/L and 100 mg/L for ZnO NPs, TiO_2 NPs and Fe_3O_4 NPs respectively. The enhanced bacterial inhibition effect of the NPs with decreasing particle size was also observed (Figs. 4 and 5). The difference was observed at 50 mg/L and 100 mg/L. The current results show clearly the role of particle size and concentration of NPs in toxicity against bacterial. Furthermore, results show that even at high concentration tested; the bacteria cells density increased after prolonged lag phase which indicates the ability of the nematode symbiotic bacteria to tolerate toxicants.

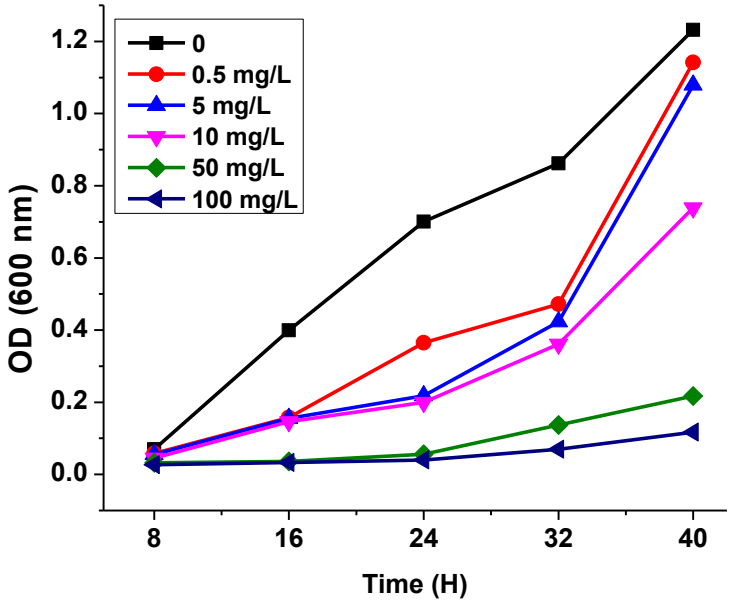


Figure 4: The growth inhibition effect of ZnO NPs against nematode symbiotic bacteria (*Xenorhabdus nematophila*) under various concentrations at different time points presented as optical density (OD 600 nm)

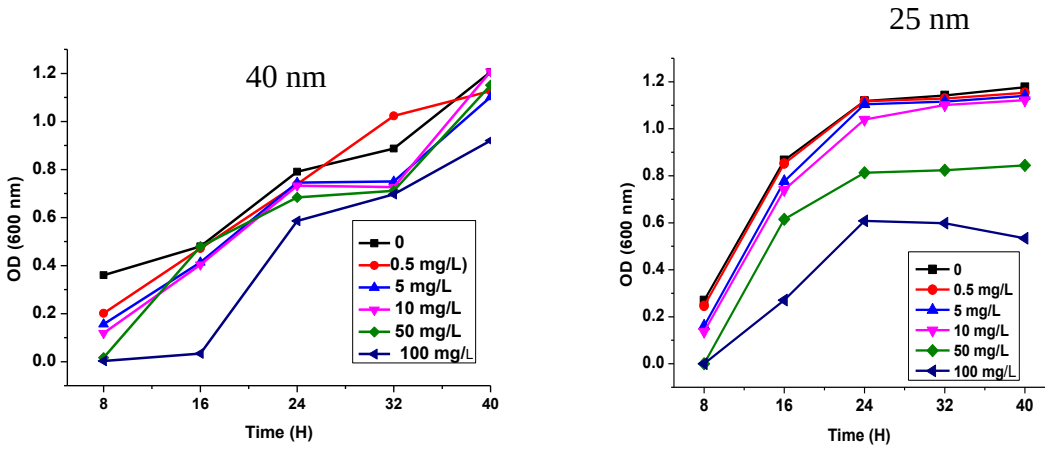


Figure 5: The growth inhibition effect of TiO_2 NPs against nematode symbiotic bacteria (*Xenorhabdus nematophila*) under various concentrations at different time points presented as optical density (OD 600 nm)

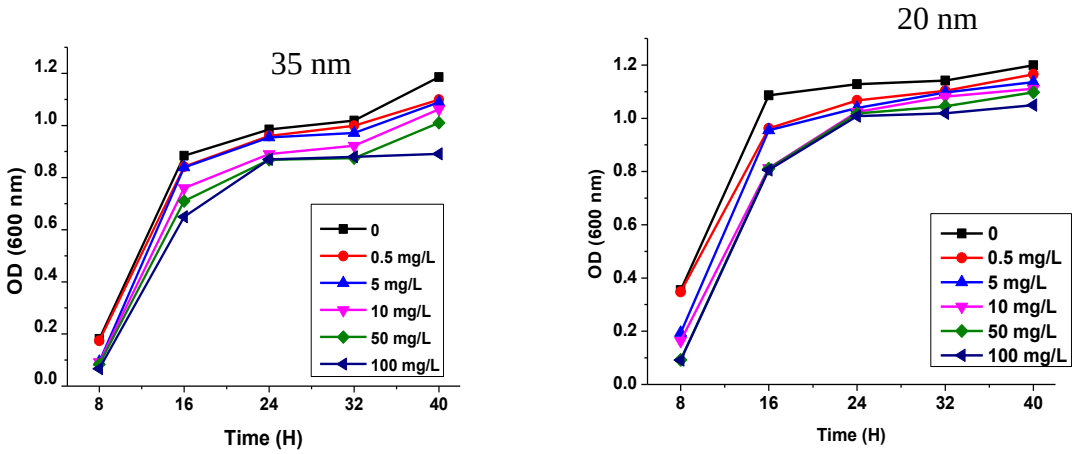


Figure 6: The growth inhibition effect of Fe_3O_4 NPs against nematode symbiotic bacteria (*Xenorhabdus nematophila*) under various concentrations at different time points presented as optical density (OD 600 nm)

(ii) Effect of NPs on the survival of entomopathogenic nematode

The effects of ZnO-NPs, TiO_2-NPs and Fe_3O_4-NPs on the survival of entomopathogenic nematode (*S. carpocapsae*) infective larvae (IJ) after five days of exposure were investigated. All nanoparticles were examined at five concentrations (0.5–10 ppm). All treatments had various degree of effect on IJs survival. Control treatment had significantly higher survival rate (> 95 %) than metal oxides treatments ($p < 0.001$). Survival of EPNs exposed to NPs of the three metal oxides depended on their concentrations, although the average survival rate was slightly nonlinear. Survival rate was observed to decrease faintly with increased concentrations (Fig. 7). Survival of nematodes exposed to 0.5 and 2 ppm of TiO_2 and Fe_3O_4-

NPs did not significantly differ from the control ($p < 0.002$). Also, for the highest concentration of 10 ppm, ZnO NPs had the lowest survival rate as compared to that of TiO_2 and Fe_3O_4-NPs. However, the survival rate was higher than 65.3 %, 78.1 % and 81.4 % for ZnO, TiO_2 and Fe_3O_4-NPs, respectively.

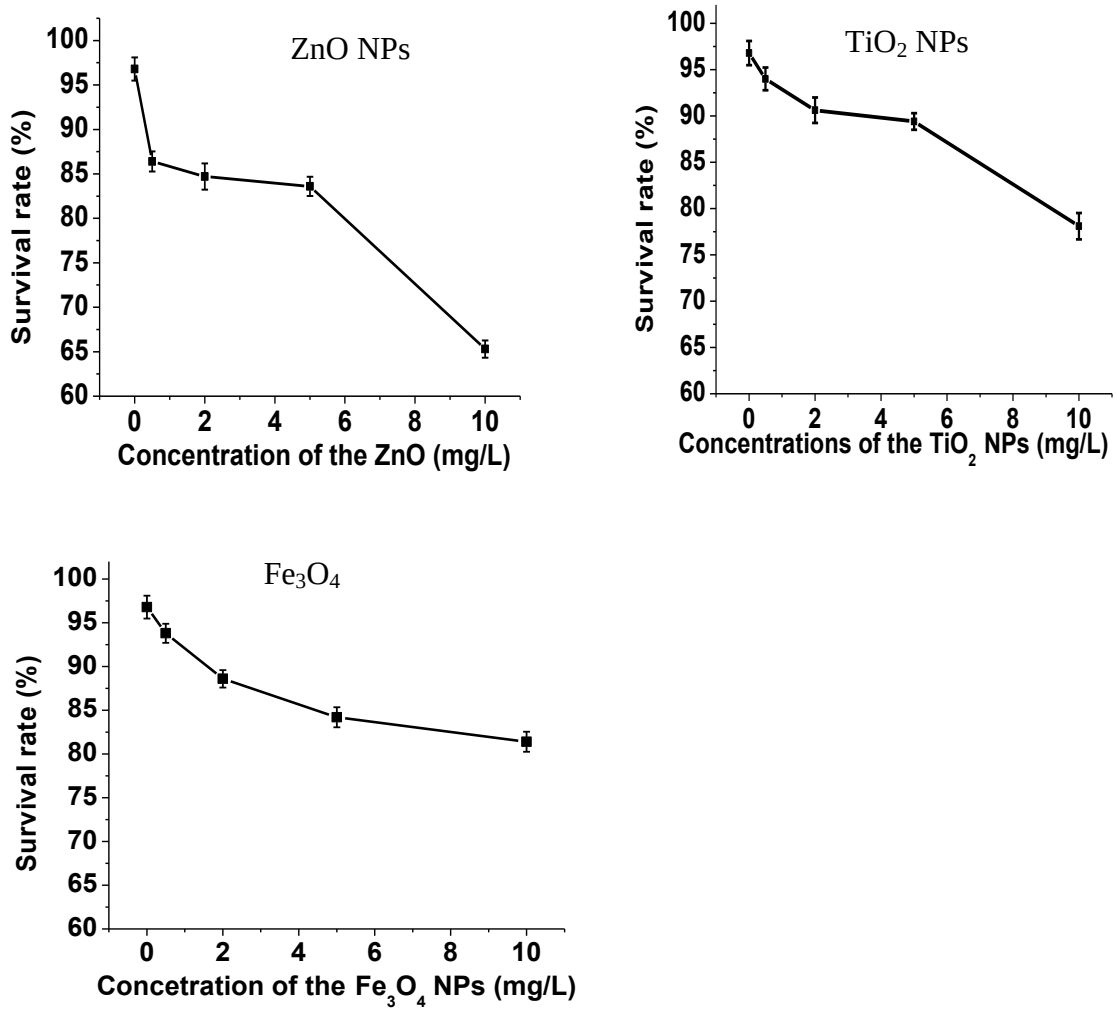


Figure 7: Effect of three metal oxides NPs on the survival of the EPNs (*Steinernema carpocapsae*) under different concentrations examined at five days' endpoints.

(iii) Efficacy of EPNs exposed into three metal oxides NPs on *G. mellonella*

Pathogenicity of nematodes that contacted various concentrations of nanoparticles on *G. mellonella* was assessed after 48 h of incubation. Treatment type however did not differ in their ability to kill *G. mellonella*, although a slight variation was observed in different concentrations (Fig. 8). The *G. mellonella* larva treated with nematodes exposed to 10 ppm of ZnO NPs experienced the lowest mortality (63.33 %), whereas larva treated with nematodes

exposed to 0.5 ppm of Fe_3O_4 NPs recorded the highest mortality (95 %) than the control (80 %). The highest or lowest mortality caused maybe associated with the level of effect that nanoparticles posed on the nematodes survival rates. This ability of the entomopathogenic nematodes to retain their pathogenic property may be associated with the immune ability of nematodes symbiotic bacteria to nanoparticles

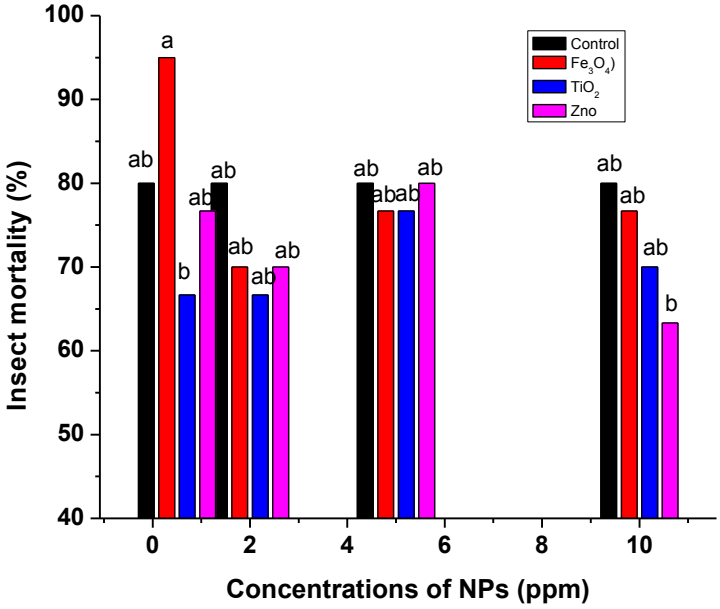


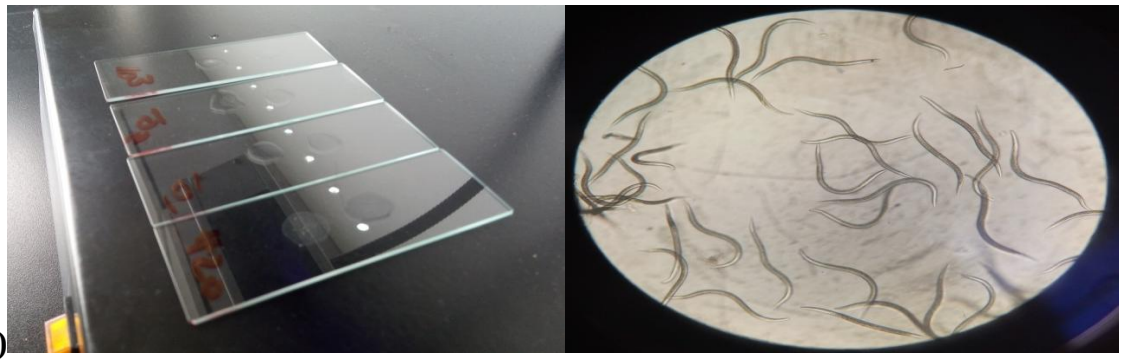
Figure 8: Effect of ZnO, TiO_2 and Fe_3O_4 nanoparticles on pathogenic properties of the *S. carpocapsae*. Bars represent means of the percentage mortality of *G. mellonella*, and bars of the given concentration coupled with the same letter(s) are not significant different from each other (p = 0.05).

Survival rate of EPNs exposed under direct UV in UV-protecting ingredients

In this experiment, the potential of nanoparticles of the three metal oxides to protect nematodes from ultraviolet radiation (380 nm) after 1, 2, 4 and 6 h of exposure were investigated (Plate 4). One hour of exposure did not significantly influenced the survival rate of nematodes in all treatments (> 90 %). However, the nematodes survival rate significantly decreased with prolonged exposure (p <0.001). Six-hours of exposure had significantly lower survival rate than the other exposure time (p < 0.001), and one hour of exposure had significantly higher survival rate than two hours (p = 0.047) in all treatments. In comparing treatment type after six-hour exposure, nematodes exposed in aqueous suspension had the lowest survival rate (35.36 %) than nematodes exposed in Fe_3O_4 (43.52 %), TiO_2 (44.48 %) and ZnO (75.98 %) (Fig. 9). The difference observed between NPs treatments and water

(control), indicates that NPs protect nematodes from ultraviolet radiation, although the degree of protection varies from one type of NPs to another.

(a)



(b)

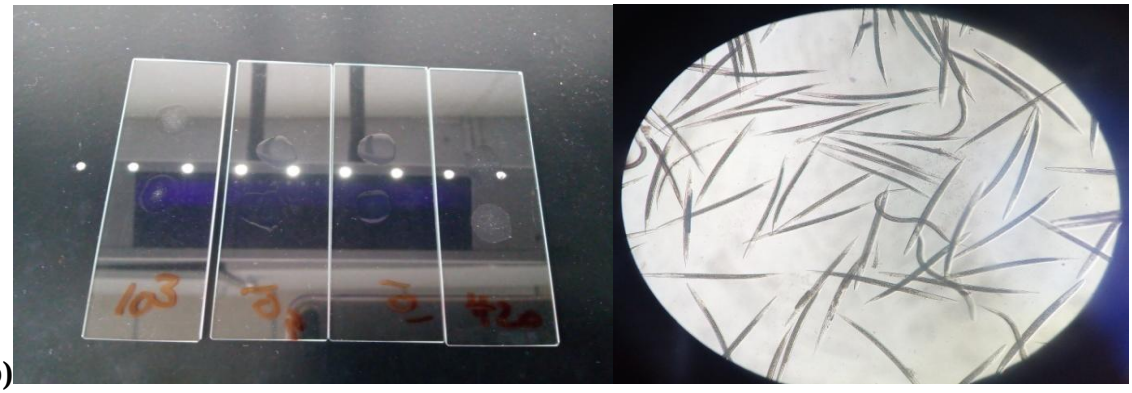


Plate 4: Exposure of nematodes to UV protecting ingredient under ultraviolet radiation (380 nm), morphology before exposure (a) and morphology after exposure (b)

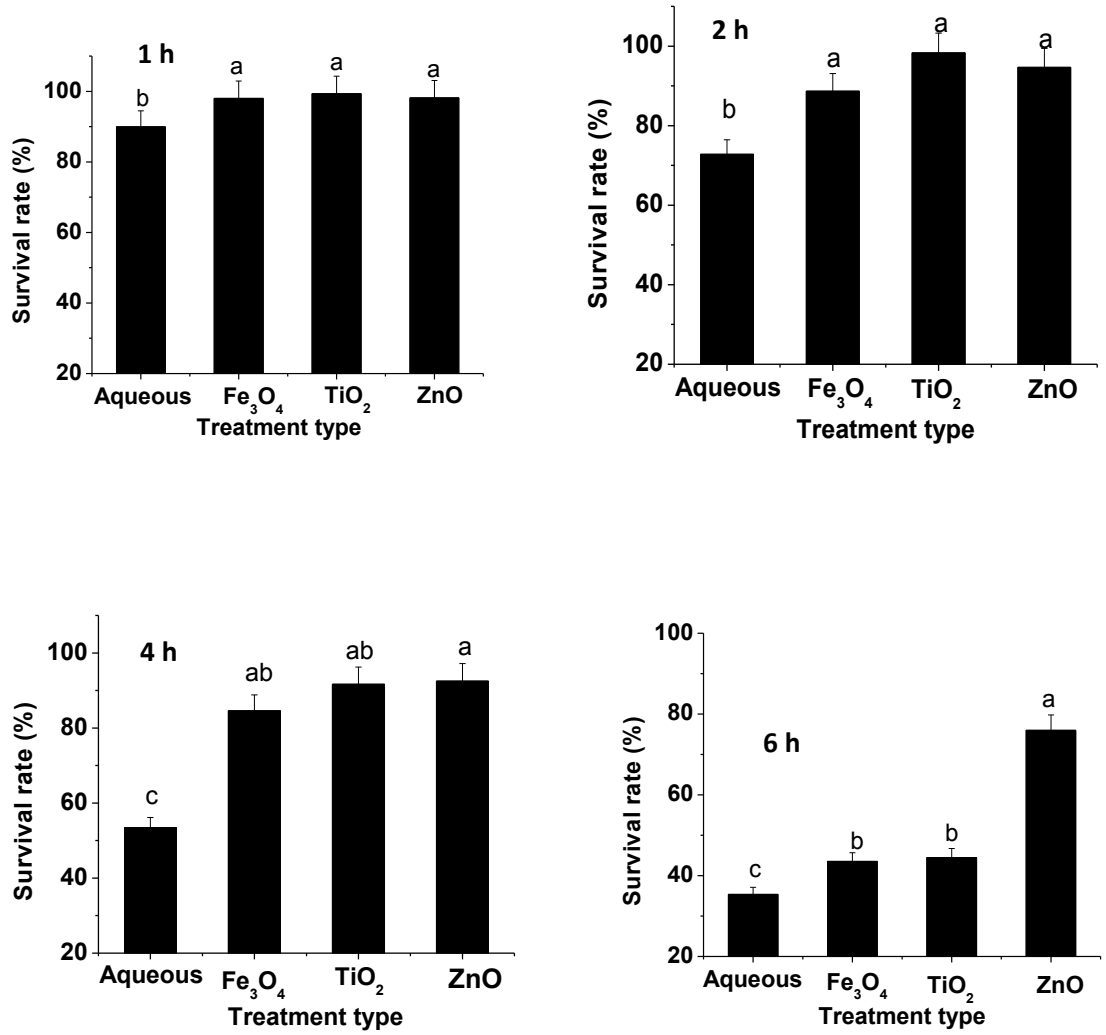


Figure 9: Survival of *Steinernema carpocapsae* in different solutions exposed to UV for 1, 2, 4 and 6 h. All formulations were made at 0.5 %. Bars represent mean survival rates and similar letters on each bar are not significantly different from each other (p =0.05).

(iv) Pathogenicity property of EPNs formulation direct exposed under sunlight in UV protection ingredient

The ability of EPNs formulations to infect and kill a host insect following exposure to sunlight for 0, 15, 30 and 60 min was assessed after 48 h (Plate 5). Insect mortality between treatments was compared. For all treatments, insect mortality significantly decreased with an increase in exposure time (p <0.001). At 30 min exposure to sunlight, all EPNs nanoparticles formulation caused higher insect mortality than aqueous treatment (Fig. 10). At 60 min, ZnO formulation was the only treatment caused higher insect mortality (46.67 %) than Fe_3O_4

(23.33 %) and TiO_2 (20 %). In general, the above results show that NPs provided significant benefit expressed as higher host mortality when compared with the aqueous EPNs suspension.

Plate 5: Pathogenic property of nematodes in UV protecting ingredient under direct sunlight

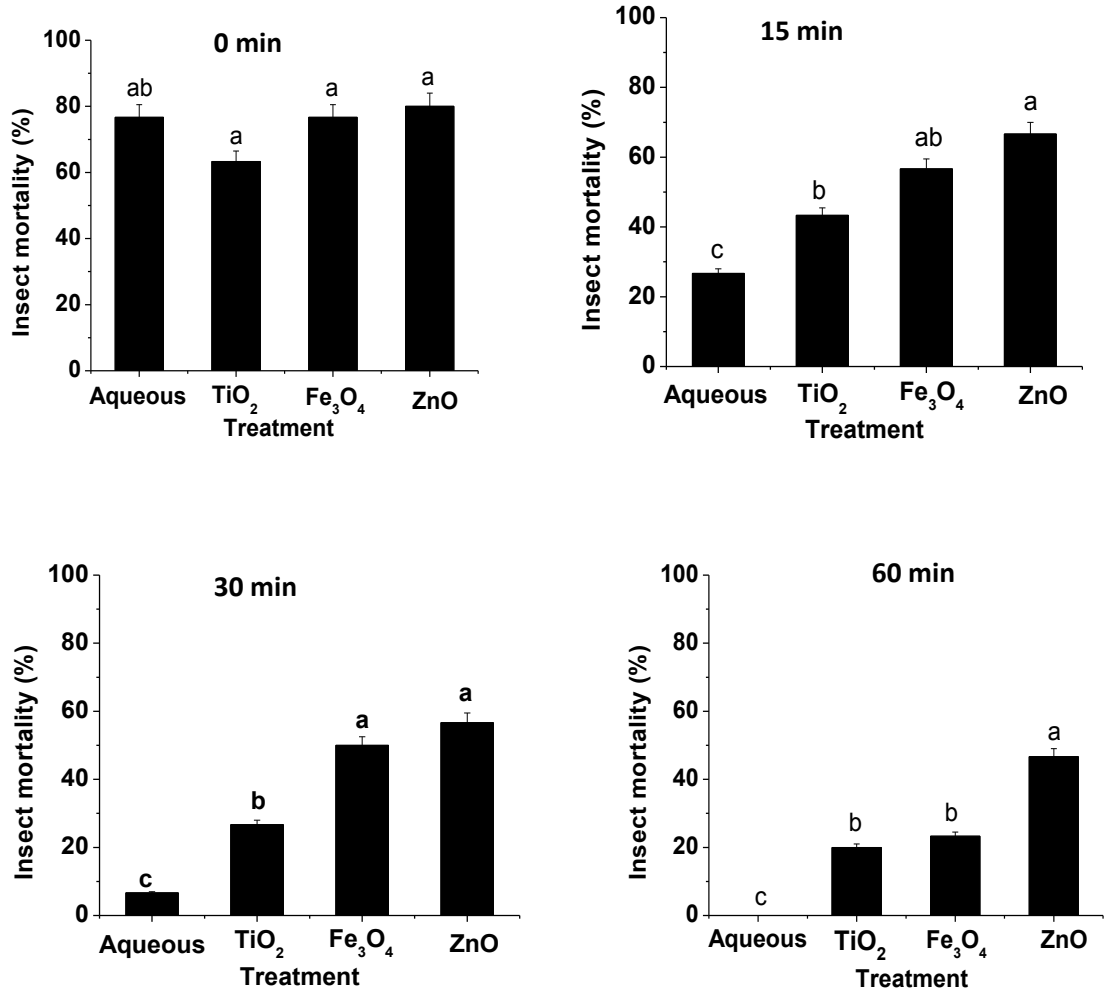


Figure 10: Mortality percentages of the *Galleria mellonella* infected by *Steinernema carpocapsae* in four formulations exposed to sunlight at different time points. Mortality was assessed 48 h after infection.

4.1.4 Effect of entomopathogenic nematodes on *Spodoptera frugiperda*

The information is protected for patenting

4.2 Discussion

Lepidopteras have been cited to be the most destructive insects worldwide (Sylvain *et al*., 2015, Harris-shultz *et al*., 2015; Juárez *et al*., 2012; Jeger *et al*., 2017). In this study, one of recently reported invasive Lepidoptera namely fall armyworm (*S. frugiperda*) has been intensively assessed in the major maize production locations in Northern Tanzania and results revealed the pest to be widely prevalent with variation between regions. It has been established that variations in pest infestations is dictmated by pest population density and the growth stage of the crops (Wiseman & Widstrom, 1984). In this study, variation of the pest infestation between locations could be due to different management practices and differences in planting dates. Variation in planting time was observed between regions whereas farmers in Manyara region planted maize early in February in the planting season of 2018 followed by Kilimanjaro and Arusha which planted their maize late in March. Similar observation on reduced pest infestation by early planting in a given cropping season was previously reported by Abrahams *et al*. (2017) and Goergen *et al*. (2016).

In managing the pest by famers, the current study revealed the availability of different management options which includes synthetic chemicals and non-synthetic chemical methods. The synthetic chemical pesticides formulations were applied by majority (86 %) of the farmers as the major option for the management of *S. frugiperda* in the area, and similar results have been reported in other countries of Africa (Abrahams *et al*., 2017; Day *et al*., 2017; Prasanna *et al*., 2018). The choice of which chemical to use was based on its availability, farmer's knowledge and purchasing power of the farmers. It was also observed that the performance of the synthetic chemicals was likely influenced by the pesticide application time, dose, and frequency as most of farmers did not follow the application instructions provided by the manufacture, and these factors have been previously reported by other scholars (DalPogetto *et al*., 2012; Hardke *et al*., 2011; Gutierrez-moreno, 2017; Kumela *et al*., 2018; Sisay, 2018). Conversely, of all chemicals reported to be used in the study area Duduba (Cypermethrin 150 g/L + Chlorpyrifos 300 g/L) appeared to be the mostly used type of synthetic chemicals. However, Duduba contain active ingredient that fall under the class of organophosphates and pyrethroids which were previously reported to have detrimental effects on human health and the environment in general (Belay *et al*., 2012; Abrahams *et al*., 2017; Togola *et al*., 2018). It has been also, established that Lepidopterans including *S. frugiperda* have developed resistance against some chemicals in these classes (Abrahams *et al*., 2017; Al-Sarar *et al*., 2006; Hardke *et al*., 2015).

In contrast, famers were also using non-synthetic chemical methods in managing *S. frugiperda* in which their applications may be associated with high cost and low efficacy of synthetic chemicals. The use of non-synthetic chemical methods was based on smallholder farmer's experience of using the same in other crop affected by other lepidopterans. The similar approaches have been reported to be used in Ethiopia and Kenya for management of the Lepidoptera (Kumela *et al*., 2018). Non-synthetic such as ash, dust, pepper and plant materials are affordable to smallholder farmers although, the method alone is not adequate to control the pest (Abrahams *et al*., 2017). The combination of different management approaches has most likely affected the level of infestation among regions. Infestation level was low in the region where different management approach was applied. Combined management approaches (IPM) improve the efficiency in managing *S. frugiperda* as compared to a single approach (Michelotto *et al*., 2017; Molina-ochoa *et al*., 1999). Thus, based on the infestation and management practices findings of this study reveals that the type of management applied influences the *S. frugiperda* infestations level.

Despite the use of the wide range of management options by famers, the pest continues to dominate their fields. Due to that, this study found it worth to evaluate effective methods such as insecticidal plants and microbes that can be used for management of *S. frugiperda* and other Lepidoptera pests.

In the present study, bioformulations evaluated against Lepidoptera pests revealed their potential in managing Lepidoptera pests, including *S. frugiperda* owing to their insecticidal properties. The *D. kilimandscharicus* and *T. vogelii* crude extracts displayed insecticidal activity against *S. frugiperda*, which was observed to increase with exposure time. The difference observed between botanical treatments and the control, suggest the potentiality of these plant extracts as a source of insecticides. The potential of using *T. vogelii* and *D. kilimandscharius* in pest management have been previously reported (Alao & Adebayo, 2015; Jacques, Safiou, Jédirfort, & Souaïbou, 2015; Nyirenda *et al*., 2011). The *T. vogelii* and *D. kilimandscharius* are reported to contain compounds with insecticidal properties such as rotenoids deguelin, tephrosin, rotenone and which have insecticidal properties. In the present study, *T. vogelii* and *D. kilimandscharius* displayed insecticidal properties that can be harnessed for further development of botanical-based pesticides for controlling *S. frugiperda* although their efficacy is subjected to long exposure time. Despite their slow efficacy, these plants are available and accessible to resource-poor farmers; thus, they can be used to reduce

insect density to lower the effect on crop damages, lthough the point of sustainability needs to be mantained (Alves *et al*., 2014; Céspedes *et al*., 2001; Risco *et al*., 2012).

The biology and behaviour of the Lepidoptera caterpillar, has limited the performance of different management options, necessitating the shift to the use of biological control agents including the application of microbes. The present study revealed the potential of using nematodes to control Lepidopterans. Unlike the botanicals, nematodes can cause insect mortality from 12 to 48 h after application. Their behaviour to invade insect, feeding and reproduce inside the host bestow their pathogenic effect against insect pests (Makirita *et al*., 2019). In the present study, nematodes caused measurable *G. mollonella* larvae mortality even at low concentrations, and mortality increased with increase in nematodes concentration. The current results agree with previous findings that insect susceptibility to nematodes increases with increase in nematodes concentration and exposure time (Belien, 2018; Kalia *et al*., 2014). Despite the infectivity of nematodes under laboratory condition, their performance in the field condition is challenged by environmental factors such as UV radiation and desiccations.

The current results show the ability of nanoparticles in protecting nematodes against UV radiations, with enhanced performance of the nematodes against Lepidoptera pest when applied with nanoparticles under direct sunlight (Makirita *et al*., 2019). The preliminary evaluation indicated that nematodes exhibited strong resistance when exposed to ZnO, TiO_2 and Fe_3O_4 NPs, although tolerance was observed to decrease with elevated concentration of the nanoparticles. Moreover, the exposure of nematodes to metal oxides nanoparticles, however, did not deprive their pathogenic properties. This ability maybe associated with immune nematode symbiotic bacteria which accelerate the EPNs performance. Previous studies assessed the toxicity of metal ion and metal oxides on different species of nematodes indicated tolerance at low concentration (Jaworska, Sepiol & Tomasik, 1996; Khare *et al*., 2011; Wu *et al*., 2013). Kucharska *et al*. (2011, 2014), found that the contact of *Steinernema feltiae* with nano-Cu and nano-Au did not affect the nematode efficiency to kill *Alphitobius diaperinus*. Moreover, the contacts of the *H. bacteriophora, S. feltiae S. arenarium, S. abbasi* and *H. indica* with nano-Ag did not, deprive the pathogenic activity of the nematodes against *G. mellonella* (Taha & Abo-Shady, 2016, Kucharska *et al*., 2014). More interestingly, metal ion and metal oxides were observed to enhance pathogenicity and reproduction of the EPNs (Jaworska & Gorczyca, 2002; Lortkipanidze *et al*., 2016; Taha & Abo-Shady, 2016). Similar results of nano-Ag, nano-Cu & nano-Au have also been reported (Kucharska *et al*., 2014;

Taha & Abo-Shady, 2016). The current observation did not contradict former findings on the insignificant effect of nanoparticles on nematodes survival and pathogenic properties. This provides the confidence of applying nematodes even in the metal polluted environment. The less toxicity effect of the NPs suggests their consideration in EPNs formulation for enhanced efficacy in controlling above-ground pests such as Diptera and Lepidoptera.

Numerous formulations have been developed to address the environmental challenges of the EPNs at the target site to enhance their performance for foliar pest. The addition of polymer or agar adjuvants during application, have been reported to improve the performance (Dito, Shapiro-Ilan, Dunlap, Behle & Lewis, 2016; Hussein & Abdel-Aty, 2012; Shapiro-Ilan, Morales-Ramos, Rojas & Tedders, 2010). Addition of UV protecting ingredients together with anti-desiccant in nematodes formulation provides a feasible approach for the field applications. The present study, also reports enhanced efficacy of the EPNs combined with nanoparticles of ZnO, TiO_2 and Fe_3O_4 on sunlight exposed surface against *G. mellonella*. The ability of nanoparticles to protect nematodes was presented in previous studies (Walia *et al*., 2008; Dito *et al*., 2016). For instance, Barricade® gel formulation combined with titanium dioxide (1 %) was reported to enhance protective property of the EPNs (Dito *et al*., 2016). Similarly, this study is currently presenting the nematodes protective properties of other metal oxides including ZnO and Fe_3O_4 NPs against UV radiation. All the same, the present study shows that even low concentration (0.5 %) of the tested nanoparticles provided protection to nematodes and enhances their pathogenic potential in a single application. Therefore, based on the current finding's nanoparticles can be manipulated for better performance of EPNs formulation against above-ground pest. Like many other biological control agents, entomopathogenic nematodes are compatible with various agricultural inputs such as chemical pesticides, fertilizer, and other biological control agents (Makirita *et al*., 2019). For instance, a combination of EPNs with entomopathogenic fungi and resistant varieties successfully controlled Lepidopteran pests including *S. frugiperda* (Ansari *et al*., 2008; Molina-ochoa *et al*., 1999). Some species of EPNs are also reported to extend their compatibility to the agrochemical for short exposure (Negrisoli, Garcia, & Negrisoli, 2010; Niekerk & Malan, 2014). This interaction of EPNs with diverse agricultural inputs provides the best option for integrated pest management. As a result, the inclusion of nanoparticles in EPNs formulation for a single application is a more viable option for growers due to reduced cost and time of application while enhancing the performance.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study revealed that *S. frugiperda* is a serious and challenging pest of maize in the study area that may also reflect or interpolate to other maize growing areas in Tanzania. In the current study, the intensity of *S. frugiperda* infestation varied between regions due to differences in planting dates and management practices by maize production farmers. This study also developed and tested the effects of an environmentally friendly biopesticide formulation from plant extracts and entomopathogenic nematodes that can be used for the management of Lepidopterans including *S. frugiperda*. The study established that *T. vogelii* and *D. kilimandscharius* extracts with concentration of 10 % w/v are effective in the management of *S. frugiperda*, but their infectivity is subjected to the exposure time of up to 9 days post-exposure indicating that the plant extracts of the studied plants have the potential for development of biopesticides. The entomopathogenic nematodes formulation at 40 IJ/ml caused high (100 %) insect mortality 2 days post-exposure indicating its potentiality in managing *S. frugiperda* and other Lepidopterans.

Furthermore, formulation results indicated the compatibility of nematodes and nematodes symbiotic bacterial with NPs of metal oxides as a result of tolerance to toxicants at (10 mg/L) low concentration (environmental relevant concentrations). This provided the potential of considering NPs for the enhanced pathogenic performance of the nematodes. Addition of NPs with anti-UV properties for the single application provides an alternative and feasible way to control notorious Lepidoptera and other above-ground pests. Based on the result of the current study, it is vital to modify EPNs formulation depending on the use and specific system for effective performance. Given the enhanced protection provided by the NPs of the metal oxides, it is of interest to determine and optimize the effect of nanoparticles formulations to other insect pest species.

5.2 Recommendations

Owing the low insecticidal activity *T. vogelii* and *D. kilimandscharius* in managing *S. frugiperda* this study recommends further characterization of these botanicals against other crop pests. Also, based on their availability and accessibility to poor resource farmers; this

study recommends use of the *T. vogelii* and *D. kilimandscharius to* reduce insect pest density where limited options for managing *S. frugiperda* is available.

The study also recommends the inclusion of the entomopathogenic nematodes in the integrated pest management approaches for management of Lepidoptera pests in Tanzania and other locations where maize suffer effects by Lepidopteras particularly *S. frugiperda*.

Further studies are required to authenticate the performance of the entomopathogenic nematode formulation under the field conditions in different agricultural systems and other Lepidoptera insects for the effectiveness of the approach. The patent application for the approach is in progress and once approved, the type, dosage and biopesticide will be availed and strongly recommended for application against *S. frugiperda* for improved maize production in Tanzania and other areas dominated by this pest.

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