2020-05

Biology, predatory activity and peoples'perceptions towards apefly (spalgis spp.) in Tanzania

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BIOLOGY, PREDATORY ACTIVITY AND PEOPLES' PERCEPTIONS TOWARDS APEFLY (*Spalgis spp*.) IN TANZANIA

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A Dissertation 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

Arusha, Tanzania

May, 2020

ABSTRACT

In June 2017, farmers in central and northern Tanzania reported the occurrence of what they called an unusual insect with a human-like facial appearance that they referred to as "*Kidudu-mtu.*" The reports prompted the need to assess the identity and occurrence of the insect in Tanzania. This research was conducted between March and August 2018 in Iringa, Morogoro, Shinyanga, Geita and Arusha regions. A total of 89 people in the study regions were purposely interviewed to determine their knowledge, perceptions and reactions towards the insect. Insect samples were collected and submitted to the Tropical Pesticide Research Institute (TPRI) for preliminary identification and toxicity test. Molecular identification was done at the Nelson Mandela African Institution of Science and Technology (NM-AIST) laboratory. The insect's predatory activity against the papaya mealybug *(Paracoccus marginatus* Williams and Granara de Willink*)* was assessed at Tanzania Agricultural Research Institute (TARI) Tengeru. The insect was preliminarily identified as a member of the genus *Spalgis* present in three regions on papaya and cassava plants. The majority (92.1%) of the respondents perceived the insect as poisonous. In the toxicity tests, no death or toxic signs were displayed by the mice and no significant differences ($P>0.05$) were observed between the control and treated mice during hematological, biochemical and histopathological examination results except increase in liver weight which was considered non-adverse based on available protocol. The molecular analysis revealed 99% similarity with *Spalgis lemolea lemolea* (Druce) commonly known as Apefly. Under laboratory conditions, the insect completed its life cycle within 23 days with 4 larval instars. The female laid an average of 68 eggs in groups of 2 to 7 at different sites after 4-5 days of emergence. The predatory activity studies showed the consumption of mealybugs by the Apefly increased as the insect developed. The average number of mealybug eggs, nymphs and adults consumed by a single Apefly larva was 1983 ± 117, 123 ± 6 and 80 ± 9 individuals, respectively. Further studies to asses biological processes associated with an increase in liver weight on mice and determine predatory potential of Apefly under field conditions are recommended.

DECLARATION

I, **Sayuni P. Nasari** 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.

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for examination of a dissertation entitled "**Biology, Predatory Activity and Peoples' Perceptions Towards Apefly (*Spalgis spp*.) in Tanzania**" in fulfilment of the requirements for the Degree of Doctor of Philosophy in the School of Life Sciences and Bioengineering (LiSBE) at Nelson Mandela African Institution of Science and Technology (NM-AIST).

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ACKNOWLEDGEMENTS

I am grateful to the Nelson Mandela African Institution of Science and Technology for providing me with the necessary support and opportunity to learn. I gratefully acknowledge the African Development Bank (AfDB) for financial support. Special gratitude to AfDB project team of NM-AIST for their great effort in ensuring smooth learning through scholarship administration. I am extremely grateful to my supervisors Dr. Ernest R. Mbega, Prof. Anna C. Treydte and Prof. Patrick A. Ndakidemi for their invaluable mentorship, support and guidance. Special thanks to Dr. Beatus Lyimo and Mr. Samson Lyimo of NM-AIST laboratory for their support during experiments as well as Dr. Petro Nagagi and Mr. Maneno Chidege of Tropical Pesticide Research Institute for their technical support. I appreciate the support from Tengeru Horticultural Research Institute for their support during my experiments.

DEDICATION

To my beloved parents, Late Philipo P. Nasari and Elietikiswa P. Nasari for their sacrifices and support. To my husband, Christian Bwaya and children Christine, Joan and Joshua for their prayers, support, perseverance and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND SYMBOLS

AfDB	African Development Bank
ALP	Alkaline Phosphate
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
BLAST	Basic Local Alignment Search Tool
Cr	Creatinine
DNA	Deoxyribonucleic Acid
EDTA	Ethylene-diamine Tetra Acetic Acid
H & E	Hematoxylin and Eosin
Hb	Hemoglobin
KNCHREC	Northern Zone Health Research Ethics Sub-Committee
LY	Lymphocytes
MCH	Mean cell hemoglobin
MCHC	Mean Cell Hemoglobin Concentration
MCV	Mean Cell Volume
MEGA	Molecular Evolutionary Genetics Analysis
NCBI	National Centre for Bioinformatics
NICRC	National Insects Collection Reference Centre
NIMR	National Institute for Medical Research
NJ	Neighbor Joining
NM-AIST	Nelson Mandela African Institution of Science and Technology

OECD	Organization for Economic Co-operation and Development
PCR	Polymerase Chain Reaction
RBC	Red Blood Cells
RH	Relative Humidity
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
SD	Standard Deviation
SEM	Standard Error of Mean
SPSS	Statistical Package for Social Sciences
TPRI	Tropical Pesticide Research Institute
WBC	White Blood Cells

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

In June 2017, reports of unusual insect with a human-like facial appearance that they referred to as "*Kidudu-mtu* spread all over Tanzania and created fear that it was poisonous if consumed with vegetables. Preliminary morphological identification of the insect indicated that it was of the genus *Spalgis*. Following that, this study was designed specifically to review and generate information with regard to the insect in Tanzania.

The genus *Spalgis* belongs to the subfamily Miletinae, (Lycaenidae family) which includes small butterflies that are commonly known as harvesters and woolly legs (Scott, 1992; Monotypy, 2017). The genus *Spalgis* was established by Frederic Moore in 1879 (Hewitson *et al.*, 1879) and has several species including *S. epeus* Westwood, *S. baiongus* Cantlie and Norman, *S. Takanamii*.

Eliot, *S. asmus* Parsons, *S. jacksoni* Stempffer, *S. lemolea* Druce, *S. pilos* Druce and *S. tintinga* Boisduval. These species are distributed in Australia, Asia and Afro-tropic zones (Ackery, 1990; Kumar, 2013). Two of these species; *S. epius* (Indian Apefly; native in India) and *S. lemolea* (African Apefly), native in Africa (Nigeria), are known to possess a unique feature in their pupa stage as they resemble the face of a monkey hence the name "Apefly" (Ackery, 1990). Indian Apefly consists of two sub-species which include *S. epeus epeus* Westwood, (Oriental Apefly) and *S. epeus nubilus* Moore and Nicobar which inhabits rainforest and humid deciduous forests at elevations between about 100-500 m (Saji, 2017). Of the members of the genus *Spalgis*, *S. lemolea lemolea* Druce has been reported in Senegal, Gambia, Guinea-Bissau, Guinea, Liberia, Ivory Coast, Ghana, Togo, Nigeria, Cameroon, Gabon to Ethiopia, Democratic Republic of the Congo, Uganda, Kenya, Malawi, Zambia, northern Zimbabwe, Botswana and Tanzania (Ackery, 1990). The *S. lemolea pilos Druce,* is commonly found in Senegal, Gambia, Guinea-Bissau, Guinea, Liberia, Ivory Coast, Ghana, Togo, Nigeria and Cameroon (D'Abrera, 1980; Lohman & Samarita, 2009). In Tanzania, *Spalgis* sp. has been reported to be associated with forests, dense savanna and thick riverine bush (Peterson, 2014). However, the available information did not provide full identity of the the *Spalgis* species in Tanzania. It was thus urgently important to identify the species of the

insect present and to assess peoples' knowledge, perceptions and reactions towards this insect in Tanzania.

Members of the genus *Spalgis* involves aphytophagous butterflies that are extremely rare and several endangered (Pierce, 1995). They represent carnivorous butterflies feeding exclusively on various species of Coccidae and Pseudococcidae families (Dinesh & Venkatesha, 2011) including mealybug species, most of which are common pests of economically-important crops (Browning, 1992; Franco *et al.,* 2001; Venkatesha, 2011). However, the literarute indicates no research has been done on the characteristics and potential of *Spalgis spp* in management of mealybugs in Africa. It was based on this knowledge gap also that this study was undertaken to assess the occurrence, studies on morphology, life cycle and development stages as well as its biological control potential against the papaya mealybug in Tanzania.

1.2 Statement of the problem

Insect predators have the potential for biocontrol against a range of insect pests that attack crops of high economic and nutritional importance worldwide (Colmenarez, *et al.,* 2020). However, little farmers' understanding of which insect predators to use for pest control can be deleterious to their conservation. For instance, In June 2017, news broke across Tanzania of what people thought to be a'deadly' insect that had an ape-like and or human-like facial appearance commonly and often referred in Kiswahili to as*"kidudu mtu"* or human insect. Based on the described morphology, it was obvious that the insect was of the genus *Spalgis* (Peterson, 2014). The insect caused unrest in the general public and it was said to cause death upon consumption of its host plants (Athumani, 2017; BongoStars, 2017; Brown, 2017; Choke, 2017; MCLDigital, 2017). The anxiety caused thereof, lead to people ceasing the consumption of affected vegetables. While the terrorizing news quickly spread through social media, the public enquired to know more about the '*kidudu mtu*.' Owing to the fact that little information exists on this ape-like facial appearing insect, it was vital to conduct research to generate information about it in Tanzania.

1.3 Rationale of the study

The obvious pre-identification of the '*Kidudu mtu'* (Peterson, 2014) prompted insights towards need for confirmation of identity and the role of the genus *Spalgis* on managing some insect pests that it feeds on including ant–tended Hemiptera such as the Homoptera,

which include different species of mealybugs (Homoptera: Pseudococcidae) (Browning, 1992).

1.4 Objectives

1.4.1 General objective

The general objective was to study the biology, predatory activity and peoples' perceptions on Apefly (*Spalgis Spp*.) and generating information useful for the conservation of the insect in Tanzania.

1.4.2 Specific objectives

(i) To determine identity, occurrence and peoples' perceptions on the reported unusual insect with a human-like facial appearance commonly referred to as "*Kidudu-mtu*.

(ii) To assess the diversity of the reported unusual insect with a human-like facial appearance "*Kidudu-mtu"* in five agro-ecological zones of Tanzania.

(iii) To study morphology, life cycle and developmental stages of the unusual insect with a human-like facial appearance "*Kidudu-mtu"* in Tanzania.

(iv) To examine the predatory activity of the unusual insect with a human-like facial appearance "*Kidudu-mtu"* against the papaya mealybug (*P. marginatus).*

(v) To determine the toxicity status of the unusual insect with a human-like facial appearance "*Kidudu-mtu"* on mice.

1.5 Research questions

(i) What is the identity and occurrence of the reported unusual insect with a human-like facial appearance?

(ii) What is the existing knowledge and perception of people towards the Apefly?

(iii) How diverse is the insect in different agro-ecological zones in Tanzania?

(iv) What is the morphology, life cycle and developmental stages of the Apefly in Tanzania?

(v) How is the predatory activity of the insect on the papaya mealybug (*P. marginatus)*?

(vi) Is the reported insect poisonous if consumed?

1.6 Significance of the study

The findings of the study contribute to the understanding of the basis of beliefs, attitudes, indigenous knowledge and practices attached to insects particularly to the current with a human-like face that has been identified in this study as Apefly. The analysis of Apefly's genetic diversity across five agro-ecological zones in Tanzania contributes to the existing body of knowledge on Tanzania butterflies and the ecosystem in general. The toxicity test conducted in this study settles the wrong perception attached to the insect and therefore, this study gives confirmation that Apefly is non-poisonous when consumed and does not have any negative health implications. The results of the biology and predatory activity of Apefly provides preliminary information necessary in utilizing the biological control capacity hold by Apefly over the mealybugs.

1.7 Delineation of the study

Since the inception of the study, it was realized that while there was a social need to understand the Apefly which was already causing public tension, literature on the same was insufficient. It was therefore important to conduct extensive literature review to achour the design of the study. As a result of lack of literature on the Apefly, the ffect of the changing climate on the availity of the Apefly was unknown. The distribution and factors affecting the abundance were unknown. In this regard, the study had to focus on the occurrence and not the abundance of the sample. Fewer samples obtained from study sites could not support the conclusion about inter and intra generic evolutionary divergence. The study focused, instead, on genetic diversity of the sample collected. While it was true that farmers were desperate to understand the Apefly, there was a need to ensure they provide reliable information. In view of this, study selected areas which were reported to have the prevalence of the Apefly. In obtaining information from farmers with regard to their perception of the Apefly and their farming practices, semi-structured questionnaire was used to keep the interview focused.

CHAPTER TWO

LITERATURE REVIEW

2.1 People's perceptions and practices towards insect species

Insects form 99% of approximately 10 million animal species on Earth (Johnson, 2003). Despite many essential and beneficial ecological services offered by insects, there is a general trend for humans to perceive insects negatively (Kellert, 1993; Bjerke *et al.*, 1998; Bjerke & Thrane, 2003; Bjerke & Østdahl, 2004; Prokop *et al.*, 2010; Prokop *et al.*, 2010 and Wagler, 2010). While some societies look at insects with the feelings of hatred, anxiety, fear, avoidance and ignorance (Kellert, 1993), others hold special beliefs and respect towards insects (Santos & Antonini, 2008 and Khan, 2013).

In Tanzania, farmers were reported to perceive Apefly (*Spalgis* spp) negatively, considering it deadly when the insect or its host plant is consumed (Athumani, 2017; Brown, 2017: Choke, 2017 and MCL Digital, 2017). Although the taxonomic and morphological information of the insect has been reported by Kroon (1978), Larsen (2005), Williams (2015) and Kaliszewska *et al.* (2015), further information on the biology, chemistry and ecology of the insect has not been reported. Such lack of research and knowledge dissemination places the Apefly not only at considerable risk, but it also brings down its potential use in biological pest control as supported by Anthes *et al.* (2008) and Celik *et al.* (2015).

Studies reveal a complex interaction of factors behind negative perceptions towards insects involving social, cultural, biological, morphological and physiological attributes such as colour, shape and size (Barua *et al.*, 2012; Wagler & Wagler, 2012 and Lemelin, 2013). Benteley (1991) highlights inadequate knowledge (ignorance of insects) as a cause of lack of appreciation for beneficial organisms resulting in overreaction to some insects such as the use of pesticides. Such a practice is reported to cause increased yield loss as a result of the suppression of the activity of natural enemies (Heong *et al.*, 1994; Heong & Escalada, 1997). Such destruction of natural enemies interferes biodiversity conservation efforts as supported by Balmford *et al.* (2002), Kawahara and Pyle (2013), Sodhi *et al.* (2004) and Snaddon *et al.* (2008).

Bentley (1991) reports that, adequate knowledge of the role played by insects is crucial for conservation efforts as people rarely protect that which they do not know. A step-wise

process of learning gaps in farmers has been suggested as an important step in biodiversity conservation, these include; (a) Learn gaps in farmers' knowledge (b) Transform gaps into research problems (c) Communicate results to farmers (d) Farmers integrate and adapt information into knowledge and practices (Bentley & Andrews, 1996).

Empirical information regarding existing perceptions, knowledge and reactions towards Apefly in Tanzania has however not been reported. Understanding the prevailing state of knowledge and peoples' reaction towards the insect is of paramount importance to allow for appropriate interventions. This study adopts the stages by Bentley and Andrews (1996) in understanding the knowledge gap on Apefly in Tanzania.

2.2 Butterfly toxins

Some insects produce or acquire biochemicals from the food they consume or through contact with insecticides and herbicides (Longley & Sotherton, 1997 and Koehler, 1999). Studies have shown that **s**ome butterflies are considered toxic or repulsive to predators due to the presence of cardiac glycosides sequestered from their larval food plants (Mebs *et al.,* 2005). For example, the monarch butterfly (*Danaus plexippus*) also represents a classic case of acquired defense, where caterpillars not only tolerate but also sequester cardenolides from their milkweed host plants (*Asclepias* spp., Apocynaceae) and transfer these toxins to the butterfly stage for their own defense against predators and parasites (Brower *et al.,* 1968; Glendinning & Brower, 1990; Stenberg *et al.,* 2012). Cardenolides (cardiac glycosides) are highly potent plant toxins that specifically inhibit Naþ/Kþ-ATPase, an essential animal cation transporter (Terness, 2001). It has not been established yet, whether Apefly contains endotoxin substances assimilated through interactions with their preys (i.e. phytotoxins from plants the preys feed on). Knowledge of Apefly chemistry is a critical step towards effective conservation and utilization of its potential.

2.3 Towards Conservation of Apefly (*Spalgis lemolea*. Druce) for managing Papaya Mealybug (*Paracoccus marginatus* Williams and Granara de Willink) in Sub Saharan Africa

The genus *Spalgis* (Order Lepidoptera, Family Lycaenidae, Subfamily Miletinae) includes insects commonly called "harvesters" and "woolly legs" (Kaliszewska *et al.,* 2015). The order Lepidoptera has about 160 000 described species and it is estimated to be the third in size among the largest groups of living things after beetles (Order Coleoptera) and flowering

plants (Kristen *et al.*, 2007). Members of the genus *Spalgis* live among their prey and often coexist with ant mutualists of the Order Hemiptera (Pierce, 1995). This genus has several species that include *S. epius* Westwood, *S. baiongus* Cantlie and Norman, *S. takanamii* Eliot, *S. asmus* Parsons, *S. jacksoni* Stempffer, *S. lemolea* Druce, *S. pilos* Druce and *S. tintinga* Boisduval, which are distributed in Australia, Asia and Afro-tropical zones (Kumar, 2013). Some members of the genus *Spalgis* are known to possess a unique feature in their pupa stage as they resemble the face of a monkey, which has given them the name "Apefly" (Ackery, 1990). The monkey-faced appearance of some lycaenid pupae has been associated with defence but the mechanism is not clearly understood (Balduf, 1939). Other authors hold the resemblance between the pupae and the head of a monkey to be only accidental because of the size and taxonomic differences between the two (Hinton, 1974).

More than 99% of Lepidoptera species are phytophagous (feed on plants only) (Cottrell, 1984; Dinesh & Venkatesha, 2016 and Kumar, 2013). Uniquely, the subfamily Miletinae (Lycaenidae) is exclusively aphytophagous (feeding on animals) (Kaliszewska *et al.*, 2015; Lohman & Samarita, 2009). Most of the aphytophagous butterflies are extremely rare and several are endangered, possibly due to climate change (Brahmaprakash *et al*., 2017; Pierce *et al.*, 2002 and Wynhoff, 1998). *Spalgis* spp, in particular, are one of the rare butterfly species, which represent a carnivorous butterfly feeding exclusively on species of the Coccidae and Pseudococcidae family (Dinesh & Venkatesha, 2011 and Lohman & Samarita, 2009). This includes different Mealybug species such as *Paracoccus marginatus* a common pest attacking economically important plant species worldwide (Saengyot & Burikam, 2012; Shajla, Vijayalakshmi & Tintumol, 2014 and Venkatesha & Dinesh, 2011).

Studies on an Indian Apefly species have indicated that the butterfly can be used for bio-control against Mealybugs (Saengyot & Burikam, 2012; Venkatesha & Dinesh, 2011). The newly hatched larvae of *S. epius* while still inside the ovisac of Mealybug have been reported to be capable of consuming eggs (Tanwar *et al*., 2010). Investigation of the daily consumption of papaya Mealy bug by the larvae showed that the third instar of *S. epius* has the ability to consume large quantities of prey compared to other larval stages (Kumar *et al*., 2006 and Lohman & Samarita, 2009). However, little is known as to how the African Apefly does interact with its prey, its biology, ecology and possible application for biological control (Leuschner & Nwanze, 1977). This review specifically focused on the potential of the African Apefly for biological control against papaya mealybug populations in sub-Saharan

Africa (Tanga, 2013). With this study, the aim was to raise awareness of this species' potential in the management of a serious pest species and to enhance the conservation and utilization of the butterfly.

2.3.1 Distribution of *Spalgis* species

Members of the genus *Spalgis* are distributed along the tropical zones of Africa, Asia and Australia (Inayoshi, 2019; Sikkim, 2004 and Williams, 2008) (Table 1).

Table 1: Global distribution of members of genus *Spalgis* based on reviewed literature (n = 84). (+) = Presence (-) = no information

SN	Species	Location														References
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	
1	*S. lemolea*	+	+	+	-	-	-	-	-	-	-	-	-	-	-	(Clench, 1965; T. Larsen, 2005; Pennington *et al*., 1978; Williams, 2015)
2	*S. Jackson*	+	+	+	-	-	-	-	-	-	-	-	-	-	-	(Williams, 2015)
3	*S. tinting*	-	-	-	+	-	-	-	-	-	-	-	-	-	-	(Druce, 1875)
4	*S. epius*	-	-	-	-	+	+	+	+	+	+	+	+	+	-	(Hinton, 1974; Inayoshi, 2019; Kumar, 2013; Kumar *et al*., 2006; Padhye *et al*., 2012; Parsons, 1999; Prabakaran *et al*., 2014)
5	*S. sgnata*	-	-	-	-	+	-	-	-	-	-	-	-	-	-	(Myers *et al*., 2000)
6	*S. baongus*	-	-	-	-	+	-	-	-	-	-	-	-	-	-	(Parsons, 1999)
7	*S. asmus*	-	-	-	-	-	-	-	-	-	-	-	-	-	+	(Parsons, 1999)
8	*S. takanami*	-	-	-	-	-	+	-	-	-	-	-	-	-	-	(Myers *et al*., 2000; Parsons, 1999; Savela, 2014)

A=East **A**frica **B**=West **A**frica **C**=Central Africa **D**=Madagascar **E**=Southern India **F**= Philippines **G**= Bangladesh **H**= Indonesia **I**=Malaysia **J**= China **K**= Vietnam **L**=Thailand **M**=Sri Lanka **N**=Papua New Guinea.

Scott (1974) reported that the warmer weather and bright sunlight in the tropics favour mating and egg-laying activities of this butterfly. The main areas in the tropics where *Spalgis* spp are found include the Australasian Ecozone (New Guinea) and Afro-tropic Ecozone, which cover sub-Saharan Africa, Madagascar and other offshore islands (Inayoshi, 2019).

2.3.2 Life stages

Similar to other butterflies and moths, Apeflies have four life stages: the egg, larva, pupa, and adult (Fig 1).

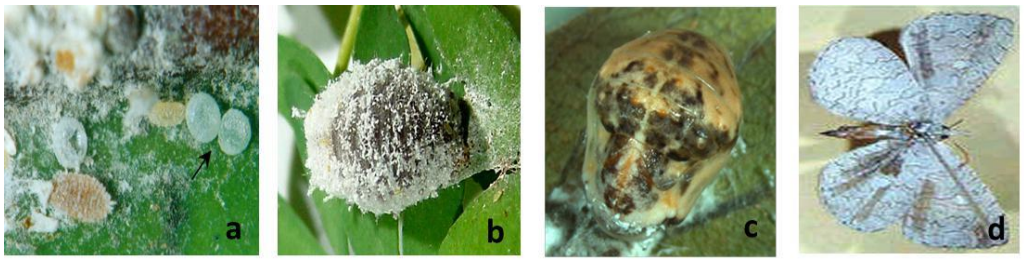

Figure 1: Life stages of Apefly spp: =Egges b= Larva c= Pupa (d) Adult. Images by ICAR-National Bureau of Agricultural Insect Resources (2013)

The Indian Apefly completes its life cycle within an average of 29 days, with four larval instars. The mean incubation period of eggs was reported to be 3.9 days and the duration of development of larval stages, pre-pupa and pupa to be 9.4, 0.9 and 9.5 days respectively (Dinesh *et al.,* 2010). The larva instars differ by size mobility and feeding habits while the pupa is a non-feeding stage (Dinesh & Venkatesha, 2011). The *S. epius* was noted to have high mortality rate at lower temperatures of about 16 °C and at higher temperatures of about 32 °C and the highest fecundity rate is at 28 °C (Jothi *et al*, 2014; Kumar *et al.,* 2006). The African *Spalgis* sp has not been biologically characterized.

2.3.3 Papaya mealybug

(i) Origin and distribution of papaya mealybugs

The papaya mealybug *Paracoccus marginatus* is native to Mexico and Central America where it is not considered as a pest (Tanwar *et al.,* 2010). It spread to the Caribbean and South America in the 1990s (Miller *et al.,* 1999) to Pacific Islands, Bangladesh, Cambodia, Philippines, Thailand and western Africa (Muniappan *et al.,* 2009). In Africa, the papaya Mealybug was observed in Ghana in 2010 and then spread to Benin, Nigeria, Togo, Gabon, Tanzania and Kenya (Muniappan *et al.,* 2009) where it is an invasive insect pest species causing a serious economic loss in infested crops.

The most destructive stage of *P. marginatus* is the adult, which sucks the fluid of the plant and weakens it (Krishnan *et al.,* 2016 and Miller *et al.,* 1999). *Paracoccus marginatus* secretes a sugary wax, which attracts ants to form mutual associations (Krishnan *et al.,* 2016). The ants spread this sugary content allowing the growth of some fungal microbes with a sooty mould appearance, which impairs the photosynthetic efficiency of the affected plants and causes large crop losses to farmers (Schneider & Lapolla, 2011). The extreme infestation

not only contaminates the yield but also leads to the destruction of the entire plant (Krishnan *et al.,* 2016).

2.8.4 Host range of papaya mealybug

The papaya mealybug is a polyphagous pest in over 55 plant species from 25 families of some of the most economically important crops and weed hosts worldwide (Bendov, 2008; Mccomie, 2000; Meyerdirk & Kauffman, 2001; Miller & Miller, 2002 and Walker *et al*., 2006) (Table 2).

Table 2: Common hosts of Papaya mealybug based on studies found during our literature review

Host Category	Botanical Name	Common Name	Reference
Cultivar	*Capsicum annuum*	Capsicum peppers	(Ahmed, Al-Helal, Khanon, & Bulbul, 2011; Macharia *et al.,* 2017; Sakthivel *et al.,* 2012; Tanwar *et al.,* 2010; Walker *et al*., 2006)
	Cajanus cajan L.	Redgram	*(Bendov, 2008; Krishnan et al., 2016; Meyerdirk et al.,*2004; Miller & Miller, 2002; Sakthivel *et al*., 2012; Tanwar *et al.*, 2010)
	Carica papaya L.	Papaya	(Macharia *et al.,* 2017; Mccomie, 2000; Meyerdirk *et al.,* 2004; Miller & Miller, 2002; Sakthivel *et al.,* 2012)
	Ceiba pentandra (L.) Gaertn.	Silk cotton	(Mccomie, 2000; Meyerdirk *et al.,* 2004; Miller & Miller, 2002; Sakthivel *et al.,* 2012; Tanwar *et al.,* 2010; Walker *et al.*, 2006)
	Citrus paradise	Grapefruit	(Ben-dov, 2008; Mccomie, 2000; Meyerdirk *et al.*, 2004;. Miller & Miller, 2002; Sakthivel *et al*., 2012; Tanwar *et al.,* 2010; Walker *et al*., 2006)
	Gossypium hirsutum L	Cotton	(Lohman & Samarita, 2009; Mccomie, 2000; Meyerdirk *et al.,* 2004; Tanwar *et al.,* 2010; Walker *et al.,* 2006)
	Hibiscus rosa sinensis L.	Shoe flower	(Mccomie, 2000; Meyerdirk *et al.,* 2004; Tanwar *et al*., 2010; Walker *et al*., 2006)
	Jatropha curcus L.	Jatropha	(Chellappan *et al.,* 2016; Mccomie, 2000; Meyerdirk *et al.,* 2004; Miller & Miller, 2002; Tanwar *et al*., 2010; Cham *et al.* 2011)
	Mangifera indica	Mango	(Ben-dov, 2008; Macharia *et al.,* 2017; Mccomie, 2000; Meyerdirk *et al.,* 2004; Miller & Miller, 2002; Sakthivel *et al.,* 2012; Tanga, 2013)
	Manihot esculenta Crantz	Sweet potato	(Bendov, 2008; Cham, Davis, Obeng, & Owu, 2011; Mccomie, 2000; Meyerdirk *et al.,* 2004; Miller & Miller, 2002; Sakthivel *et al*., 2012; Tanwar *et al.,* 2010)
	Morus alba L.	Mulberry	(Krishnan *et al.,* 2016; Mccomie, 2000; Meyerdirk *et al.,* 2004; Sakthivel *et al.,* 2012; Tanwar *et al.,* 2010)
	Nerium oleander	Oleander	(Ben-dov, 2008; Krishnan *et al.,* 2016; Mccomie, 2000; Meyerdirk *et al.,* 2004; Miller & Miller, 2002; Tanwar *et al.,* 2010)
	Persea Americana	Avocado	(Mccomie, 2000; Meyerdirk *et al.,* 2004; Tanwar *et al.,* 2010)
	Pisum sativum	Pea	(Chellappan *et al.,* 2016; Krishnan *et al.,* 2016; Sakthivel *et al.,* 2012)
	Plumeria sp.	Frangipanj	(Cham *et al*., 2011; Mccomie, 2000; Meyerdirk *et al.,* 2004)
	Prunus avium	Cherry	(Krishnan *et al.,* 2016)
	Psidium guajava L.	Guava	(Ben-dov, 2008; Krishnan *et al.,* 2016; Macharia *et al.,* 2017; Mccomie, 2000; Meyerdirk *et al*., 2004; Miller & Miller, 2002)
	Punicum granatum	Pomegranate	(Krishnan *et al.,* 2016; Mccomie, 2000; Meyerdirk *et al.,* 2004; Tanwar *et al.,* 2010)
	Lycopersicon	Tomato	(Ahmed *et al.,* 2011; Krishnan *et al.,* 2016; Mccomie,

Host Category	Botanical Name	Common Name	Reference
	esculentum Mill		2000; Meyerdirk *et al.*, 2004; Miller & Miller, 2002; Tanwar *et al.*, 2010)
	Solanaum torvum Sw	Turkey berry	(Krishnan *et al*., 2016; Meyerdirk *et al*., 2004; Tanwar *et al.*, 2010)
	Solanum melongena L.	Eggplant	(Ben-dov, 2008; Krishnan *et al.*, 2016; Macharia *et al*., 2017; Mccomie, 2000; Meyerdirk *et al*., 2004; Miller & Miller, 2002)
	Tectona grandis L.	Teak	(Ben-dov, 2008; Krishnan *et al.*, 2016; Mccomie, 2000; Meyerdirk *et al.*, 2004; Miller & Miller, 2002; Tanwar *et al.*, 2010)
Weeds	*Abutilon indicum L*	Country mallow	(Cham *et al.*, 2011; Krishnan *et al.*, 2016; Miller & Miller, 2002; Tanwar *et al.*, 2010; Walker *et al*., 2006)
	Achyranthus aspera L	Latjira	(Krishnan *et al*., 2016; Tanwar *et al*., 2010; Walker *et al*., 2006)
	Canthium inerme (L.f.)	Kuntze Turkey-berry	(Krishnan *et al*., 2016; Tanwar *et al*., 2010)
	Cleome viscosa L.	Wild mustard	(Chellappan *et al.*, 2016; Krishnan *et al.*, 2016; Mccomie, 2000; Tanwar *et al.*, 2010; Walker *et al*., 2006)
	Commelina benghalensis L.	Chandvel	(Krishnan *et al*., 2016; Tanwar *et al*., 2010; Walker *et al*., 2006)
	Convolvulus arvens L.	Chandvel	(Krishnan *et al*., 2016; Tanwar *et al*., 2010; Walker *et al*., 2006)
	Euphorbia hirta L.	Asthma plant	(Krishnan *et al*., 2016; Tanwar *et al*., 2010)
	Leucas aspera (Wild)	Dronapushpi	(Krishnan *et al*., 2016; Miller & Miller, 2002; Tanwar *et al.*, 2010)
	Ocimum sanctum L.	Tulas	(Krishnan *et al*., 2016; Tanwar *et al*., 2010)
	Parthenium hysterophorus L	Congress grass	(Chellappan *et al*., 2016; Krishnan *et al*., 2016; Mccomie, 2000; Tanwar *et al.*, 2010)]
	Phyllanthus niruri L	Hazardani	(Krishnan *et al*., 2016; Tanwar *et al*., 2010)
	Trianthema Portulacastrum L	Pig weed	(Krishnan *et al*., 2016; Tanwar *et al*., 2010)
	Tridax Procumbens L	Ghamra	(Krishnan *et al*., 2016; Miller & Miller, 2002; Tanwar *et al.*, 2010; Selvaraju & Sakthivel, 2012)

The papaya mealybug infests a wide variety of crop and weed species (Table 2) where it is able to survive and lead to severe losses of both food and cash crops. Since most of the control methods in use have not been successful (Tanwar *et al.*, 2010) and they are costly to ordinary farmers this raises the need for further research on sustainable solutions to reduce the losses.

2.3.5 Infestation level and yield loss

It has been observed that farmers can incur mean yield loss of up to 91% due to papaya mealybug infestations (Macharia *et al.,* 2017). The pest attacks and damages various parts of the host plant including the leaves, stems, flowers and fruits (Sharma & Pati, 2013). The infested plants usually have a thick white wax and soot mould covering part or all the plant parts and usually with other associated effects including deformed fruits and fruit abortion (Tanwar *et al.*, 2010). Heavy infestation by the pest on papaya occurs along the veins and the

midribs of the older leaves and all areas of the tender leaves and fruits (Tanwar *et al.*, 2010). Severely affected older leaves turn yellow and dry up (Meyerdirk *et al.*, 2004).

Table 3: The pest status of papaya mealybugs in some Sub-Saharan African countries

Country	Presence	Yield loss (%)	Infestation level	Author
Kenya	+	91	High	Macharia *et al.* (2017)
Tanzania	+	NA	High	IITA (2015)
Ghana	+	65	-	Hintenou (2015)
Cameroon	+	NA	High	Fand and Suroshe (2015)
Benin	+	NA	High	Goergen *et al.* (2014)
Gabon	+	Not quantified	High	Goergen *et al. (*2014)
Nigeria	+	Not quantified	High	Nébié *et al.* (2016)

+ = Present**,** NA = not assessed

The leaf damage is through curling, crinkling, twisting and general leaf distortion, reduced leaf size and surface area. The stem and shoot damage are manifested through shoots and young stem distortion and malformation, arrested growth at the shoot terminals leading to shortened internodes and rotting at the shoot tip (Meyerdirk *et al.*, 2004 and Tanwar *et al.*, 2010). Infested flowers are distorted and fail to open, and when they open, petals become twisted or malformed or show various types of blemishes, premature flowers drop resulting in poor fruit sets, fruits shrivel and drop (Sharma & Pati, 2013). The yield losses in most sub-Saharan African countries have not been quantified, although infestation has been reported and thus this calls for effective and sustainable intervention to effectively manage the pest (Table 3).

2.3.6 Preference for biological pest management methods for papaya mealybug

Management of Papaya Mealybug has not been very successful due to the species' high reproduction rate, protection by ants and a wide host range (Rasheed *et al.*, 2014). From our reviewed literature, authors identified different methods and the frequency of their recommendation is as shown in Fig. 2. Literature search revealed that the most recommended method for managing Papaya mealybug is the use of biological control in about 70% of the literature sources. The preference for biological control methods has been due to the belief that they are economically viable and environmentally friendly compared to the common synthetic chemical control approach and that they can be species-specific, only targeting the

papaya Mealybug (Macharia *et al.,* 2017; Sakthivel *et al.,* 2012 and Thangamalar *et al*., 2010). The method is also considered a long-term solution to the mealybug infestation problem as the pest and predators are self-perpetuating. The predator is able to persist even when the mealybug population is low, thus keeping the pest populations below economic injury levels (Mani *et al.,* 2012 and Suresh *et al.,* 2010).

Different natural enemies of the papaya mealybugs are commercially available including mealybug destroyer (*Cryptolaemus montrouzieri*), ladybird beetles, lacewings and hoverflies, all with a potential impact on Mealybug populations (Macharia *et al.,* 2017; Tanwar *et al.,* 2010 and Walker *et al.,* 2006). In addition to predators, several parasitoids have been tried and confirmed to have the ability to attack papaya Mealybug in 95 to 100% of the cases (Walker *et al.,* 2006). In some countries such as the USA, parasitoids such as *Acerophagus papaya, Anagyrus loecki, Anagyrus californicus, Pseudleptomastix mexicana* and *Pseudaphycus* spp are used for biological control of papaya mealybug (Meyerdirk & Kauffman, 2001; Meyerdirk *et al.,* 2004 and NoYes & Schauff, 2003). In other countries including India, the Lepidopteran predator *S. epius* is a well-known representative of carnivorous butterflies feeding on various species of Mealybugs (Chatterjee & Halder, 2017; Franco, Zada & Mendel, 2009; Lohman & Samarita, 2009; Rich, 2008 and Tanwar *et al*., 2010). This highlights the possibility of using the African *Spalgis* species in biological control although studies must be conducted to substantiate their efficacy against mealybugs.

Surprisingly, a chemical option to suppress the mealybug was supported by only 16% of authors, though it is the most common method used by farmers (Krishnan *et al.,* 2016; Sakthivel *et al*., 2012 and Sharma & Pati, 2013). The commonly used chemicals are profenophos 50 EC (2 mL/L), chlorpyriphos 20 EC (2 mL/L), buprofezin 25 EC (2 mL/L), dimethoate 30 EC (2 mL/L), acephate, carbaryl, cypermethrin, diazinon, malathion and white mineral oils (Macharia *et al*., 2017 and Walker *et al.,* 2006).

Although the chemical method had the second-highest literature support, it had been reported to be unable to control papaya mealybugs to below an economically-significant level (Walker *et al.,* 2006). This necessitates the application of twice the usual dose in treating the pest due to the thick waxy coat on the mealy bug's body cottony sacs and their tendency to hide inside damaged leaves and buds (Krishnan *et al.,* 2016; Sakthivel *et al.,* 2012 and Tanwar *et al.,* 2010). As a result, this makes chemical control methods only partially effective and requires multiple applications, leading to the problems of insecticide resistance, effects on non-target

organisms including the natural enemies and micro-organisms as well as pollution to the environment (Sakthivel *et al.*, 2012; Jalali *et al.*, 2016). Furthermore, excessive pesticides can cause both short- and long-term effects on human health (Charles, 1996; Horrigan *et al.*, 2002).

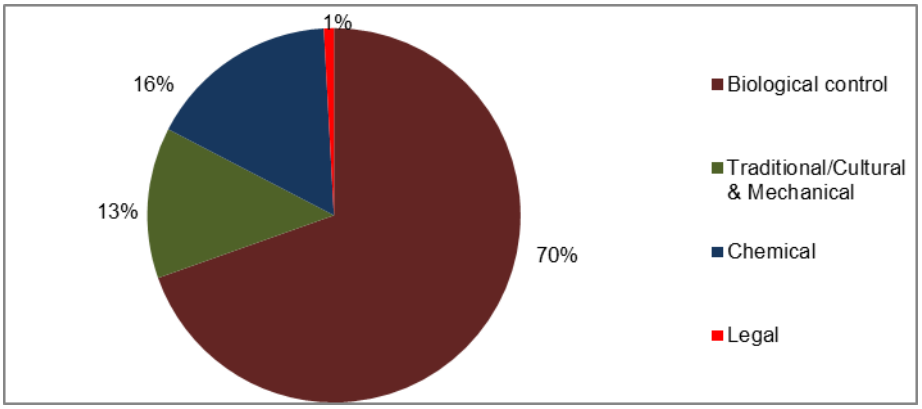

Figure 2: Papaya mealybug management methods and support frequency by authors cited in this review

2.3.7 Efficacy of biological control through Apeflies

Using the Indian Apefly *S. epius* as a model system, five larvae of this species showed the potential of consuming a mean number of 4117± 553 eggs, 281 ± 45 nymphs and 77± 16 adults of papaya mealybug under laboratory conditions (Dinesh & Venkatesha, 2011) as reported in Fig 3.

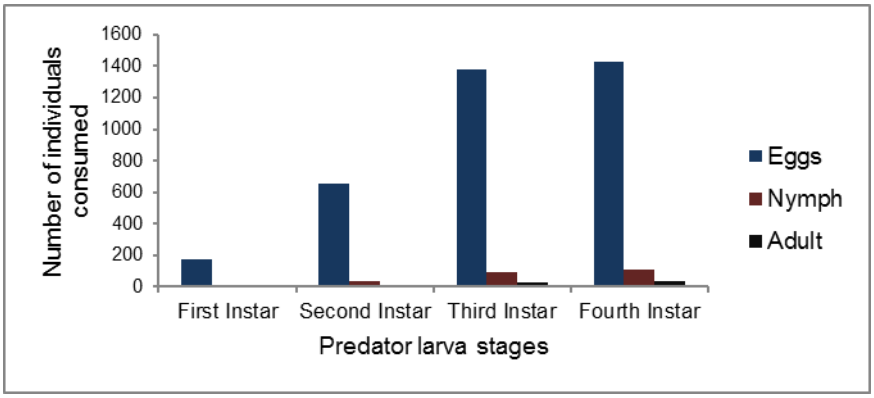

Figure 3: Efficacy of *Spalgis epius* in the management of different life stages of mealybugs according to studies by Dinesh and Venkatesha (2011)

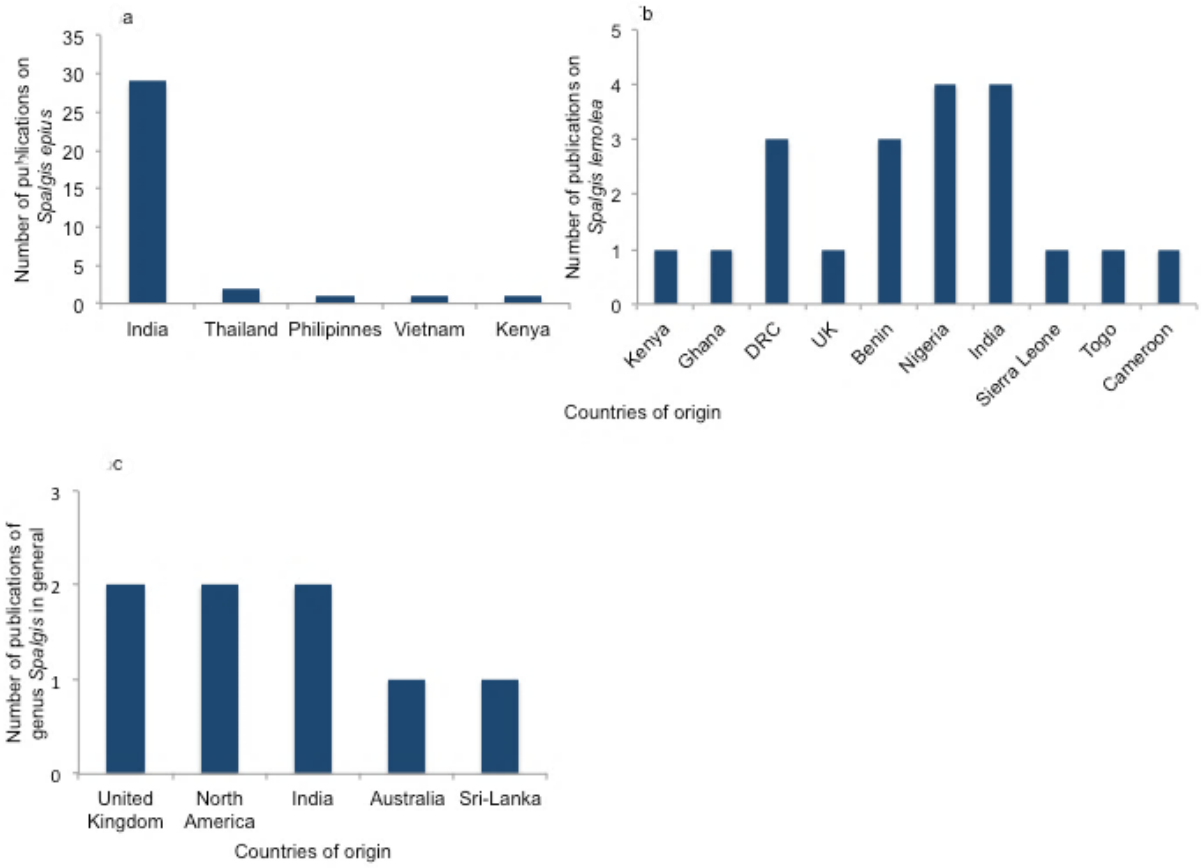

Figure 4: Publications between years 1989-2018 in support of *Spalgis epius, Spalgis lemolea* and genus *Spalgis* respectively in biological control of papaya mealybugs

Adult *S. epius* lay eggs on the mealybug masses and the newly hatched larvae remain inside the mealybug ovisac to consume the eggs of the Mealybugs (Tanwar *et al.*, 2010). As this happens the population of mealybug is restrained to their minimum levels in favour of the crops (Dinesh & Venkatesha, 2011). Due to this biological ability, out of 84 publications reviewed several authors have recommended members of the genus *Spalgis* in biological control programs against papaya mealybug and *Spalgis epius* is the most studied, particularly in India (Fig. 4 a, b and c).

Studies report the existence of the African Apefly (*S. lemolea)* (Bennett & Greathead, 1978) but there is no indication of how the species can be used for the management of mealybugs. This calls for further research to understand how African Apefly could be used in African countries as a biological control agent. The model is hereby proposed in studying the African *Spalgis* to aid stepwise research and utilization of the same in managing papaya mealybug in Sub Saharan Africa (Fig. 5).

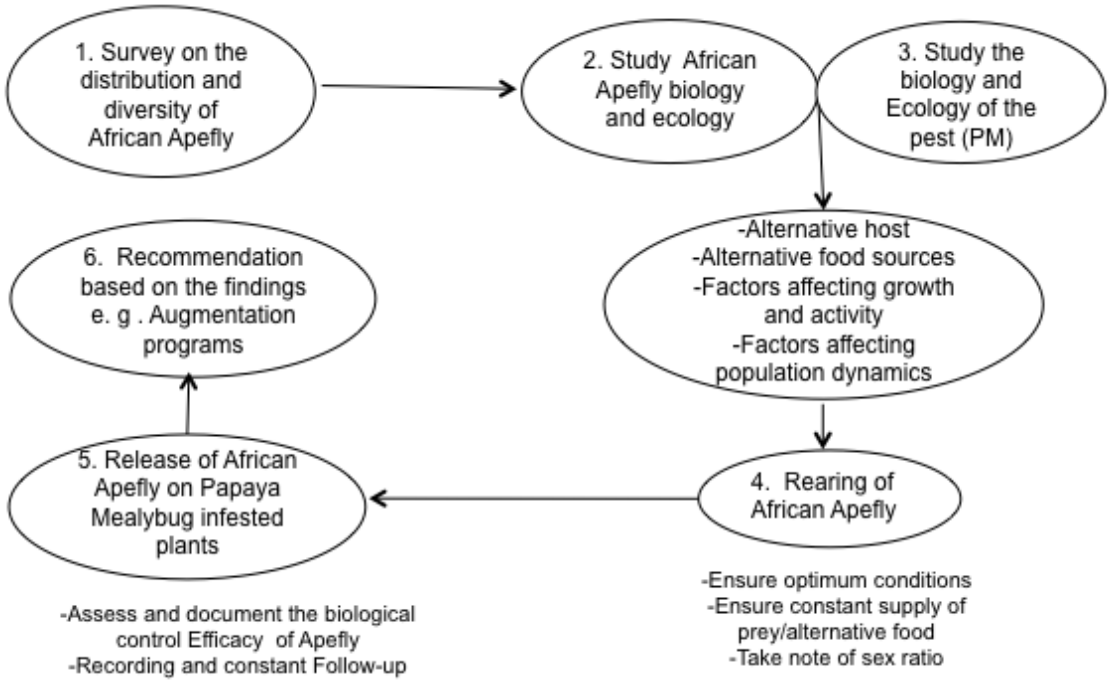

Figure 5: Proposed stepwise study and research model for *S. lemolea* in Sub Saharan Africa

This literature review investigated members of genus *Spalgis*, focusing on distribution, diversity, chemistry and the possibility of applying them as biological control agents on mealybugs in Sub-Saharan Africa. Successful application of the Indian *S. epius* for biological control of papaya mealybug has been described and the possibility of applying the African Apefly *(S. lemolea)* in biological control of papaya Mealybug in Africa have been highlighted. Inadequate information on *S. lemolea's* predatory ability and a possibility of its integration in biological control of mealybug species calls for further studies.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The survey of the occurrence, host range and farmers' perceptions on what they referred to as unusual insect that was then identified as Apefly (Nassari *et al.*, 2020) was conducted in five regions of Tanzania namely Shinyanga, Morogoro, Geita, Arusha and Iringa from March to October 2018 (Fig. 6). Four sites were purposefully selected from each district following previous reports of Apefly emergence through news channels.

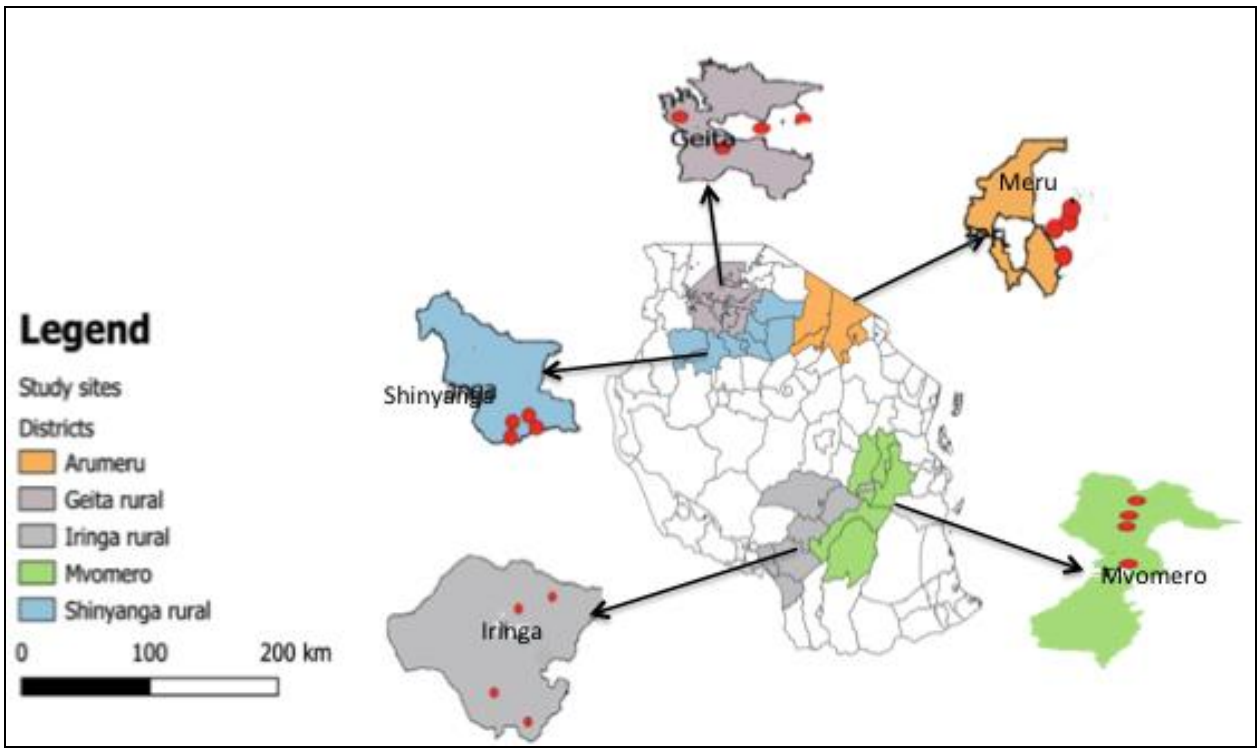

Figure 6: Map of Tanzania indicating the sites where the study was carried out

The sites were Mvomero district, Iringa district, Geita district, Meru district and Shinyanga district as shown in Table 4 below. For areas where records were lacking, the selection was based on mealybug infestations and/or the presence of crops prone to mealybug infestation.

Table 4: GPS points for the study sites

District	Latitude	Longitude
Arumeru	06˚30’ 310’S	037˚33’ 541’E
	06˚08’ 397’S	037˚35’ 519’E
	06˚08’ 337’S	037˚35’ 490’E
	06˚30’ 260’S	037˚34’ 163’E
Iringa	07˚31’ 428’S	035˚28’ 508’E
	07˚37’ 268’S	035˚37’ 208’E
	07˚38’ 325’S	035˚36’ 072’E
	07˚46’ 471’S	035˚41’ 349’E
Geita	02˚43’ 187’S	031˚50’ 599’E
	02˚43’ 186’S	031˚50’ 596’E
	02˚44’ 196’S	031˚56’ 460’E
	02°53' 614’S	032°13' 529’E
Arumeru	03˚24’ 312’S	036˚48’ 515’E
	03˚20’ 585’S	037˚18’ 590’E
	03˚23’ 451’S	036˚47’ 511’E
	03°20’ 315’S	033°46’ 303’E
Shinyanga	03˚40’ 516’S	033˚24’ 550’E
	03°37′ 523’S	033°50′ 499’E
	03°54’ 107’S	033°13’ 334’E
	03°48’ 365’S	033°20’ 400’E

3.2 Assessment of Apefly occurrence

A total of 20 farms (four farms per district) with reports of the occurrence of the ‘*kidudu mtu*” were randomly visited. Geographical information such as latitude, altitude and longitude were collected in each location. In each visited farm, numbers of insect pupa were collected from four randomly selected host plants. As pupas were mainly found in association with papaya mealybug, the infestation levels of papaya mealybug were quantified as *** = High (> 10 colonies per infected tissue), ** moderate (five to ten colonies per infected leaf), *=low (1-4 colonies per infected tissue) and - = no infestation. Information on the history of pesticide use was collected as well. Pupal were collected by handpicking them off a plant part (mainly leaves) and placing them in plastic containers (dimensions 4 x 15 x 21 cm), covered

with a fine screen from which the adult could not escape upon emergence. In total, 17 insect pupas were collected.

The collected pupas were carried in different containers and labeled basing on-site and host plants. The samples were transported to the Tanzania Agriculture Research Institute Tengeru Centre, Arusha Tanzania. The specimens were kept in glass insect cages 30 x 30 x 30 cm with two fine mesh sides at 27-29 ^{o}C and adult emergence was recorded. Emerged adults were provided with 10% honey diluted in water-soaked in cotton wool for feeding (Dinesh *et al.,* 2010). The specimens were sent to the Tropical Pesticide Research Institute (TPRI) for identification to the genus level. The Representative specimens were deposited in the insect specimen box at the National Insects Collection Reference Center (NICRC), Arusha, Tanzania.

3.3 Knowledge Survey

A survey of the existing knowledge, perceptions, and reactions of farmers in Tanzania with respect to Apefly was carried out between January and September 2018. A total of 100 key informants (20 respondents from each district) were purposefully selected for interviews. Trained enumerators administered semi-structured questionnaires after pre-testing the questionnaire for its validity among households in the selected areas and to familiarize the enumerator with the questionnaires as well as the handling of the survey. The information collected included: Participant's socio-economic profiles and people's knowledge, perception and reaction towards the African Apefly. Respondents were interviewed in the Swahili language. The questionnaires were discussed during face-to-face interviews and non-verbal communications were noted.

3.4 Molecular identification and diversity of Apefly species available in Tanzania

3.4.1 Primer design

Primers targeting ribosomal ribonucleic acid (rRNA) were designed using Primer3 software available for free on the internet using sequences from gene bank accessions KP215790.1 and KP215813.1, KP215790.1 and KP215813.1, KP215816.1 and KP215791.1, KP215816.1 and KP215791.1, and KP216182.1 and KP216184.1. In total five primers sets were designed, and primer sequences were sent to Inqaba biotechnical industries (pty) Ltd (Pretoria). The resultant primers were tested using deoxyribonucleic acid (DNA) samples of insects collected

from different locations in Tanzania using a conventional Polymerase Chain Reaction (PCR) (C1000 touch, thermal cycler, Bio-rad, Singapore). Of the tested primers, one set of primers i.e. P2F-5'CGGCGTGCACTTCTCTCTTA3' and P2R-5'GCCCGAAACAGAATCATCGC3' was selected due to its ability to amplify the target region of the insect.

3.4.2 DNA extraction and PCR reactions

The adult Apefly (*Spalgis spp*) that emerged from pupa were homogenized by using a bead raptor followed by total genomic DNA extraction using Quick-DNA tissue/insect Mini-prep kit (Zymo Research Corp) following the manufacturer's instructions. The DNA purity and concentration were assessed by Nanodrop lite spectrophotometer (Thermo fisher scientific). The target gene was amplified by PCR with the following composition for each single reaction tube; 12.5 µl of 2 x OneTaq master mixes with standard buffer (New England Biolabs, UK), 0.5 µl of forward and reverse primers and 9.5 µl of nuclease-free water to bring the total reaction volume to 25 µl. The PCR conditions were set as follows; initial denaturation at 94 ^{o}C for 30 sec followed by 30 cycles of 94 ^{o}C for 30 sec, 58 ^{o}C for 45 sec and 68 ^{o}C for 60 sec and a final extension at 68 ^{o}C for 5 min. Gel electrophoresis was used to confirm the presence of the expected band size.

3.4.3 Sequencing analysis and species identity

The PCR amplicons were purified and sequenced by Inqaba biotechnical industries (pty) Ltd (Pretoria). The 16s rRNA sequences were obtained as forward and reverse sequences. PreGap 4 (V. 1.6-r) was used to evaluate the quality of the sequences and gene editing was conducted in Gap 4 (V. 11.2-r).

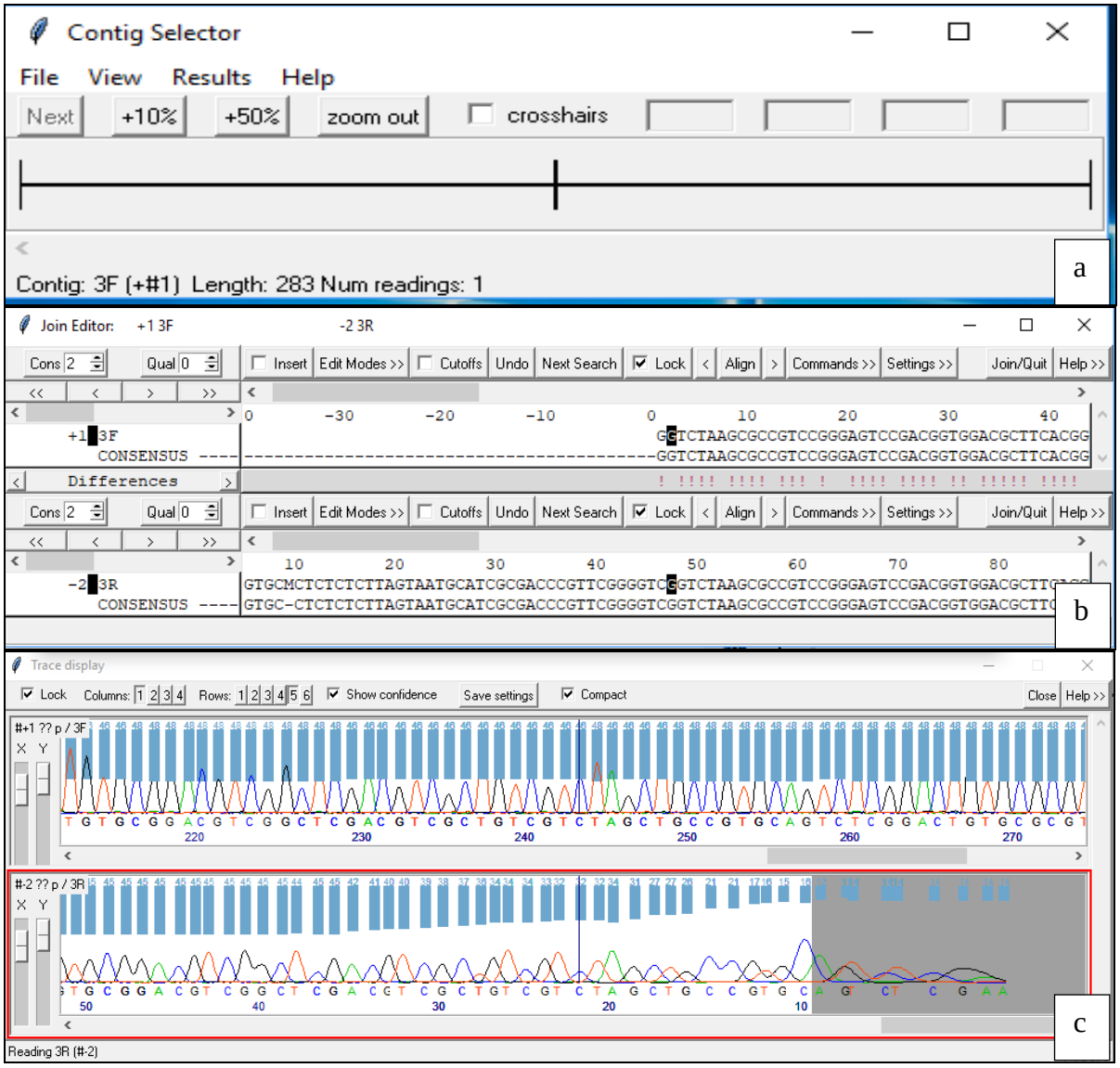

Figure 7: Processing the forward and reverse gene sequences in the software Gap4

In Gap4, the sequences were first opened in the contig selector (Fig. 7 a), and then viewed and edited in the contig editor (Fig. 7 b) to eliminate gene ambiguity with the help of DNA chromatograms and confidence levels displayed simultaneously in the trace display window (Fig. 8) and by checking the base allocations on the forward and reverse sequences. The extreme ends of the sequences where base allocation ambiguity could not be corrected were trimmed off in Gap4 during gene-editing and the editing process continued until a mismatch of 0.00% was obtained between the forward and the reverse sequences. This was followed by joining the forward and reverse sequences on the contig editor to generate the consensus sequences per strain as portrayed in Fig. 8. The obtained consensus sequences for all the 10 Apefly strains are provided in Appendix 1.

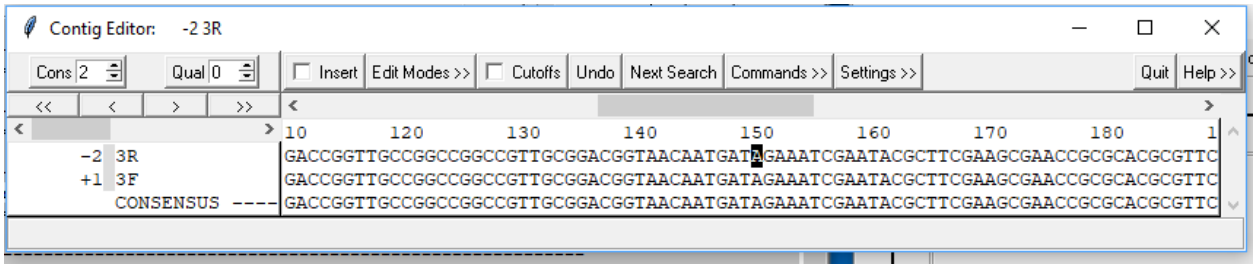

Figure 8: Generation of consensus sequences by joining the forward and reverse gene sequences

The Megabalst program of the Basic Local Alignment Search Tool (BLAST) (V. 9.2.0) (Zhang, Schwartz, Wagner & Miller, 2000) was used to obtain the Apefly species identities at the National Centre for Bioinformatics (NCBI) Genbank server (www.ncbi.nlm.nih.gov/BLAST). The identification decisions were made based on sequence similarity of over 99%.

3.4.4 Phylogenetics

Phylogenetics was conducted using the Molecular Evolutionary Genetics Analysis (MEGA) X (V. 10.0.5) (Kumar, Stecher, Li, Knyaz & Tamura, 2018). The process of multiple aligning the gene sequences of the 10 *S. lemolea lemolea* isolates is displayed in Fig. 10 both before (Fig. 9 a) and after (Fig. 9 b) the gene alignment in MEGAX.

To evaluate the relationship between *S. lemolea lemolea* species and related genera and species in the NCBI gene bank, using the sequence of one of the *S. lemolea lemolea* isolates (STR10), a search was conducted on the blastin suite of the NCBI gene bank and the search was optimized for highly similar sequences.

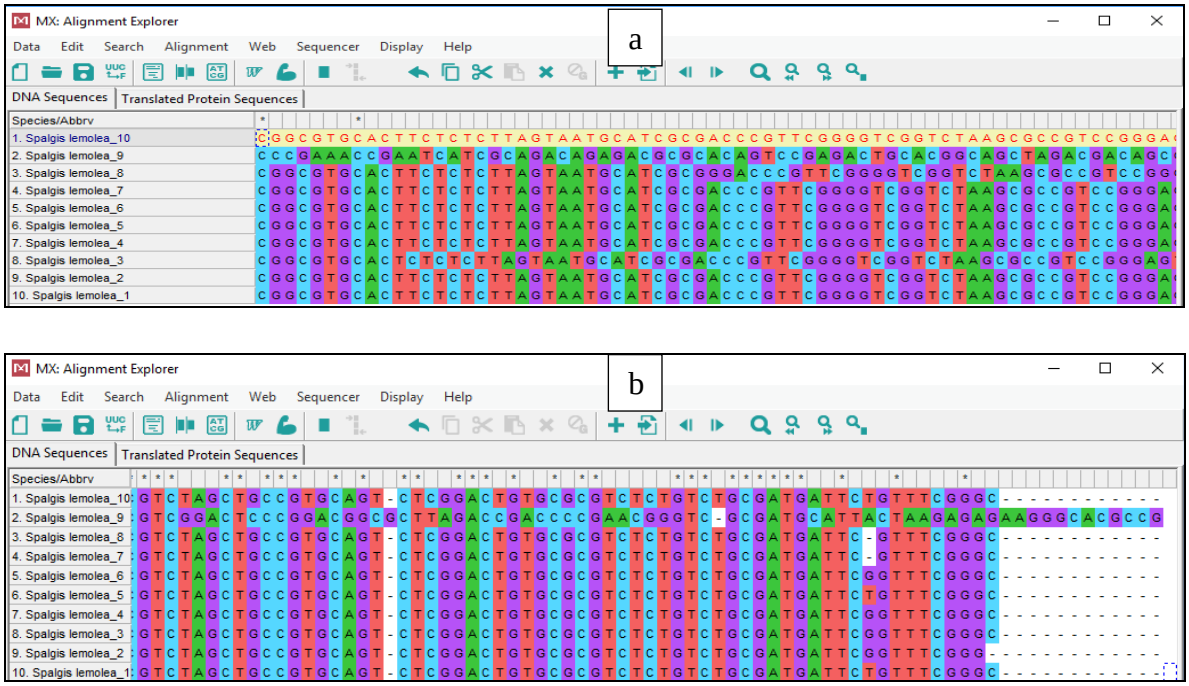

Figure 9: Processing gene sequences in the MEGA software during the phylogenetic analyses of the 10 *Splagis lemolea lemolea* 16S rRNA sequences

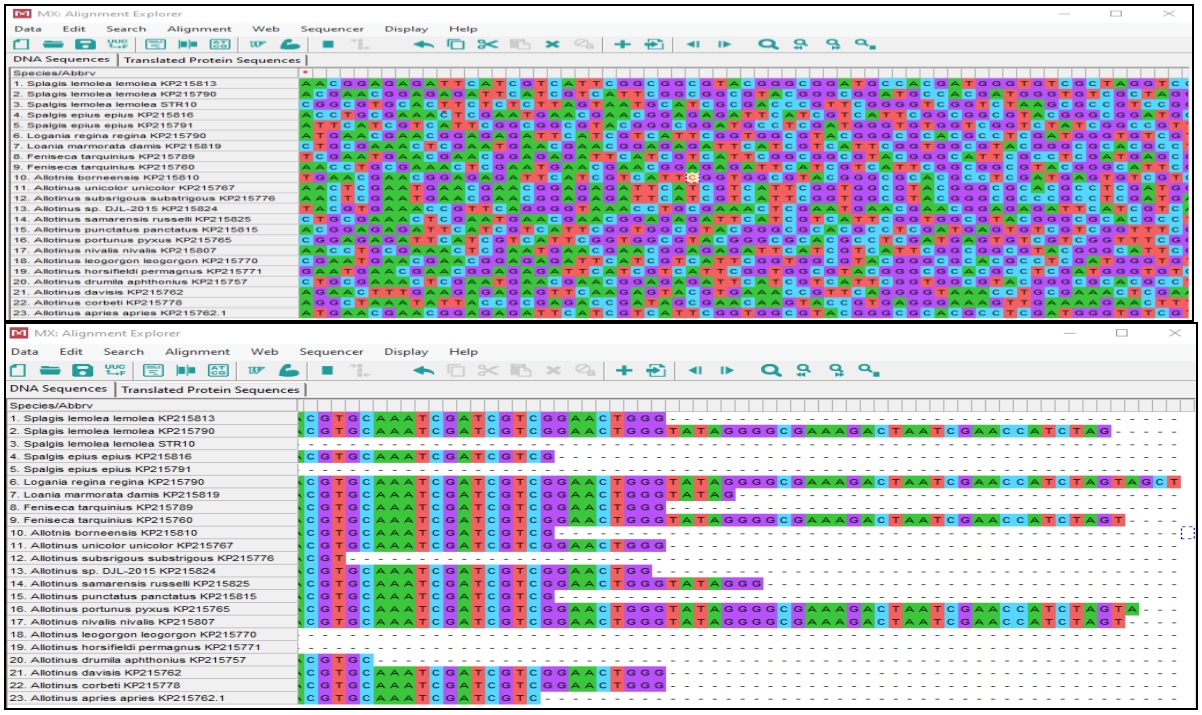

Figure 10: Processing gene sequences in the MEGA software during the phylogenetic analyses of evolutionary relationships between *Spalgis lemolea lemolea* STR10 and related gene sequences in the NCBI genebank

From the results obtained, the search was filtered to include only sequences with similarity above 91%. A total of 22 gene sequences were obtained in this manner. The sequences were

downloaded in FASTA formats and aligned with the gene sequence of *S. lemolea lemolea* STR10 on MEGAX software. Figure 10 displays the gene sequences of *S. lemolea lemolea* STR10 and the 22 related gene sequences on the alignment explorer of MEGAX. The blank spaces demonstrate areas of DNA base similarities.

3.5 The life cycle and developmental stages of the Apefly

3.5.1 Lab rearing of the prey –papaya mealybugs

Papaya mealybug eggs, nymphs and adults were initially collected by using a camel hairbrush from an infested pawpaw plant (*C. papaya*) in the Pawpaw field of Tengeru Horticultural Research Institute in Arusha, Tanzania. Adopting the Technology for Production of Natural Enemies (Singh, 1994) the mealybugs were reared in the laboratory at the temperature of 25 - 27 ^{o}C in a relative humidity (RH) of 55-65% on potted sprouted Irish potatoes (*Solanum tuberosum).* For constant availability of the prey, fresh potato sprouts were infested with mealybugs whenever required.

3.5.2 Laboratory rearing of the predator - Apefly

The Apefly (*S. lemolea lemolea*) larvae were reared in the laboratory on potato plants (*S. tuberosum)* infested with *P. marginatus* following the method described by (Dinesh *et al*., 2010) with minor modifications. The predator larvae completed their development on the mealybug-infested potato plants. The emerged adults of Apefly were allowed to mate and fresh mealybug-infested potato plants were provided for egg oviposition in the same cage. The eggs laid in the mass of mealybugs on potato leaves were carefully separated and kept individually in Petri dishes on a filter paper. The newly emerged larvae from these eggs were maintained in the same Petri dishes and provided with egg masses and nymphs of mealybug as food until they reached the pre-pupa stage. A few larvae from each instar were stored in 70% alcohol for morphometric purposes (Saengyot & Burikam, 2012).

3.5.3 Evaluation of the morphology and life cycle of Apefly

The moulting of larvae was confirmed by examining the Petri dishes daily for exuvial. We recorded for the developmental period from egg to adult (in days) and the number of larval instars in a life cycle, were recorded. The size of the egg, each larval instar, pre-pupa, pupa and adult Apefly were measured with an optical microscope (Optika B-350 –

Italy) (Nasari *et al.*, 2020). The external morphologies of each stage were also studied including observation of colour, measurement of body and wing size. Observations of the eclosion timing, larval feeding habits, as well as the mating and egg-laying behaviours of the adults were recorded. The duration of the pre-oviposition and oviposition periods was also documented.

3.6 The predatory and biological control potential of the Apefly against the papaya mealybug

The study assessed the potential of the Apefly (*S. lemolea lemolea)* as a papaya mealybug predator by quantifying the prey consumption and preference in the laboratory at 25-27 ºC, under a 55-65% RH and 12 h L:12 h D photoperiod. This method, though slitely modified, was proposed by Dinesh *et al.* (2010). The daily and stage-wise consumptions were used as measures and were employed to determine the predatory potential of the Apefly on the papaya mealybugs (*P. marginatus)* under laboratory conditions.

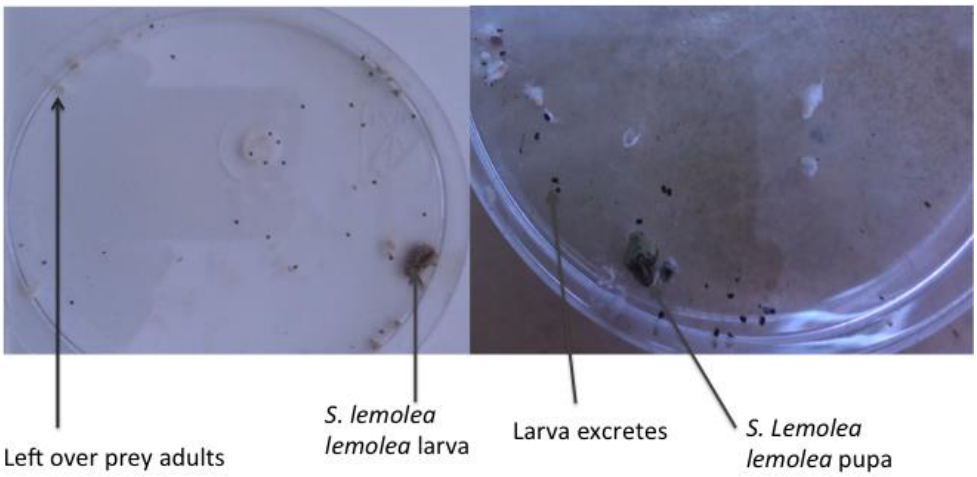

Figure 11: Apefly (*Spalgis lemolea lemolea*) larvae feeding on mealybugs (*Paracoccus margnatus)* during the laboratory experiment on its biological control potential

To determine the daily consumption of mealybugs by the Apefly, the study assessed the daily prey consumption by the Apefly from hatching to pupation. The Apefly eggs laid on the mealybug masses were collected with a fine camel hairbrush and kept individually in Petri dishes (5 cm diameter) on filter papers to hatch. This experiment was replicated five times adapting the methodology by Saengyot and Burikam (2012).

In each replicate, the newly emerged larvae from the eggs were maintained in the same Petri dish and provided with a daily egg masses (up to 500), nymphs (200-250) and adults (100-150) of mealybugs as food until they reached the pre-pupa stage. Before providing the prey stages, the eggs, nymphs and adults of mealybugs were counted using a stereo zoom microscope. Based on preliminary observations, the prey stages were offered to the Apefly larva daily in pre-determined quantities to ensure that the available prey exceeded the amount that the predator could consume in a day.

The number of eggs, nymphs and adults of prey consumed by the Apefly larvae was recorded at 24 h intervals by counting the number of leftover preys in the Petri dishes in each life stage category. The larval excretes and leftover prey stages (Fig. 11) were removed daily and the predators were fed with fresh prey stages. The collected data was used to determine the number of preys consumed during each instar.

3.7 Toxicity of Apefly meal on mice

3.7.1 Collection and preparation of Apefly meal

Pupa Apefly were collected from papaya plants in an organic garden located at Tengeru in Arusha Tanzania (S 03˚24’31. 2” and E 036˚48’51.5”).

Identification of the African Apefly up to the genus level was done at the TPRI and the specimens were deposited at the NICRC, Arusha, Tanzania. Molecular identification of the collected samples was carried out at the Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania, and the samples were confirmed to be African Apefly (*S. lemolea lemolea).* The collected samples were pulverized into fine particles. The preparation of Apefly is as summarized in Fig. 12.

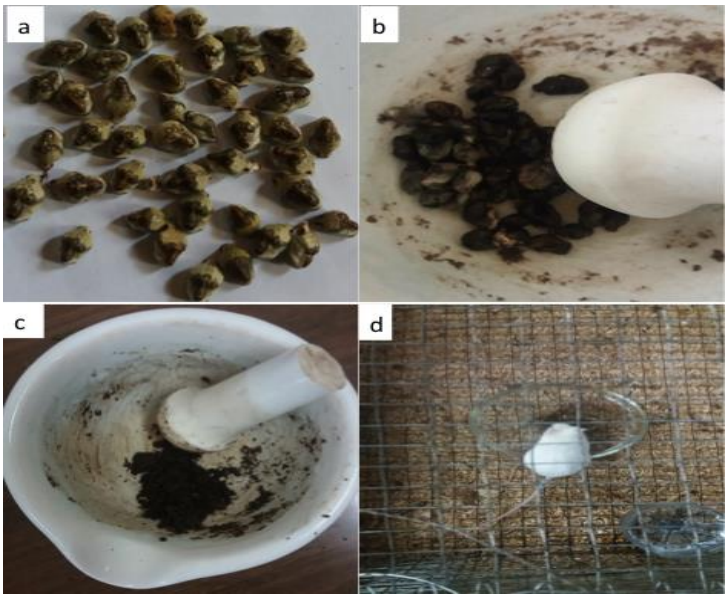

Figure 12: The preparation and handling of Apefly meal (a) Apefly pupa (b) Grinding of Apefly pupa (c) Apefly powder (d) A mouse feeding on 100% Apefly powder

3.7.2 The experimental animals

Female Swiss albino mice 8-10 weeks old, with mean weight 27.12 ± 0.54 were randomly obtained from the Plant Protection Department of the TPRI. The selection of female mice was based on their sensitivity to the toxic effects of chemicals than males (OECD, 1992). An experiment was conducted to determine mice's daily food consumption rates prior to the experiment and their 24 h Apefly meal intake was obtained as a difference in weight between the food put into the cage and that remaining after 24 h following the method described by Moran (2003). The mice were weighed and marked and randomly allocated to specific experimental groups. The mice were fed with broiler mash and clean drinking water for five days prior to treatment to acclimatize to the laboratory conditions and the experimental conditions were 25-30 ^{o}C and 40-60% RH and 12 h light/dark. The mice that participated in the acute toxicity test were continually provided with feeds and water adequately even after termination of the experiment.

3.7.3 Ethical consideration

An ethical clearance with notification number KNCHREC0006 was given by the Northern Zone Health Research Ethics Sub-Committee (KNCHREC) of the National Institute for Medical Research (NIMR) in Tanzania.

3.7.4 Acute toxicity study

A total of nine healthy female albino mice were involved in the acute toxicity test following the guidelines of the Organization of Economic Cooperation and Development (OECD) (2008). The mice were kept in wire mesh cages 39 x 17.5 x 17.5 cm, one mouse per cage and were provided with wood shavings as bedding. Since no toxicity test of the Apefly was done before, mice were randomly allocated into three groups of three mice each, where one group was control and two treatment groups. The control group received normal food (broiler mash) without Apefly while the second group received 50% Apefly meal plus 50% broiler mash and the third group 100% Apefly meal. All mice were fasted for four hours before being exposed to the treatments. After administration of the doses the mice were individually examined in the first 30 min, and after 1 h, 4 h, 12 h; 24 h over a period of 14 days. All symptoms of toxicity and recovery were noted; observations included changes in skin and fur, eyes, respiratory activity and behaviour pattern. Furthermore, attention was directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep, coma and mortality. The principles and criteria summarized in the Humane Endpoints Guidance Document (Demers *et al.*, 2006) were taken into consideration. Individual data was recorded in tabular form, and numerical results for the control and treated groups were compared to determine any health implications of Apefly consumption by the mice.

3.7.5 Sub-acute toxicity tests

The sub-acute toxicity test was carried out following OECD number 407. A total of 20 female albino mice were randomly allocated into seven cages of three mice each. The mice were starved for four h and their weights were determined before treatment. Apefly meal was given at 0%, 50%, 75% and 100% daily for 28 days. The mice were carefully observed in the first 30 min, and after 1 h, 4 h, 12 h and 24 h over a period of 28 days. Their body weights were determined after every 7 days and symptoms of toxicity such as changes in the skin, fur, and eyes, respiratory activities and behaviour patterns were noted. Further attention was directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep, coma and mortality.

3.7.6 Haematological and biochemical examination

On the 28^{th} day, all mice were individually weighed and subjected to chloroform anaesthesia. Blood samples were collected from each of them by cardiac puncture into two types of tubes,

with and without the anti-coagulant substance: ethylenediaminetetraacetic acid (EDTA). Haematological parameters including; white blood cell (WBC), red blood cell (RBC), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and haemoglobin concentration (Hb) were determined using the blood samples in the EDTA tubes by an automatic haematology analyzer. The blood samples contained in the tubes without EDTA were centrifuged at 4000 rpm for 10 min and the obtained serum subjected to biochemical and liver function analysis for parameters such as alkaline phosphate (ALP), Creatinine (cr), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST). After blood collection, all mice were sacrificed and dissected and their organs such as spleen, liver, kidney and heart were collected. The organs were cross-examined, and comparisons made between the control and treated mice groups. The organs were then weighed and preserved in 10% neutral buffered formalin for histopathological examination.

3.7.7 Histopathological analysis

The internal organs such as spleen, heart, kidney and liver, were prepared for histopathology assessments. Three replicates of the liver, kidney and spleen sections of 5 μm per treatment were cut and processed by rapid manual tissue processing as described in Culling (1974). The processed sections were stained with hematoxylin and eosin (H & E) and cover-slipped following pre-described methodologies (Culling, 1974). The slides were then observed under a light microscope and photomicrographs captured for documentation.

3.8 Data analyses

Knowledge survey data were summarized, frequencies and percentages were calculated using the Statistical Package for Social Science (SPSS) version 20. For each question, the percentage of farmers who gave similar responses was calculated for each site. Chi-square was conducted to assess the association of responses with their location. The level of significance was set at $P = 0.05$. One-way analysis of variance (ANOVA) with posthoc tests were conducted to test if there was any significant difference in the weight of mice that received different concentrations of Apefly during acute and sub-acute toxicity tests. Toxicity data such as body and organ weight, haematological and biochemical parameters were analyzed using measures of central tendency (Mean & SD). To determine if there were differences between sexes, an independent-samples t-test was used to compare the mean body

weight and the wingspan of the Apefly at P = 0.05. Descriptive statistics were used to compare the developmental stages of the Apefly as well as daily prey consumption by considering measures of central tendency.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Results

4.1.1 The occurrence and host range of Apefly in Tanzania

Pupa Apefly was present in 10 out of 20 visited sites especially in sites and hosts that were heavily infested by papaya mealybug. Out of 17-pupa Apefly collected, 10 emerged to adults (Table 5). Pupation was noted to occur outside the mealybug colony, under the leaves and fruits and other plant areas where there are fewer chances of interaction with other insects.

Table 5: Apefly occurrence, host plants and pesticide use in the study sites

Districts	Study Sites	Pupa collected	Adult emergence	Host Plant	Pesticide use	MIL
Mvomero	Mvomero	2	1	Cassava	X	***
	Sungaji	2	1	Cassava	X	***
	Muhonda	1	0	Cassava	X	*
	Diangoya	0	0	Cassava	X	-
Iringa	Ilolo	0	0	Cassava	X	*
	Luganga	0	0	Cassava	X	-
	Kiwele	0	0	Cassava	X	**
	Matondo	0	0	Papaya	/	-
Geita	Busanda	3	2	Papaya	X	***
	Butundwe	1	1	Cassava	X	***
	Kasamwa	0	0	Cassava	X	-
	Kalangalala	2	1	Cassava	X	**
Meru	Imbaseni	1	0	Papaya	X	*
	Ngongongare	0	0	Papaya	X	-
	Nambala	3	2	Cassava	X	**
	Leganga	2	2	Papaya	X	***
Shinyanga	Tinde	0	0	Papaya	X	-
	Malasa	0	0	Papaya	X	-
	Nganganurwa	0	0	Papaya	/	-
	Shabuluba	0	0	Papaya	/	-

x = No pesticide applied and /= pesticide applied, MIL= mealybug infestation level; *** = High (> 10 colonies per infected tissue), ** moderate (5-10 colonies per infected leaf), * = low (1-4 colonies per infected tissue) and - = no infestation

Apefly pupa was available on plant species mainly in association with mealybug on host plants. In proportion, the highest Apefly collection was on cassava (*Manihot esculenta*) compared with papaya (*C. papaya*).

4.1.2 Survey of the knowledge, perception and reactions towards the Apefly

(i) Socio-economic profiles

Table 6 summarizes the social-economic profiles of the participants (N=100). Most of the respondents (60%) were males and a majority (54%) of them were aged between 41-60 years while 72% had professional training from either colleges or universities. About 52% of them were leaders at village and ward levels.

Table 6: The socio-economic profile of the participants

Characteristics	N	%
Sex		
Male	60	60.0
Female	40	40.0
Age (Years)		
21-40	35	35.0
41-60	54	54.0
≥61	11	11.0
Education		
No Professional training	28	28.0
College	54	54.0
University	18	18.0
Occupation		
Community leader/elders	29	29.0
Ward/Village staff	52	52.0
District staff	17	17.0
Regional staff	2	2.00

(ii) Survey results

Out of the 100 participants, 89 were included in the analysis of knowledge and Table 7 summarizes the knowledge assessment responses. Among the respondents, only 38.2% reported having encountered a living Apefly while 61.8% had heard of the insect. A majority (79.8%) of them reported having known the insect between 2010-2018 for the first time, with the highest frequency in 2017 while a few (9%) had known the insect since the 1990s and 1980s.

Table 7: Assessment of the existing knowledge, perceptions and reactions of people about the Apefly

Characteristics	N	Percentage (%)
How did you know?		
Encountered the insect	34	38.20
Heard about the insect	55	61.81
When?		
2010-2018	71	79.82
2000-2009	10	11.20
Before 2000	8	9.04
In your farm?		
Yes	13	14.60
No	76	85.43
In which season?		
Wet season	5	5.62
Dry season	84	94.41
Interaction with other insects?		
Yes	61	68.50
No	4	4.51
I don't know	24	27.02
Apefly useful in Agriculture?		
Yes	3	3.41
No	2	2.22
I don't know	84	94.41
Heard any sick/dead of Apefly?		
Yes	81	91.03
No	8	9.03
Source of information?		
Experts	3	3.44
Media	74	83.12
Farmers	12	13.53
Is Apefly poisonous?		
No	7	7.91
Yes	82	92.12
Any Intervention?		
Yes	12	13.53
No	77	86.54
How do you deal with Apefly?		
Chemical spray	6	6.72
Biological	4	4.51
Avoidance	79	88.80
Farmers affected by Apefly?		
Yes	54	60.72
No	35	39.34
How was your first reaction?		
No reaction	10	11.22
Scared	79	88.85

The media was the main source of information about the Apefly 83.1% of the respondents (n=89). Most respondents (68.5%) had knowledge of other insects that are associated with the Apefly, describing them as "white waxy insects" and "sticky insects", meaning mealybugs.

However, 94.4% did not know the relationship between the mentioned insects and the Apefly.

Table 8: Knowledge, perception and reactions in association with the studied districts

	Districts n (%)					
	Meru	Geita	Mvomero	Shinyanga	Iringa	$\chi 2$ (**ρ**-value)
How did you know?						7.401(0.114)
I saw	7 (35)	9 (45)	11 (61.1)	5 (23.8)	2 (20)	
I heard	13 (65)	11 (55)	7 (38.9)	16 (76.2)	8 (80)	
When?						18.550(0.002) *
2010-2018	18 (90)	16 (80)	11 (61.1)	20 (95.2)	6 (60)	
2000-2009	1 (5)	-	6 (33.3)	-	3 (30)	
Before 2000	1 (5)	4 (20)	1 (5.6)	1 (4.8)	1 (10)	
In your farm?						18.422(<.001) *
Yes	2 (10)	2 (10)	9 (50)	-	-	
No	18 (90)	18 (90)	9 (50)	21 (100)	10 (100)	
Interaction with other insects?						37.171(<.001) *
Yes	15 (75)	20 (100)	14 (77.8)	8 (38.1)	4 (40)	
No	4 (20)	-	-	-	-	
I don't know	1 (5)	-	4 (22.2)	13 (61.9)	6 (60)	
In which season?						8.162(0.012) *
Wet season	4 (20)	-	-	-	1 (10)	
Dry season	16 (80)	20 (100)	18 (100)	21 (100)	9 (90)	
Is Apefly useful?						6.020(0.755)
Yes	1 (5)	1 (5)	1 (5.6)	-	-	
No	-	-	-	1 (4.8)	1 (10)	
I don't know	19 (95)	19 (95)	17 (94.4)	20 (95.2)	9 (90)	
Heard of a sick/dead of Apefly?						6.989(0.069)
Yes	20 (100)	19 (95)	16 (88.9)	16 (76.2)	10 (100)	
No	-	1 (5)	2 (11.1)	5 (23.8)	-	
Source of information?						14.162(0.048) *
Experts	-	3 (15)	-	-	-	
Media	18 (90)	12 (60)	14 (77.8)	20 (95.2)	10 (100)	
Farmers	2 (10)	4 (20)	2 (11.1)	1 (4.8)	-	
No information	-	1 (5)	2 (11.1)	-	-	
Is Apefly poisonous?						1.148(1.000)
No	1 (5)	2 (10)	1 (5.6)	2 (9.5)	1 (10)	
I don't know	19 (95)	18 (90)	17 (94.4)	19 (90.5)	9 (90)	
Farmers affected by Apefly						49.554(<0.001) *
Yes	2 (10)	20 (100)	6 (33.3)	7 (33.3)	-	
No	18 (90)	-	12 (66.7)	14 (66.7)	10 (100)	
Interventions						16.262(<0.001) *
Yes	1 (5)	3 (15)	8 (44.4)	-	-	
No	19 (95)	17 (85)	10 (55.6)	20 (100)	10 (100)	
How do you deal with Apefly?						37.378(<0.001) *
Chemical spray	-	3 (15)	3 (16.7)	-	-	
Biological	-	-	4 (22.2)	-	-	
Avoidance	20 (100)	17 (85)	11 (61)	20 (100)	11 (100)	

*P<0.05, $\chi 2$ – Chi-square value (Fisher Exact)

When respondents were asked about their opinion on potential dangers of the Apefly to human health, 92.1 of them said that the Apefly is dangerous based on what they heard through media (83.1%) and other sources (16.9%). When asked about their emotional reactions towards the insect, 88.8% reported that the insect was scarily and cause anxiety. About 86.5% were not satisfied with the intervention made by experts to address the anxiety when the insect availability was at its peak. Generally, a majority of the participants

perceived the insect negatively mainly due to its unusual appearance and the spreading news about its toxicity. The findings were similar across the districts indicating that the insect was generally perceived negatively regardless of the location. Despite the existence of negative perception towards Apefly, 88.8% of the surveyed respondents did not aggressively deal with Apefly but rather avoided them. The avoidance was described as abstinence from vegetables associated with Apefly for a period of about 3-5 months, especially in most pronounced areas. This reportedly reduced vegetable consumption by about 60.7% which caused high losses to vegetable farmers, especially in Geita (Table 8)**.** The responses of the participants on the knowledge, perceptions and reactions were associated with respective districts in a bivariate analysis as cross-tabulation ($P < 0.05$). The findings are summarized in Table 8.

4.1.3 Molecular identification of the Apefly samples

(i) PCR-based identification of the collected Apefly samples

The PCR using P2F/P2R amplified the target gene and produced a product of about 225 bp (Fig 13).

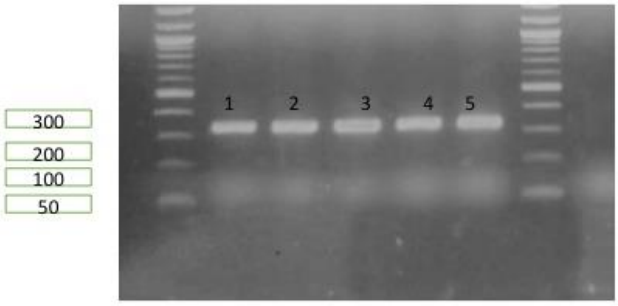

Figure 13: Electrophoresis of PCR samples of rRNA Apefly genes from representative host plants

1 = *S. lemolea lemolea* (Papaya) from Geita, 2 = *S. lemolea lemolea* (Cassava) from Geita, 3 = *S. lemolea lemolea* (Papaya) from Meru, 4 = *S. lemolea lemolea* (Cassava) from Meru, 5 = *S. lemolea lemolea* (Cassava) from Mvomero.

Sequencing of the PCR amplicons and comparison of the resulting sequences with the NCBI collections indicated the Apefly samples collected from different locations in Tanzania were 99% similar to *S. lemolea lemolea* of Gene Bank accession numbers KP215813.1 and KP215790.1. Table 9 displays the Apefly species identities of the 10 insect samples aster sequencing and analysis if the 16S rRNA genes. All the 10 Apefly isolates were identified as *S. lemolea lemolea*.

Table 9: Identity of genes on the NCBI website (blastin-nucleotide suite for somewhat similar sequences on the nucleotide collection database)

	Strain identity	Closest match from Nucleotide suite	Query length	Query cover	E value	% Identity	Accession no
1	*S. lemolea lemolea*	*Spalgis lemolea lemolea MCZ: AJG-07-N803*	330	100%	2e-166	100%	KP215813.1
2	*S. lemolea lemolea*	*Spalgis lemolea lemolea MCZ: AJG-07-N803*	329	100%	3e-164	99.7%	KP215813.1
3	*S. lemolea lemolea*	*Spalgis lemolea lemolea MCZ: AJG-07-N803*	329	100%	1e-162	99.39%	KP215813.1
4	*S. lemolea lemolea*	*Spalgis lemolea lemolea MCZ: AJG-07-N803*	330	100%	9e-165	99.70%	KP215813.1
5	*S. lemolea lemolea*	*Spalgis lemolea lemolea MCZ: AJG-07-N803*	330	100%	2e-166	100%	KP215813.1
6	*S. lemolea lemolea*	*Spalgis lemolea lemolea MCZ: AJG-07-N803*	329	100%	1e-162	99.39%	KP215813.1
7	*S. lemolea lemolea*	*Spalgis lemolea lemolea MCZ: AJG-07-N803*	329	100%	1e-163	99.70%	KP215813.1
8	*S. lemolea lemolea*	*Spalgis lemolea lemolea MCZ: AJG-07-N803*	331	100%	2e-161	99.10%	KP215813.1
9	*S. lemolea lemolea*	*Spalgis lemolea lemolea MCZ: AJG-07-N803*	329	100%	4e-163	99.39%	KP215813.1
10	*S. lemolea lemolea*	*Spalgis lemolea lemolea MCZ: AJG-07-N803*	330	100%	2e-166	100.00%	KP215813.1

(ii) Phylogenetics

Genetic diversity of the S. lemolea lemolea gene sequences

The phylogenetic analyses of the identified Apefly isolates in this study were conducted based on the estimates of genetic divergence between the respective sequences. The numbers of base differences per site from between the different sequences as calculated using the p-distance method are shown in Table 10. The analysis involved 10 nucleotide sequences corresponding to the 10 Apefly isolates which were studied. All ambiguous positions were removed for each sequence pair (pairwise deletion option) and there was a total of 348 positions in the final dataset.

Table 10: Estimates of genetic divergences between the 10 gene sequences of *S. lemolea lemolea*

***Spalgis*_lemolea_10**									
***Spalgis*_lemolea_9**	0.535								
***Spalgis*_lemolea_8**	0.000	0.532							
***Spalgis*_lemolea_7**	0.000	0.534	0.000						
***Spalgis*_lemolea_6**	0.003	0.534	0.000	0.000					
***Spalgis*_lemolea_5**	0.000	0.535	0.000	0.000	0.003				
***Spalgis*_lemolea_4**	0.003	0.535	0.000	0.000	0.000	0.003			
***Spalgis*_lemolea_3**	0.003	0.535	0.000	0.000	0.000	0.003	0.000		
***Spalgis*_lemolea_2**	0.003	0.534	0.000	0.000	0.000	0.003	0.000	0.000	
***Spalgis*_lemolea_1**	0.000	0.535	0.000	0.000	0.003	0.000	0.003	0.003	0.003

A Genetic relationship of the 10 Apefly species that were studied is displayed in Fig. 14.

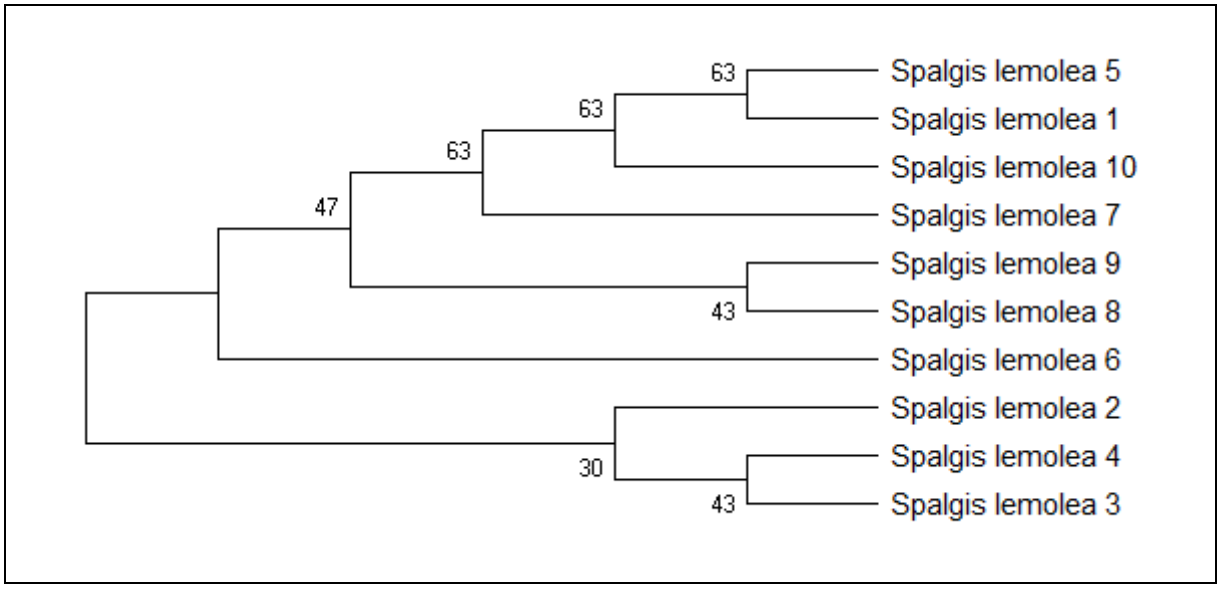

Figure 14: Genetic relationships of the 10 Apefly taxa collected from different areas in Tanzania

Table 11: Estimates of genetic divergences between sequences of *Spalgis lemolea lemolea* STR10 and related sequences in the NCBI database

Splagis_lemolea_lemolea_KP215813																						
Splagis_lemolea_lemolea_KP215790	0.000																					
Spalgis_lemolea_lemolea_STR10	0.000	0.000																				
Spalgis_epius_epius_KP215816	0.053	0.053	0.046																			
Spalgis_epius_epius_KP215791	0.061	0.061	0.046	0.005																		
Logania_regina_regina_KP215790	0.052	0.050	0.073	0.057	0.064																	
Loania_marmorata_damis_KP215819	0.056	0.056	0.076	0.059	0.068	0.006																
Feniseca_tarquinius_KP215789	0.062	0.061	0.073	0.065	0.074	0.035	0.031															
Feniseca_tarquinius_KP215760	0.062	0.059	0.073	0.064	0.074	0.033	0.030	0.000														
Allotnis_borneensis_KP215810	0.057	0.057	0.067	0.060	0.067	0.018	0.020	0.034	0.034													
Allotinus_unicolor_unicolor_KP215767	0.055	0.055	0.076	0.060	0.070	0.016	0.019	0.036	0.036	0.018												
Allotinus_subsrigous_substrigous_KP215776	0.061	0.061	0.080	0.066	0.074	0.020	0.024	0.036	0.036	0.016	0.013											
Allotinus_sp._DJL-2015_KP215824	0.054	0.054	0.076	0.058	0.068	0.014	0.018	0.035	0.034	0.017	0.001	0.012										
Allotinus_samarensis_russelli_KP215825	0.051	0.051	0.070	0.056	0.065	0.012	0.015	0.032	0.032	0.014	0.004	0.012	0.003									
Allotinus_punctatus_panctatus_KP215815	0.056	0.056	0.064	0.061	0.067	0.018	0.020	0.034	0.034	0.003	0.018	0.013	0.017	0.015								
Allotinus_portunus_pyxus_KP215765	0.059	0.057	0.073	0.065	0.071	0.021	0.023	0.038	0.036	0.005	0.022	0.018	0.021	0.018	0.004							
Allotinus_nivalis_nivalis_KP215807	0.062	0.059	0.073	0.064	0.074	0.033	0.030	0.000	0.000	0.034	0.036	0.036	0.034	0.032	0.034	0.036						
Allotinus_leogorgon_leogorgon_KP215770	0.058	0.058	0.076	0.062	0.070	0.016	0.020	0.038	0.038	0.019	0.003	0.013	0.001	0.004	0.019	0.023	0.038					
Allotinus_horsifieldi_permagnus_KP215771	0.056	0.056	0.076	0.061	0.068	0.015	0.019	0.037	0.037	0.018	0.001	0.012	0.000	0.003	0.018	0.022	0.037	0.001				
Allotinus_drumila_aphthonius_KP215757	0.059	0.059	0.073	0.059	0.068	0.016	0.020	0.036	0.035	0.017	0.013	0.017	0.012	0.009	0.017	0.022	0.035	0.013	0.012			
Allotinus_davisis_KP215762	0.060	0.060	0.083	0.066	0.076	0.017	0.020	0.033	0.033	0.018	0.013	0.007	0.011	0.011	0.016	0.020	0.033	0.013	0.012	0.017		
Allotinus_corbeti_KP215778	0.055	0.055	0.076	0.059	0.070	0.016	0.019	0.036	0.035	0.018	0.000	0.013	0.001	0.004	0.018	0.022	0.035	0.003	0.001	0.013	0.012	
Allotinus_apries_apries_KP215762.1	0.058	0.058	0.080	0.063	0.073	0.018	0.022	0.039	0.039	0.021	0.003	0.016	0.004	0.007	0.021	0.025	0.039	0.005	0.004	0.016	0.016	0.003

The evolutionary history was inferred using the Neighbor-Joining (NJ) method (Saitou & Nei, 1987). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the p-distance method (Nei & Kumar, 2000) and are in the units of the number of base differences per site. This analysis involved 10 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 348 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al.,* 2018).

Evolutionary relationships between Spalgis lemolea lemolea STR10 gene sequence and related gene sequences in the NCBI genebank

The numbers of base differences per site from between sequences are shown in Table 9 for *Splagis lemolea lemolea* STR10 and related gene sequences in the NCBI genebank (>91% similarity). A phylogenetic diagram was developed to illustrate the evolutionary relationships among the 10 *S. lemolea lemolea* isolates and the related gene sequences in the NCBI genebank (Fig. 14). The identified *S. lemolea lemolea* strains used in the phylogenetic analysis were all closely related to other *S. lemolea lemolea* strains in the genebank and closely related to two *S. epius epius* strains in the genebank.

The evolutionary analyses were conducted in MEGAX and the evolutionary history was inferred using the NJ method. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the p-distance method (Nei & Kumar, 2000) and are in the units of the number of base differences per site. This analysis involved 32 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 946 positions in the final dataset.

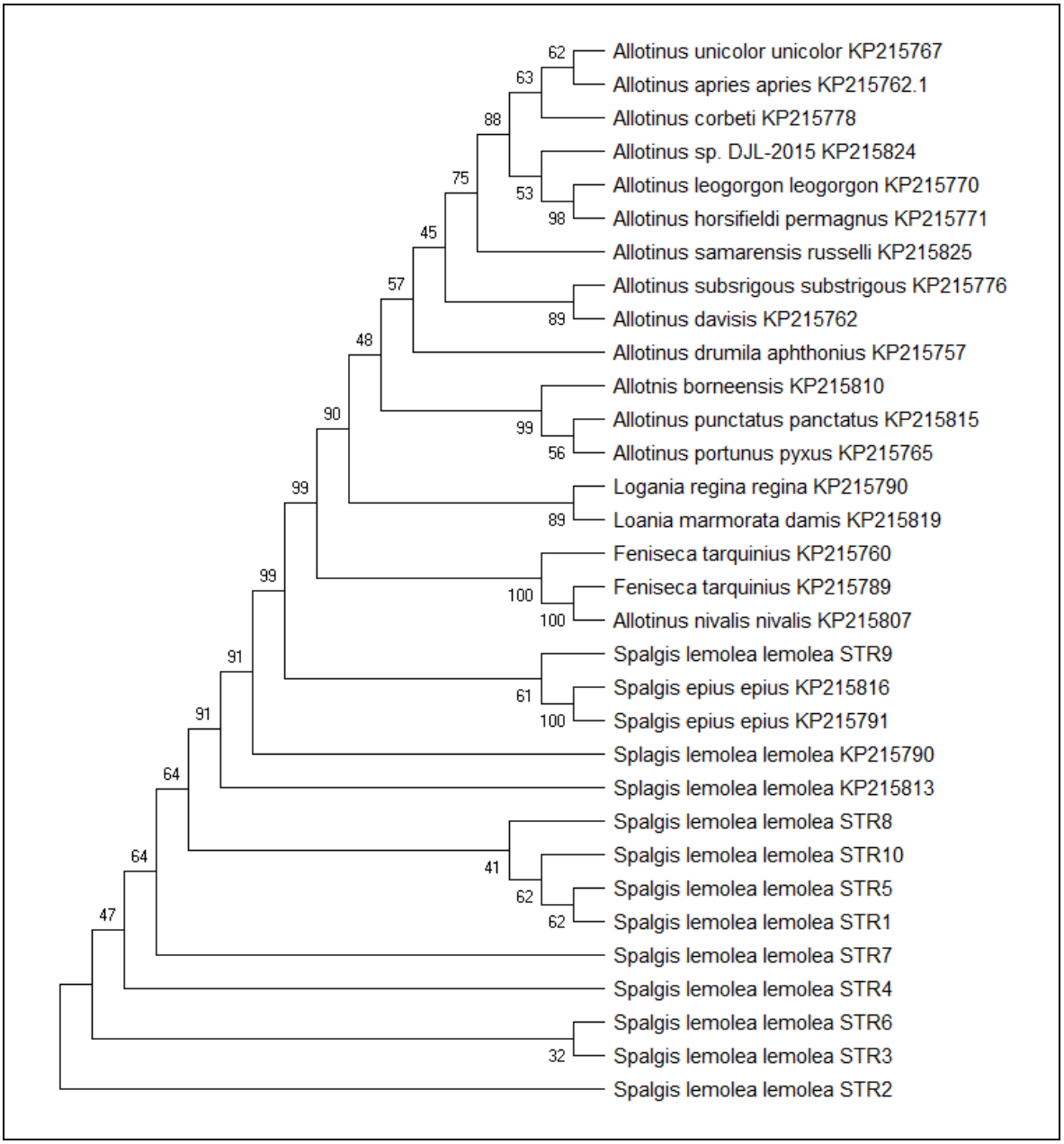

Figure 15: Genetic relationships of 10 *Splagis lemolea lemolea* sequences and 22 related taxa in the NCBI gene bank

4.1.4 Life cycle and developmental stages of the Apefly

(i) The morphology and life cycle of the Apefly (*S. lemolea lemolea)*

Eggs

The eggs of the Apefly were laid singly interspersed in small groups of 2-8 eggs, with many short flights in between different spots (Fig 16). The eggs were disc-shaped, flat on both sides

with depressions on the tops. The eggs were creamy in color, with a mean diameter of 0.51 ± 0.02 mm.

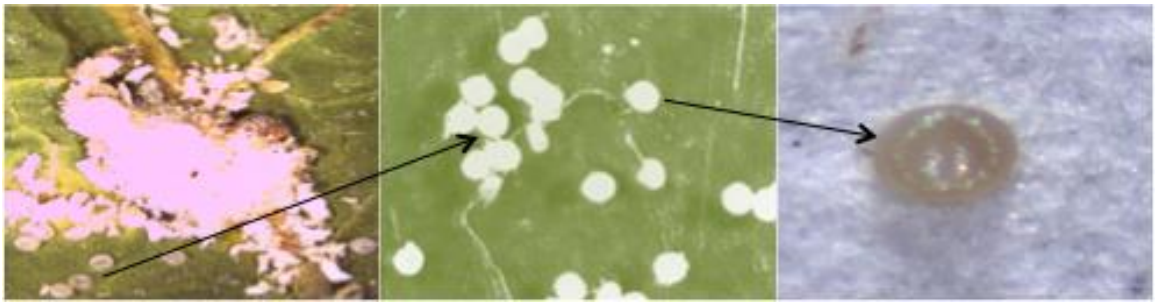

Figure 16: Eggs of the Apefly (*S. lemolea lemolea*) laid on potato leaves infested with mealybug colonies

Larvae

There were four larval instars of the Apefly (Fig. 17). The 1st larval instar was inactive, residing in the mealybug colony and made a feeding chamber in mealybug egg masses. It also fed on small mealybug nymphs when the eggs were not available. After moulting, the 2nd instar larvae moved out of its egg chamber for feeding and its body was covered with mealybug wax and mealybug eggs attached to it. The 3rd and 4th instar larva instars crawled while feeding and ate all mealybug stages. The length and width of the 4 larval instars are displayed in Table 10. The freshly-eclosed larva was creamy in colour with dark-brown setae (Fig. 17 b).

Figure 17: Larval instars of the Apefly (*S. lemolea lemolea)*

(a) Larval instars before molting (b) Freshly enclosed larval instars. Photographs by author

The freshly-eclosed 4th larval instar had much shorter setae compared to the previous stages, with a pale-brown segmented body. All larva instars had a hard dark-brown hairy cuticle

covered with thick waxy coating before molting (Fig. 13 a) as a result of its close association with the mealybugs.

Pre-Pupa and Pupa

The pre-pupa larva stopped eating, shrunk and its setae disappeared (Fig. 18 a). It then moved away from the mealybug colony and firmly attached itself to the leaf or stem of the host plant and produced a small amount of silk for attachment to the plant. The pupa was light brown on the dorso-lateral side and whitish on the ventral side. However, the color darkened gradually as development progressed. The dorsal side of the pupa resembled the face of a monkey (Fig. 18 b). The pupa showed clear spots of eyes, nose, and cheeks on the dorsal side.

Figure 18: The pupa stages of the Apefly (*S. lemolea lemolea)*

(a) Pre-pupa and (b) Pupa of the Apefly

Adults

The adults Apefly had whitish-grey wings with thin black stripes on the inner side of the wings (Fig. 19 b, c, h and i) and bold black stripes on the outer edges of the forewings (Fig. 19 f and g). There were no significant colour differences between male and female adults (Fig. 19 a). The abdomen of the male was slender (Fig. 19 d) but broader in females compared, the former with an ovipositor for egg-laying (Fig. 19 e). Observation of the pre-mating behaviours revealed a prolonged physical contact whereby the females pushed underneath the males to mate (Fig. 19 a).

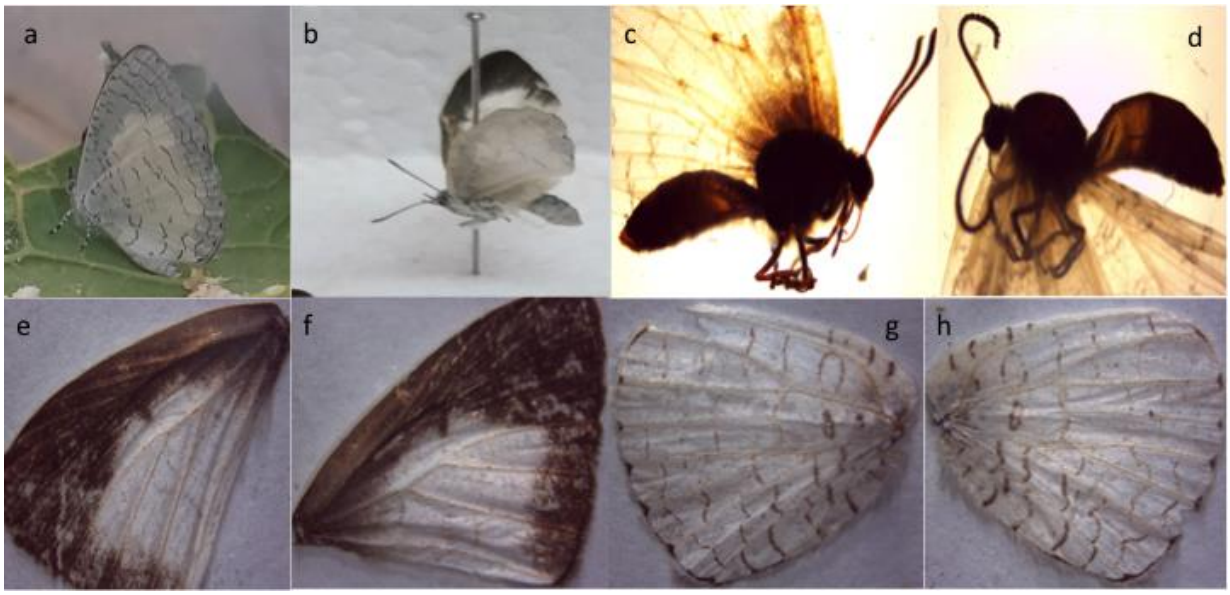

Figure 19: Adult male and female Apefly (*S. lemolea lemolea*)

(a) Adult female of Apefly laying eggs, (b, c) Male Apefly, (d) Female Apefly, (e) Left forewing, (f) Right forewing, (g) Left hind wing and (h) Right hind wing

(ii) The body lengths and developmental periods of the Apefly

The 1st and the last larval instars measured 1.9 ± 0.17 mm and 10.24 ± 0.23 mm in length respectively and 0.64 ± 0.03 mm and 6.08 ± 0.61 mm in width respectively (Table 12). The mean body length of the male and female adults was 10.10 ± 0.43 mm and 11.03 ± 0.84 mm respectively, and the average wingspan was 27.15 ± 0.65 mm for males and 29.76 ± 1.01 mm for females.

Table 12: Body length measurements (mm) of the larva, pre-pupa and pupa of *Spalgis lemolea lemolea*

Stage of development	**Length (mm)**			**Width (mm)**		
	Mean ± SD	Range	Median	Mean ± SD	Range	Median
Larva 1st Instar	1.90 ±0.17	1.60-2.20	1.90	0.64 ± 0.03	0.58-0.72	0.63
2nd Instar	3.26 ±0.18	2.80-3.61	3.31	1.61 ± 0.11	1.40-1.81	1.62
3rd Instar	5.77 ± 0.21	5.20-6.20	5.82	3.67 ± 0.28	3.00-4.35	3.59
4th Instar	10.24 ± 0.23	9.70-10.70	10.3	6.08 ± 0.61	5.30-7.40	5.78
Pre-pupa	7.41 ± 0.22	6.82-7.8.01	7.42	4.59 ± 0.34	4.00-5.30	4.51
Pupa	6.73 ± 0.22	6.21-7.20	6.71	3.94 ± 0.06	3.82-4.02	3.92

There was a significant difference in body length ($t = -4.384$, $P < 0.001$) and in wingspan ($t = -9.684$, $P < 0.001$) between the male and female (Table 13). The female laid an average of 68 eggs after 4-5 days of eclosion, whereby 2 - 7 eggs were laid in a group at different spots. The duration of each developmental stage of the Apefly was determined under laboratory

conditions with the temperature at 25-27 ^{o}C, under 55-65% RH and 12 h L: 12 h D photoperiod.

Table 13: Body length measurements (mm) of the adult *S. lemolea lemolea*

Body length (mm)	Mean ± SD	Wingspan (mm)	Mean ± SD
Male	10.10 ± 0.43	Male	27.15 ± 0.65
Female	11.03 ± 0.84	Female	29.76 ± 1.01

[t (38) = -4.384, P < 0.001 t (38) = -9.684, P < 0.001] n=25

The duration of each developmental stage of the Apefly was determined under laboratory conditions with the temperature ranging between 25 to 27 ^{o}C, RH ranging between 55-65% and photoperiod of 12 h L: 12 hr D. The mean duration of development of the eggs, larva instars, pre-pupa and pupa were 3.66, 10, 0.95 and 9.48 days respectively as presented in Table 14. The life span of the Apefly from egg to adult emergence was 23.5 days.

Table 14: Duration of developmental stages of the Apefly (*S. lemolea lemolea)* reared on mealybugs (*P. marginatus)* under laboratory condition

Stage of development	Duration (days)		
	Mean ± SD	Range	Median
Egg	3.66 ± 0.41	3.11-4.53	3.60
Larva 1st Instar	2.27 ± 0.62	1.50-3.91	2.21
2nd Instar	2.06 ± 0.29	1.72-2.60	2.01
3rd Instar	2.01 ±0.29	1.61-2.52	1.91
4th Instar	3.31 ± 0.51	2.40-4.21	3.30
Pre-pupa	0.95 ± 0.16	0.71-1.60	0.91
Pupa	9.48 ± 1.19	8.01-12.11	9.01

4.1.5 The predatory activity of the Apefly on the papaya mealybug

The Apefly (*S. lemolea lemolea*) with 4 larval instars completed their developmental stages in 10 days in the laboratory. The freshly hatched 1st instar larvae consumed a mean of 77.4 ± 6.5 mealybug eggs on the 1st day and consumption increased as development progressed. The highest consumption of the mealybug eggs (311.2 ± 20.3) was reached on the 9th day but decreased to 288.8 ± 19.5 on the 10th day (Fig. 20).

The consumption of nymphs of the mealybugs by a newly hatched Apefly on the 1st day was 1.20 ± 0.4 nymphs, increasing as development progressed. The maximum number of nymph consumption (24.6 ± 1.1), was reached on the 9th day but decreased to 20.8 ± 1.1 on the 10th day. When mealybug adults were provided as prey, the consumption by a newly- hatched

Apefly larva was 0.21 ± 0.4 adults on the 1st day but increased thereafter to 16.6 ± 1.8 adults on the 9th day and then decreased to 14.80 ± 2.8 on the 10th day (Table 15).

Table 15: The daily consumption of mealybug (*P. marginatus*) stages by Apefly (*S. lemolea lemolea*) larvae

Days	Eggs		Nymph		Adult	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
1	77.4 ± 6.5	69 - 85	1.20 ± 0.45	1 - 2	0.21 ± 0.45	0 – 1
2	98.8 ± 6.61	92 - 108	2.21 ± 1.10	1 – 3	1.01 ± 0.71	0 – 2
3	119 ± 5.57	112 – 126	4.01 ± 0.71	3 – 5	2.40 ± 0.55	2 – 3
4	146.1 ± 6.6	139 – 152	6.61 ± 1.14	5 – 8	4.61 ±0.89	4 – 6
5	176.2 ± 9.96	159 – 182	10.63 ± 1.14	9-12	6.42 ±1.34	5 – 8
6	213.2 ± 26.29	183 – 255	14.61 ± 1.14	13 – 16	8.81 ±1.30	7 – 10
7	249.4 ± 29.31	211 – 291	17.42 ± 1.52	16 – 20	11.42 ± 0.89	10 – 12
8	302.6 ± 28.04	262 – 335	21.01 ± 1.58	19 – 23	13.80± 1.31	12 – 15
9	311.2 ± 20.28	287 – 342	24.60 ± 1.14	23 – 26	16.61 ± 1.82	15 – 19
10	288.8 ± 19.51	262 - 311	20.81 ± 1.10	19 – 22	14.80 ± 2.77	12 – 19

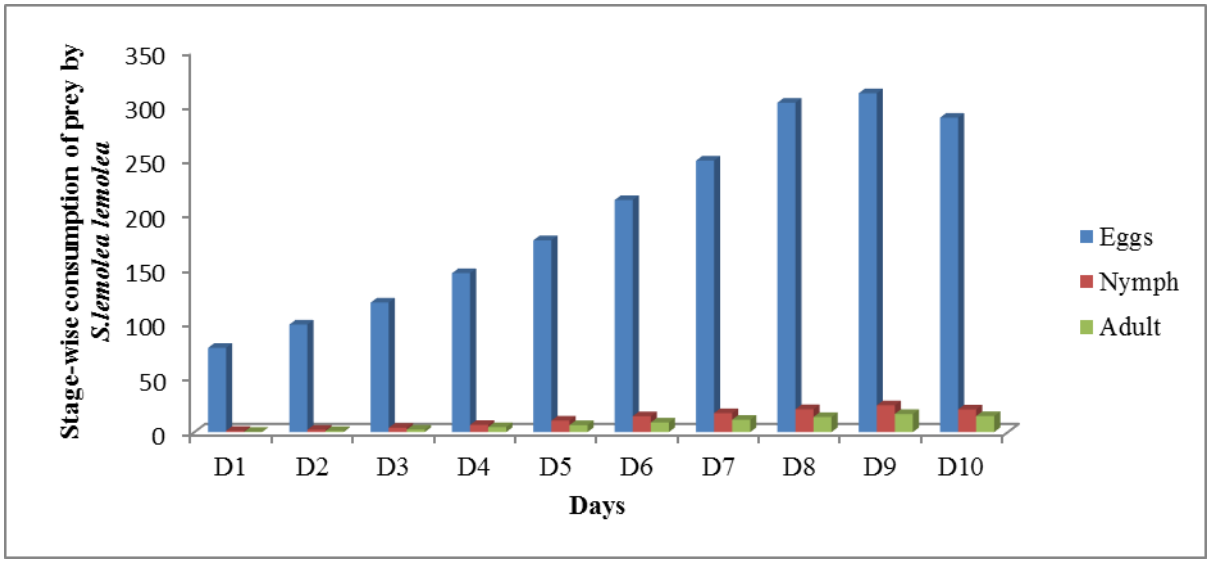

Figure 20: Daily consumption of mealybugs (*P. marginatus*) by Apefly (*S. lemolea lemolea*) larvae under laboratory condition

On average, a single Apefly larva consumed 1982.6 ± 117 eggs, 123 ± 5.8 nymphs and 80 ± 8.5 adults of mealybugs during its entire larval development period. The consumption of prey increased as the development of the Apefly progressed (Fig. 21).

The consumption of mealybugs by the Apefly larval instars differed in the mean number of the prey eggs, nymphs and adults consumed across different instars. The 1st instar larvae of the Apefly were almost sedentary, while the 2nd, 3rd and 4th instar larvae crawled while feeding (Fig. 17).

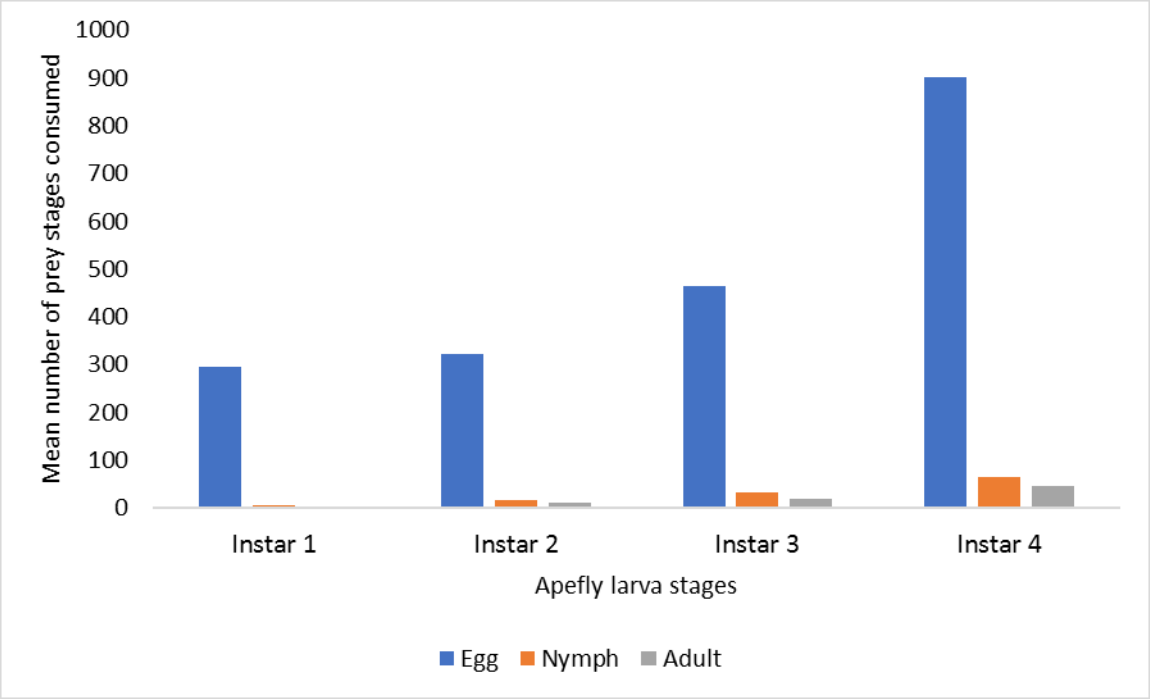

Figure 21: Stage-wise consumption of mealybugs (*P. marginatus*) by Apefly (*S. lemolea lemolea*) larvae in different instars under laboratory conditions

4.1.6 Toxicity of Apefly meal on mice

(i) Acute toxicity tests

Behavioural observations

Acute toxicity test of the Apefly meal on albino mice revealed that the behaviour of treated and control groups in the first 30 min, and after 4 h, 24 h and daily up to the 14th day did not show any visible signs of acute toxicity. There was no decrease in weight or abnormal growth resulting from the consumption of the Apefly meal even at a 100% dose. Detailed observations are presented in Table 16.

Table 16: Behavioural observations of a cute toxicity study of the Apefly meal on mice

Observation	Control (0% Apefly meal)	Treatment 1 (50% Apefly meal)	Treatment 2 (100% Apefly meal)
Changes in skin and fur	Null	Null	Null
Eyes	Normal	Normal	Normal
Respiratory activity	Normal	Normal	Normal
Tremors	Not observed	Not observed	Not observed
Convulsion	Not occurred	Not occurred	Not occurred
Salivation	Normal	Normal	Normal
Drowsiness	Not occurred	Not occurred	Not occurred
Comma	Not occurred	Not occurred	Not occurred
Death	Not occurred	Not occurred	Not occurred

Bodyweight changes

One-way ANOVA and post-hoc tests were conducted to assess for significant differences in the weight of mice at different concentrations of the Apefly meal. The findings showed significant differences ($p = 0.030$) in mice weight at day 0 but no significant differences ($p = 0.149$) were noted at day 14 (Table 17). The results generally revealed a gradual increase in the weight of mice for both control and treated groups.

Table 17: Bodyweight (g) of the control and mice treated with Apefly meal in the acute toxicity test

Weight (g)	Control (0% Apefly meal)	Treatment 1 (50% Apefly meal)	Treatment 2 (75% Apefly meal)	P-value
Day 0	29.77 ± 0.25	30.20 ± 0.66	31.20 ± 0.66	0.130
Day14	33.23 ± 0.68	33.60 ± 1.21	31.83 ± 1.01	0.149

Values are an average of three mice fed with the Apefly diet, expressed as mean ±SEM

(ii) Sub-acute toxicity tests

Behavioural observation

The results of the sub-acute toxicity study of the Apefly meal on mice showed that there were no signs of toxicity in mice of both control and treated groups even at 100% Apefly consumption. All animals were normal throughout the study period and they all survived until the end of the 28th day of experimentation.

Haematological parameters

The results of the sub-acute toxicity study of the Apefly meal on mice showed that there were no signs of toxicity in mice from both control and treated groups even at 100% Apefly meal concentrations. All animals were normal throughout the study period and all survived until the 28th day of experimentation. The values of all haematological parameters remained within normal limits as summarized in Table 18. The results of haematological parameters of the control and treated mice showed no significant differences ($P > 0.05$) in all haematological parameters after 28 days of treatment with the Apefly meal.

Table 18: Hematological values of control and mice treated with Apefly meal in the sub-acute toxicity test

Parameters	Control (0% Apefly)	Treatment 1 (50% Apefly)	Treatment 2 (75% Apefly)	Treatment 3 (100% Apefly	P-value
WBC M/mm3	4.81 ± 0.22	4.54 ± 0.68	4.62 ± 0.18	4.97 ± 0.57	0.474
LYM %	80.8 ± 1.31	82.2 ± 3.77	85.8 ± 7.09	80.4 ± 3.21	0.233
RBC M/mm3	5.3 ± 2.03	5.72 ± 2.64	4.16 ± 0.57	4.37 ± 0.51	0.440
MCV (pg)	32.16 ± 9.49	36.82 ± 11.26	32.66 ± 7.83	41.1 ± 18.59	0.650
MCH (pg)	31 ± 1.67	31.46 ± 2.01	31.26 ± 0.67	30.08 ± 2.38	0.639
MCHC (g/dl)	32.5 ± 0.38	32.62 ± 0.68	33.12 ± 2.35	31.96 ± 1.43	0.652
Hb (g/dl)	14.1 ± 1.58	12.94 ± 1.11	13.7 ± 2.37	13.18 ± 1.38	0.699

Values are expressed as mean ± SEM, WBC=White blood cell, RBC= Red blood cell, MCV= Mean Corpuscular Volume, MCH= Mean corpuscular hemoglobin, MCHC= Mean corpuscular hemoglobin concentration, Hb= Hemoglobin

Body and organ weight changes

The results showed a gradual increase in the bodyweight of mice from day 0 to 28. There was no significant difference ($P > 0.05$) in the means between the control and treatment groups as can be seen in Table 18. Similarly, the organ weights relative to body weights of the mice did not show any significant differences in weight changes of organs such as spleen, kidney and heart between the control and mice treated with the Apefly meal at all doses except for the liver which however did not show any toxicity signs when subjected to histopathological examinations (Table 19 and 20). The average percentage increase of the relative liver weight with reference to the control was 24.61%. Using a flow-chart diagram showing a decision tree for adverse versus non-adverse effects induced by compounds which increase liver weight, the increase in liver weights of mice treated with Apefly meal seem to be non-adverse (Hall *et al.*, 2012).

Table 19: Bodyweight (g) of the control and mice fed on Apefly diet in the sub-acute toxicity test

Weight (g)	Control (0% Apefly meal)	Treatment 1 (50% Apefly meal)	Treatment 2 (75% Apefly meal)	Treatment 3 (100% Apefly meal)	P-value
Day 0	27.12 ± 0.54	27.92 ± 0.62	27.76 ± 0.64	27.92 ± 0.62	0.158
Day14	33.66 ± 0.87	33.32 ± 1.22	33.72 ± 1.80	32.40 ± 1.07	0.369
Day28	42.20 ± 1.38	40.72 ± 2.22	41.68 ± 3.36	38.88 ± 2.36	0.187

Values are an average of five mice fed with the Apefly diet, expressed as mean ± SEM

Table 20: Average organ weight values of control and mice treated with Apefly measured during sub-acute toxicity study

Organ	Control (0% Apefly meal)	Treatment 1 (50% Apefly meal)	Treatment 2 (75% Apefly meal)	Treatment 3 (100% Apefly meal)	P-value
Spleen	0.21 ± 0.01	0.18 ± 0.02	0.18 ± 0.11	0.16 ± 0.03	0.807
Liver	1.96 ± 0.01	1.97 ± 0.01	1.99 ± 0.03	1.97 ± 0.02	0.001
Kidney	0.57 ± 0.01	0.56 ± 0.01	0.57 ± 0.02	0.55 ± 0.01	0.281
Heart	0.17 ± 0.01	0.17 ± 0.01	0.34 ± 0.38	0.17 ± 0.01	0.425

Values are an average of five mice fed with the Apefly diet expressed as mean ± SEM

Table 21: Relative organ weight of control and mice treated with Apefly meal in the sub-acute toxicity tests

Organ	Control (0% Apefly meal)	Treatment 1 (50% Apefly meal)	Treatment 2 (75% Apefly meal)	Treatment 3 (100% Apefly meal)	P-value
Spleen	0.46 ± 0.01	0.44 ± 0.04	0.41 ± 0.2	0.42 ± 0.05	0.859
Liver	3.94 ± 0.11	4.86 ± 0.24	4.79 ± 0.32	5.08 ± 0.27	<0.001
Kidney	1.35 ± 0.03	1.38 ± 0.05	1.36 ± 0.07	1.43 ± 0.07	0.174
Heart	0.41 ± 0.01	0.43 ± 0.01	0.77 ± 0.78	0.43 ± 0.01	0.422

Values are an average of five mice fed with the Apefly diet expressed as mean ±SEM

Biochemical examinations

The results of the kidney and liver function tests revealed no significant differences ($P > 0.05$) for both concentrations in alkaline phosphate, creatinine and liver hepatic enzymes AST and ALT (Table 22). Consumption of the Apefly meal was found to maintain the biochemical parameters within reasonable limits.

Table 22: Biochemical parameters of control and mice treated with Apefly during sub-acute toxicity test

Parameters	Control (0% Apefly meal)	Treatment 1 (50% Apefly meal)	Treatment 2 (75% Apefly meal)	Treatment 3 (100% Apefly meal)	P-value
Ap	64.80 ± 1.30	65.40 ± 2.70	64.8 ± 0.84	64.2 ± 1.10	0.727
Cr	0.88 ± 0.12	0.98 ± 0.24	0.80 ± 0.05	0.86 ± 0.17	0.373
AST	22.84 ± 3.00	18.26 ± 3.04	23.18 ± 11.04	23.00 ± 4.48	0.562
ALT	21.24 ± 2.57	18.68 ± 1.17	19.52 ± 1.53	18.86 ± 1.31	0.120

Values are an average of five mice fed with the Apefly diet expressed as mean ± SEM

Histopathological examinations

Microscopic examination of the main internal organs of animals such as liver, kidney, spleen, and heart also revealed no differences between the control and treated groups of mice even after administration of 100% Apefly meal for 28 days. The photomicrographs of some organs are displayed in Fig. 22 and 23.

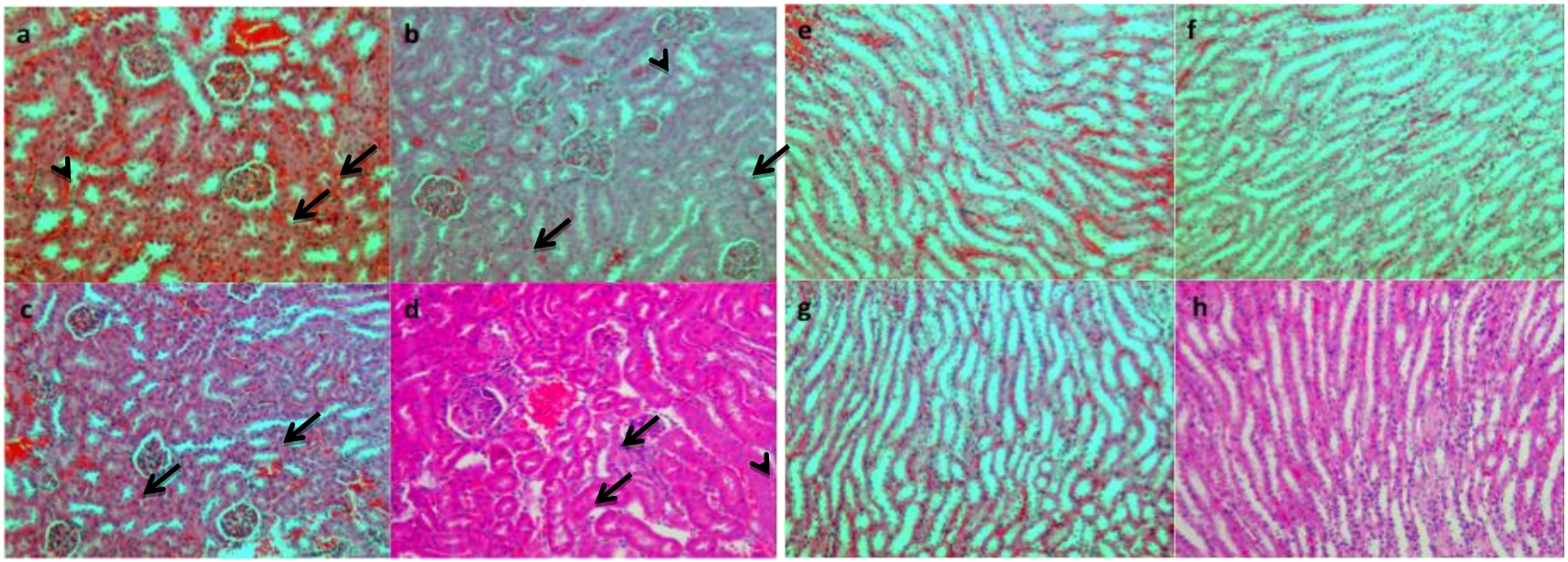

Figure 22: Photomicrographs of the renal cortex

(a) Control, (b) 50% Apefly meal, (c) 75% Apefly meal and (d) 100% Apefly meal) and renal medulla (e) Control, (f) 50% Apefly meal, (g) 75% Apefly meal and (h) 100% Apefly meal. The renal cortex of both controls and treated groups showed normal glomeruli (arrowheads) with mild congestion (arrows). Congestion was also seen in medulla of controls and treated groups (Magnification 10x)

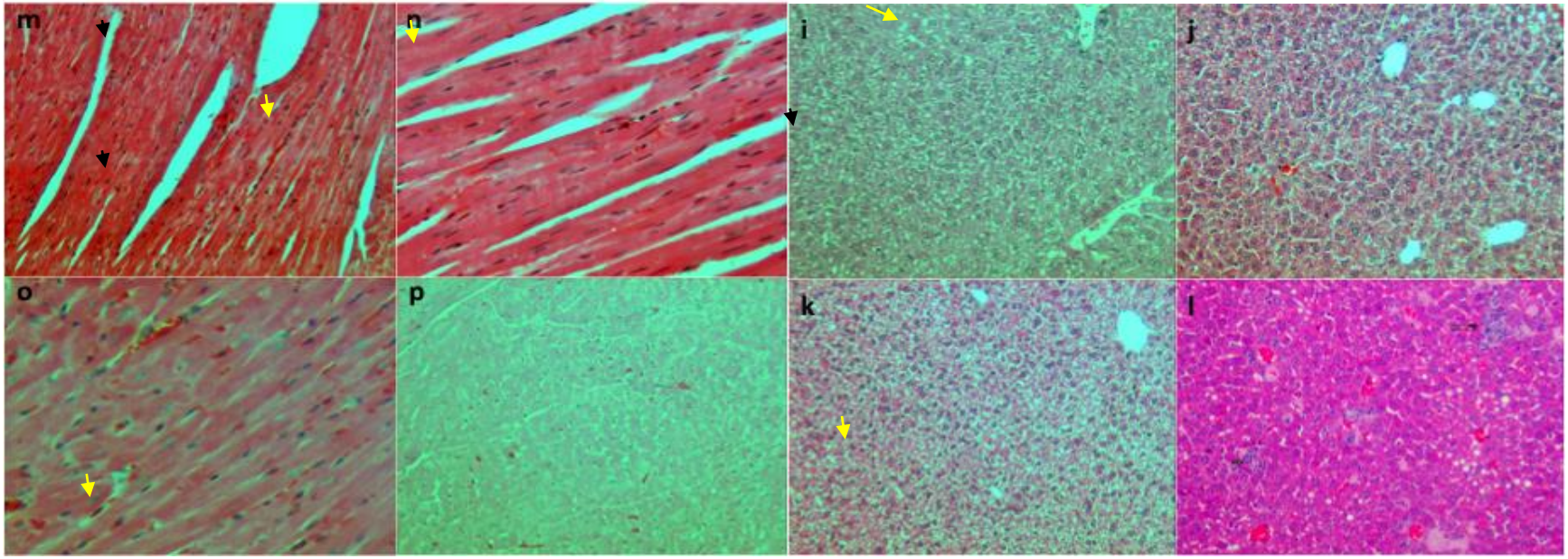

Figure 23: Representative photomicrographs of the liver section

(i = Control, j = 50% Apefly meal, k = 75% Apefly meal and l = 100% Apefly meal) and heart section (m = Control, n = 50% Apefly meal, o = 75% Apefly meal and p = 100% Apefly meal. The liver and cardiac muscles of both controls and treated groups have similar microscopic morphologies that appear to be normal. Distention of sinusoidal and deranged cytoplasm observed in tissue sections of the liver is considered to be artifactual

4.2 Discussions

4.2.1 Occurrence and host range of the *S. lemolea lemolea* in Tanzania

The survey carried out in five regions of Tanzania revealed that *S. lemolea lemolea* is present and is associated with mealybug species that harbour different host plants. This association is similar to *S. epius* in India, as reported by Dinesh and Venkatesha (2014). The availability of *S. lemolea lemolea* was affected by pesticide use, climatic factors and abundance of its prey (mealybugs) as in *S. epius* by Lohman and Sumarita (2009), Dinesh (2010), Dinesh and Venkatesha (2011) and, Saengyot and Burikam (2012). In the surveyed areas, the availability of *S. lemolea lemolea* is greatly influenced by the presence of mealybug (Dinesh & Venkatesha, 2026) which is again determined by availability of most preferred host plants such as *Manihot esculeta* and *Carica papaya* (Mccomie, 2000; Meyerdirk *et al.*, 2004; Krishnan *et al.*, 2016; Macharia *et al.*, 2017). *Splagis lemolea lemolea olea* was only reported in forests and highlands such as Usambara mountains, Uluguru mountains, Nguru mountains and Pugu hills (Kielland, 1990). However, in this study, *S. lemolea lemolea* has been found present in various cultivated crops infested with different mealybugs in the low lands.

Two of the five regions Shinyanga and Iringa exhibited low mealybug infestation which as a result led to unavailability of *S. lemolea lemolea*. Such observation is associated not only with the use of pesticides as observed but also weather conditions particularly temperature and rainfall are reported as important factors for the development of mealybugs (Hemiptera-Pseudococcidae) (Amarasekare *et al.*, 2008). This study revealed that *S. lemolea lemolea* was available during the dry season due to the availability of mealybugs as supported by Tanwar *et al.* (2010) and, Kanagaraj and Kathirvelu (2018). The results of the present study therefore, suggest proper pest management methods, those which deals with the pest without destroying the natural enemies. This calls for the development of a sound integrated pest management strategy that includes other biological agents such as other predators of mealybug and entomopathogenic fungi.

4.2.2 Knowledge, perceptions and reactions towards the Apefly in Tanzania

The results revealed a lack of knowledge of this insect due to inadequate information. This can be attributed to the lack of research and the “uncommonness” of the Apefly, which was reported by a majority of respondents in the field. However, this “uncommonness” was not always the case, since the Apefly samples were collected from 65% of the respondents’

fields, revealing their ignorance of the presence of this insect in their fields. The respondents' attention was centered on the pupal Apefly which has a monkey-face appearance, but none of them showed awareness of the pre-and post-pupal life stages of the Apefly. The respondents identified cassava and papaya as the plant species that harbored *S. lemolea lemolea*. Although the host plants differed slightly in different localities, the common factor for all of them was the mealybug infestation. None of the respondents was aware of the predatory nature of *S. lemolea lemolea* larvae and their potential in pest control.

Most respondents falsely believed that the *S. lemolea lemolea* pupal stage is poisonous. Their primary source of information was the media and fellow farmers in their localities. However, the negative attitude towards the insect had no supportive evidence from the respondents and could only be linked to its strange appearance, as supported by Wagler and Wagler (2012). The spreading information was noted to have significantly impacted the farmers' perceptions and decision-making, creating anxiety, especially in remote areas where vegetables are consumed daily. However, it was observed that, despite the negative attitude towards the Apefly, no aggressive response towards the insect had been reported. For example, about 88.8% of the respondents avoided the consumption of vegetables associated with the Apefly, as supported by Curtis and Mannheimer (2005).

4.2.3 Molecular identification, diversity and phylogenetics of the Apefly in Tanzania

The collected Apefly samples were identified using their 16S rRNA genes. The results of this study confirmed that all the 10 analyzed samples resembled the species *S. lemole lemolea* (99-100%). Studies on the evolutionary divergences among these 10 Apefly sequences showed that there was a close evolutionary relationship among STR 1, 10 and 7 and the rest of the Apefly sequences were more closely related to STR6. Nevertheless, all 10 Apefly sequences originated from the same root, indicating their common ancestry. Confirmation of the species is important as it allows further studies on the known species.

The analysis of inter and intrageneric pairwise distances (p-distances) revealed that the genetic distances between the Apefly species were similar in magnitude. In comparison to related genera and species of butterflies in the NCBI genebank, *S. lemolea lemolea* was closely related to *S. epius*. However, there were several distant relatives such as *Allotinus rivalis rivalis, Feniseca tarquinius, Logarnia marmorata* and several other *Allonitus* species. The monophyletic *Allotinus* and *Logania* clade are reported to have initially been in Africa

but migrated out about 30 million years ago while *S. lemolea lemolea* dispersed back into Africa from Asia about 20 million years ago (Kaliszewska *et al.,* 2015). The closely related *Feniseca* lineage dispersed into North America from Asia around the same time (Zachos *et al.,* 2001).

4.2.4 Morphology, life cycle and developmental stages of the Apefly

The eggs of *S. lemolea lemolea* was creamy in colour, disc-shaped, flat on both sides with depressions on the tops and bottom flattened with a depression on the top showing greater similarity with predatory lycaenid *S. epius* (Dinesh *et al.,* 2010) and *Feniseca tarquinius* (Hall, Minno & Butler, 2007) as well as several other phytophagous lycaenids, *Paralucia pyrodiscus lucida* Crosby (Braby, 1990), *Rapala takasagonis* Matsumura (Hsu *et al.,* 2005), and *Lampides boeticus* L. (Vijayachander & Arivudainambi, 2007).

The larvae underwent four larval instars, pre-pupa and pupa stages to reach the adult stage as reported in carnivorous lycaenid butterflies *F. tarquinius* and *S. epius* by Hall *et al.* (2007) and Dinesh and Venkatesha (2011) respectively and other phytophagous lycaenids like *R. takasagonis* Matsumura (Hsu *et al.,* 2005) and *L. boeticus* L. (Vijayachander & Arivudainambi, 2007). The 1st instar larva of *S. lemolea lemolea* was whitish colour, and mostly stationary while other instars were mobile and covered with white wax which camouflaged them with the mealybugs (Lamborn, 1914), and as reported in other species such as *S. substrigata* (Snell) in the Philippines (Smith, 1914) and *S. epius* (Dinesh *et al.,* 2010; Saengyot & Burikam, 2012 and Venkatesha & Dinesh, 2011), *F. tarquinius* (Hall *et al.,* 2007). The sizes of the four larval instars of *S. lemolea lemolea* are comparatively similar to those reported for other species of lycaenids such as *S. epius* (Dinesh *et al.,* 2010), *P. pyrodiscusblucida* (Braby, 1990) and *Lycaeides Melissa samuelis* Nabokov (Herms *et al*., 1996).

(i) Body lengths and developmental periods of the Apefly

The mean total larval period in *S. lemolea lemolea* was 10 days, whereas in a predatory *S. epius* in India it was 9.4 days (Dinesh *et al.,* 2010) and 11.9 days in phytophagous lycaenid *L. boeticus* (Vijayachander & Arivudainambi, 2007). It is reported that aphytophagous lycaenid larvae spend less time in the larval stage as compared to the phytophagous lycaenids (Banno, 1990; Clark, 1926). The monkey-faced pupa was similar to that of S. *epius* (Dinesh & Venkatesha, 2011; A. Dinesh *et al.,* 2010) and that of *F. tarquinius* (Hall *et al.,* 2007). It is

reported that the monkey-faced appearance of some lycaenid pupae is for self-defense (Balduf, 1939). The male and female butterflies emerged randomly from the pupae of the same age in the laboratory. The external morphology of *S. lemolea lemolea* adult was similar to that of *S. epius* (Dinesh *et al.,* 2010) except for the colour patterns. The intermittent flight and egg-laying patterns demonstrated by *S. lemolea lemolea* have also been reported in *C. xami* (Cordero *et al.,* 2000). The average duration of *S. lemolea lemolea* adult was 9 to 13 days from emergence. The observed pre-mating behaviour included prolonged antenna contact and physical contact whereby the females pushed underneath the males to mate as supported by Myers (1972). Oviposition by the adult females was witnessed near the mealybug colonies and the eggs of *S. lemolea lemolea* were creamy in colour, disk-shaped and both the top and bottom flattened with a depression on top as reported in predatory lycaenid *S. epius* (Dinesh & Venkatesha, 2011), *Feniseca tarquinius* (Hall *et al.,* 2007) as well as several other phytophagous lycaenids, *Paralucia pyrodiscus lucida* Cros by Braby (1990), *Rapala takasagonis* Matsumura (Hsu *et al.,* 2005), and *Lampides boeticus* L. (Vijayachander & Arivudainambi, 2007).

The larvae of the Apefly underwent 4 instars, prepupa and pupa stage to reach the adult stage as reported in carnivorous lycaenid butterflies *F. tarquinius* and *S. epius* (Dinesh *et al.,* 2010; Hall *et al.,* 2007) respectively and other phytophagous lycaenids *R. takasagonis* Matsumura (Hsu *et al.*, 2005) and *L. boeticus* L. (Vijayachander & Arivudainambi, 2007). The sizes of the four larval instars of the Apefly are comparatively similar to those reported in other species of lycaenids such as *S. epius* (Dinesh *et al.,* 2010), *P. pyrodiscusblucida* (Braby, 1990) and *Lycaeides Melissa samuelis* Nabokov (Herms *et al.,* 1996). The first instar larva of *S. lemolea lemolea* was whitish in colour, and mostly stationary while other instars were mobile covered with white waxy which camouflaged them with the mealybugs as supported by Lamborn (1914) and as reported in other species such as *S. substrigata* (Snell) in the Philippines (Smith, 1914) and *S. epius* (Dinesh & Venkatesha, 2011; Dinesh *et al.,* 2010; Saengyot & Burikam, 2012), *F. tarquinius* (Hall *et al.,* 2007).

4.2.5 The predatory potential of the Apefly against the papaya mealybug

The 1st instar mostly fed on the mealybug eggs, while the 2nd instar fed on the eggs and young nymphs. The 3rd and 4th instars fed intensively on all stages. However, all four larva instars of *S. lemolea lemolea* consumed higher numbers of eggs than the nymphs and adults of *P. marginatus* due to the size of eggs, which are very small compared to the nymph and adult

stages of *P. marginatus* as reported in the predation of *Planococcus citri* (Risso) by *S. epius* (Dinesh *et al.,* 2010), *M. hirsutus* by *C. montrouzieri* (Mani, 1995). The daily and instar-wise prey consumption of *P. marginatus* by *S. lemolea lemolea* increased as development progressed as also reported with *S. epius* on *M. hirsutus* (Dinesh & Venkatesha, 2011; Mani, 1995). The consumption of mealybug eggs was higher than other stages as reported by Saengyot and Burikam (2012) for *S. epius,* a phenomenon that can be associated with the inability of the prey to escape from the *S. lemolea lemolea* larvae. On average, a single *S. lemolea lemolea* larva consumed 1982.6 ± 117 eggs, 123 ± 5.8 nymphs and 80 ± 8.5 adults of *P. marginatus* during its entire larval development period, which is high compared with the findings on *S. epius* by Saengyot and Burikam (2012) which shows that the total number of prey consumed during the larval instars by the five larvae were 4115.75 ± 553.28 eggs, 281.25 ± 45.08 nymphs and 77.50 ± 16.52 adults. These preliminary findings suggest that *S. lemolea lemolea* can be an effective biological control agent of *P. marginatus* however, it calls for further study that will use available standards and advanced statistics to reach into conclusion. Literature shows that Apefly larvae feed on mealybug species (Lohman & Samarita, 2009; Dinesh *et al.,* 2010; Venkatesha & Dinesh, 2011; Saengyot & Burikam, 2012 and Dinesh & Venkatesha, 2014).

The first instar mostly fed on eggs of mealybugs, while the second instar fed on the eggs and young nymphs of the mealybug. The third and fourth instars fed intensively in all stages; however, all four larval instars of the Apefly consumed more eggs than nymphs and adults. This could be due to the size of eggs, which are very small compared with the nymph and adult stages of *P. marginatus* as reported in the predation of *Planococcus citri* (Risso) by *S. epius* (Dinesh *et al.,* 2010), *M. hirsutus* by *C. montrouzieri* (Mani *et al.,* 1987). The daily and instar-wise prey consumption of *P. marginatus* by *S. lemolea lemolea* increased as they progressed in development as reported with *S. epius* on *M. hirsutus* (Mani *et al.*, 1987; Venkatesha & Dinesh, 2011). On average, a single Apefly larva consumed 1982.6 ± 117 eggs, 123 ± 5.8 nymphs and 80 ± 8.5 adults of mealybugs during its entire larva development period.

The mean total larval period in *S. lemolea lemolea* was ten days, whereas in a predatory *S. epius* in India it was 9.4 days (Dinesh *et al.,* 2010) and 11.9 days in phytophagous lycaenid *L. boeticus* (Vijayachander & Arivudainambi, 2007). The aphytophagous lycaenid larvae spend less time in the larval stage as compared to phytophagous lycaenids (Banno, 1990; Clark,

1926). The monkey-faced pupa was similar to that of S. *epius* (Dinesh & Venkatesha, 2011 and Dinesh *et al.,* 2010) and that of *F. Tarquinius* (Hall *et al.,* 2007). It is reported that the monkey-faced appearance of some lycaenid pupae is for self-defence (Balduf, 1939). The male and female butterflies emerged randomly from the pupae of the same age in the laboratory. The external morphology of the Apefly adult was similar to that of *S. epius* (Dinesh *et al.,* 2010) except colour patterns. The adults were active between 11:00 to 15.00 h when it was warm with bright sunlight, which are necessary conditions for mating and egg-laying. Similar conditions were reported in a predatory *S. epius* by Dinesh *et al.* (2010). The intermittent flight and egg-laying pattern demonstrated by the Apefly has also been published by Cordero *et al.* (2000).

4.2.6 Toxicity of Apefly meal on mice

Several arthropods are poisonous, and their toxins arouse complex and sometimes fatal manifestations in human beings (Haddad *et al.,* 2015). They produce toxins for defence when touched, pressed or crushed while others inject venom by using a specialized apparatus. Literature shows that insects can acquire bio-chemicals from the food they consume or through contact with insecticides and herbicides (Longley & Sotherton, 1997). For instance, some lepidopterans such as monarch butterflies (*Danaus plesippus*) accumulate certain poisons called cardiac glycosides from their host plants (Schreiner & Nafus, 1997). This study evaluated the *in vivo* effects of the Apefly on mice upon ingestion to determine whether it contains endotoxins assimilated by interacting with their prey i.e. phytotoxins from plants the preys feed on.

Investigations of the weight of the mice on consumption of the Apefly did not indicate any change in body weight as compared to the control mice even at 100% Apefly meal concentration. The relative liver weight of the mice treated with apefly meal, however, increased by an average of 24.61% as compared to the control mice. The result provides evidence that Apefly consumption had no effect on the body weight and therefore as reported by Teo *et al.* (2002) the consumption did not affect the growth of the mice. The increase in liver weight upon exposure to different Apefly meal doses is considered as an indication of biological response (Ajagbonna *et al.,* 1999). Toxicological reports suggest that an increase in relative liver weight up to 50% is usually considered adaptive provided that it is not associated with other signs of liver toxicity (European Society of Toxicological Pathology, 2012). Other studies argue that isolated liver weight increase of up to 20% is only adverse if

it is accompanied by histopathological changes or clinical-chemistry changes (Hall *et al.,* 2012). Similarly, an increase in relative (to body weight) liver weights in rats and mice ≤ 15% without further effects observed at (histo) pathology cannot be considered adverse (Palazzi *et al.,* 2016). In some studies, slight increase in liver weight is recorded in controls and is considered part of normal biological variation. Using a flow-chart diagram showing a decision tree for adverse versus non-adverse effects induced by compounds which increase liver weight, the increase in liver weights of mice treated with Apefly meal in this study seems to be non-adverse since it is not accompanied by any biochemical, hematological or histopathological clinical-chemistry changes.

Blood analysis was done to determine the physiological and pathological status in the haematological system. Parameters such as RBC, WBC, LYM and Hb were screened to investigate if the normal ranges of these parameters were altered from the intake of Apefly meal. Studies have revealed that the normal ranges of these parameters can be altered by the intake of toxic substances (Ajagbonna *et al.,* 1999). The results from this study showed that acute and sub-acute ingestion of the Apefly meal did not cause any change in these haematological parameters for both the control and treated mice. Similarly, ingestion of toxic substances is manifested in the alteration of biochemical parameters that are sensitive indicators of metabolic defects (Reddy *et al.,* 2013). In this study, parameters such as ALP, Creatinine, and liver hepatic enzymes AST and ALT showed no significant deviations from the normal ranges in both control and treatment groups, suggesting that Apefly had no effects on the liver function of the mice. Examination of internal organs such as the liver, lungs, hearts, and kidneys showed no organ abnormalities between normal and treatment groups. Similarly, the organ weights were compared to diagnose whether they were exposed to injuries or infections (Shah *et al.,* 2011). The results showed that the differences in weights of internal organs were not statistically significant in both the control and treated groups of mice. These findings inform the general public about the non-toxic nature of Apefly, bringing the fear of Apefly and economic loss related to it to an end.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study reports the occurrence of *S. lemolea lemolea* and provides detailed information on the biology, predatory activity and people's perception towards *S. lemolea lemolea.* The findings of the present study show that *S. lemolea lemolea* is present in different agro-ecological zones of Tanzania living in close association with diverse species of mealybug. Furthermore, the study reveals that *S. lemolea lemolea* incidences were high where mealybug infestations were high indicating prey-predator relationship, and this is essential information to consider in the design of an IPM strategy for mealybugs. Despite this assurance, the insect `is at risk of exposure to pesticides due tonegative perceptions caused by insufficient knowledge of the insect. The negative perceptions were fueled by the appearance of the pupal and lack of adequate information about the insect from agricultural extension officers.

The preliminary study of predatory activity of *S. lemolea lemolea* has shown that, larva preyed on all stages of *P. marginatus*, demonstrating the ability to reduce the pest population. This information can be utilized to develop *S. lemolea lemolea* as an effective biocontrol agent of mealybugs in Tanzania and Africa. Furthermore, non-adverse nature of the Apefly revealed by this study avails the possibility for conservation and utilization of the potential it holds.

5.2 Recommendations

Information of non-adverse effects of Apefly that has been generated here is hereby provided and recommended in solving the fear that exists against the Apefly identified in Tanzania. Nevertheless, further study is recommended to understand the causes of the increase in liver weight of the mice upon exposure to different Apefly meal doses. Also, further research is recommended on the efficacy of *Spalgis* species identified in Tanzania in suppressing mealybug populations under field conditions while comparing it with available standards by using advanced statistics. There is a need to create an understanding of the biology and ecology of *Spalgis lemolea's* prey to generate more information on its interaction with prey for successful utilization in biological control programs. The community is hereby urged to protect and conserve the identified *Spalgis* species due to its potential role in pest control.

REFERENCES

Ackery, P. R. (1990). Biocontrol potential of African lycaenid butterflies entomophagous on Homopterous. *Journal of African Zoology*, 104, 582–591.

Ahmed, N., Al-Helal, A., Khanon, P., & Bulbul, S. (2011). Control strategies of papaya mealybug Paracoccus marginatus Williams and Willink infesting vegetable crops in Bangladesh. *The Journal of Plant Protection Sciences*, 3, 44–47.

Ajagbonna, O. P., Onifade, K. I., & Suleiman, U. (1999). Hematological and biochemical changes in rats given an extract of Calotropis procera Accessed from http://oer.udusok.edu. ng:8080/xmlui/handle/123456789/312 om 24/5/2020, 1(1), 36–42.

Amarasekare, K. G., Chong, J. H., Epsky, N. D., & Mannion, C. (2008). Effect of temperature on the life history of the mealybug Paracoccus marginatus (Hemiptera: Pseudococcidae). *Journal of Economic Entomology*, 101(6), 1798–1804.

Athumani, M. (2017). *Kidudu mtu ndio hiki*. Retrieved 30 June 2019 from https://www.youtube.com/watch?v=XzUFO-MHn5A

Balduf, W. V. (1939). *The Bionomics of Entomophagous Insect. Part II*. St. Louis: J. S. Swift & Co.

Banno, H. (1990). Plasticity of size and relative fecundity in the aphidophagous lycaenidbutterfly, *Taraka hamada*, 15, 111–113.

Banwo, O. O., & Adamu, R. S. (2003). Insect pest management in African agriculture: Challenges in the current millenium. *Archives of Phytopathology and Plant Protection*, 36(1), 59–68. Retrieved from https://doi.org/10.1080/032354003100010 6723

BenDov, Y. (2008). Scale Net *Paracoccus marginatus*. Retrieved from http://www.sel.barc. usda.gov/catalogs/pseudoco/Paracoccusmarginatus.htm>

Bennett, F., & Greathead, D. (1978). Biological control of the mealybug Phenacoccus manihoti Matile-Ferrero: Prospects and necessity. In *Proceedings cassava protection workshop,* (pp. 181–194). Cali Colombia: CIAT. Retrieved from https://www.cabdirect.org/cabdirect/abstract/19786723005

Braby, M. F. (1990). The life history and biology of Paralucia pyrodiscus lucida Crosby Lepidoptera: Lycaenidae. *Australian Journal of Entomology*, 29(1), 41–50.

Brahmaprakash, G. P., Sahu, P. K., Lavanya, G., Nair, S. S., Gangaraddi, V. K., & Gupta, A. (2017). Microbial Functions of the Rhizosphere. In D. P. Singh, H. B. Singh, & R. Prabha (Eds.), *Plant-Microbe Interactions in Agro-Ecological Perspectives: Volume 1: Fundamental Mechanisms, Methods and Functions* (pp. 177–210). Singapore: Springer Singapore. Retrieved from https://doi.org/10.1007/978-981-10-5813-4_10

Brodeur, J., Abram, P. K., Heimpel, G. E., & Messing, R. H. (2018). Trends in biological control: Public interest, international networking and research direction. *Journal of the Iinternational Organisation for Biological Control*, 63(1), 11–26. Retrieved from https://doi.org/10.1007/s10526-017-9850-8

Brower, L. P., Ryerson, W. N., Coppinger, L. L., & Glazier, S. C. (1968). Ecological Chemistry and the Palatability Spectrum. *Science*, 161(3848), 1349-1350.

Browning, H. W. (1992). Overview of biological control of homopterous pests in the Caribbean. *Florida Entomologists*, 75, 440–446.

Cham, D., Davis, H., Obeng-Ofori, D., & Owu, E. (2011). Host range of the newly invasive mealybug species ER7890 Williams and Granara De Willink (Hemiptera: Pseudococcidae) in two ecological zones of Ghana. *Research in Zoology*, 8, 11–17.

Charles, J. G. (1996). Mealybug resistance management strategy. In G. W. Bourdot & D. M. Suckling (Eds.), *Pesticide Resistance: Prevention & Management* (pp. 172–176). Lincoln, New Zealand: New Zealand Plant Protection Society. Retrieved 26 November 2019 from www.resistance.nzpps.org/index.php?p=insecticides/mealybug

Chatterjee, M., & Halder, J. (2017). Occurrence of Papaya mealybug, Paracoccus marginatus as an emerging pest of Hibiscus mutabilis and its predator from Terai region of West Bengal. *Pest Management in Horticultural Ecosystems*, 23(1), 37–40.

Chellappan, K., Dinesh, G. S., Batumalai, S., Candasamy, M., Krishnappa, P., Dua, K., … Gupta, G. (2016). The Protective Action of the Aqueous Extract of Auricularia polytricha in Paracetamol Induced Hepatotoxicity in Rats. *Recent Patents on Drug Delivery and Formulation*, 10(1), 72–76.

Choke, S. (2017). *www geita info mboga*. Retrieved 9 June 2019 from https://www.youtube.com/watch?v=OCfDbRAR678

Clark, A. H. (1926). *Carnivorous butterflies* (Annual Reports No. 2856) (pp. 439–508). Retrieved from Washington DC, USA: Smithsonian Institute.

Clench, H. (1965). Variation and distribution of *Hemiargus huntingto*ni (Lepidoptera: Lycaenidae). *Journal of the New York Entomological Society*, 73 (1) 41–45.

Colmenarez, Y. C., Corniani, N., Jahnke, S. M., Sampaio, M. V., & Vásquez, C. (2020). Use of parasitoids as a biocontrol agent in the neotropical region: Challenges and potential. *Horticultural crops [recurso eletrônico]. London: IntechOpen, 2020. Cap.*9, 171-193.

Cordero, C., Marcias, R., & Jimenez, G. (2000). The number of copulations of territorial males of the butterfly Callophrys xami (Lycaenidae), *Journal of Research on the Lepidoptera*, 35, 78–89.

Cottrell, C. (1984). Aphytophagy in butterflies: Its relationship to myrmecophily. *Zoological Journal of the Linnean Society*, 80 (1), 1–57.

Culling, C. (1974). *Handbook of Histopathological and Histochemical techniques (including museum techniques)* (3rd ed.). London: Butterworth and Co. (Publishers) Ltd.

Curtis, B. A., Mannheimer, C. A., & Loutit, B. (2005). *Tree Atlas of Namibia*. Windhoek: National Botanical Research Institute, Ministry of Agriculture, Water and Forestry. 356-357

Day, J. F., Edman, J. D., Kunz, S. E., & Wikel, S. K. (2000). Direct Injury: Phobias, Psychoses, Annoyance, Allergies, Toxins Bruce F. EldridgeJohn D. Edman. In B. F. Eldrige & J. D. Edman (Eds.), *Medical Entomology: A Textbook on Public Health and Veterinary Problems Caused by Arthropods* (pp. 99–149). Dordrecht: Kluwer Academic Publishers. Retrieved from https://books.google.co.tz/books?id=61vuCAAAQBAJ&lpg=PA99&ots=AbI8rJnGtZ&dq=Day%20et%20al.%2C%202000%20%2Bentomophobia&lr&pg=PA99#v=onepage&q&f=false

Dinesh, A., & Venkatesha, M. (2011). Prey consumption by the mealybug predator *Spalgis* epius on pink hibiscus mealybug (Maconellicoccus hirsutus). *Phytoparasitica*, 39, 11–17.

Dinesh, A., & Venkatesha, M. (2016). Population dynamics of *Spalgis* epius (Lepidoptera: Lycaenidae) a candidate biocontrol agent of mealybugs and its interaction with mealybug-attendant ants. *Biocotnrol Science and Technology*, 26, 337–350.

Dinesh, A., & Venkatesha, M. G. (2014). Inter-and intraspecific interactions in twomealybug predators *Spalgis* epius and Cryptolaemus montrouzieri in the presence and absence of prey. *Bulletin of Entomological Research*, 104(1), 48–55.

Dinesh, A., Venkatesha, M. G., & Ramakrishna, S. (2010). Development, life history characteristics and behaviour of mealybug predator, *Spalgis* epius (Westwood) (Lepidoptera: Lycaenidae) on Planococcus citri (Risso) (Homoptera: Pseudococcidae). *Journal of Pest Science*, 83, 339–345.

Drews, C. (2002). Attitudes, knowledge and wild animals as pets in Costa Rica. *Anthrozoös*, 15(2), 119–138.

Druce, H. (1875). A List of the Collection of Diurnal Lepidoptera Made by Mr. J. J. Monteiro, in Angola, with Descriptions of Some New Species. *Proceedings of the Zoological Society of London*, 406–417.

Fabres, G. (1981). *Entomophagous insects associated with the cassava mealybug in the Peoplés Republic of Congo*. Presented at the First Triennual Root CRops Symbosim of the International Society for Tropicla Root CRops-Africa Branch, Ibadan, Nigeria. Retrieved from https://idl-bnc-idrc.dspacedirect.org/bitstream/handle/10625/17334/IDL-17334.pdf?sequence=1

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39 783–791. *Evolution*, 39, 783–791.

Franco, J., Zada, A., & Mendel, Z. (2009). Novel approaches for the management of mealybug pests. In I. Ishaaya & R. Horowitz (Eds), *Biological Control of Arthropod Pests* (pp. 233–278). Dordrecht: Springer. Retrieved from https://link.springer.com/chapter/10.1007/978-90-481-2316-2_10

Gautam, M. K., & Goel, R. K. (2014). Toxicological Study of Ocimum sanctum Linn Leaves: Hematological, Biochemical, and Histopathological Studies. *Journal of Toxicology*, 2014, 1 - 9.

Gavkare, O. M., K. A. R., & Sharma, P. L. (2014). New record of Nesidiocoris tenuis (Reuter)(Hemiptera: Miridae) associated with Bemisia tabaci Gennadius (Hemiptera: Aleyrodidae) on tomato from Maharashtra, India. *Journal of Biologial Control*, 28(2), 53–54.

Getanjaly, V. L. R., Sharma, P., & Kushwaha, P. (2015). Beneficial insects and their value to agriculture. *Research Journal of Agriculture and Forestry Sciences*, 3, 25–30.

Glendinning, J. I., & Brower, L. P. (1990). Feeding and breeding responses of five mice species to overwintering aggregations of the Monarch butterfly. *Journal of Animal Ecology*, 59(3), 1091–1112.

Goergen, G., Ajuonu, O., Kyofa, M., Umeh, V., Bokonon, A., Tamo, M., & Neunschwander, P. (2014). Classical biological control of papaya mealybug in West Africa. *Biocontrol News and Information*, 35(1), 5–6.

Gredler, G. (2001). *Encouraging beneficial insects in your garden* (Technical Report No. PNW 550) (pp. 1–4). Retrieved from Covallis, Oregon: Oregon State University Extension Service: https://ir.library.oregonstate.edu/concern/administrative_report_or_publications/6108vb585

Gurung, A. B. (2003). Insects–a mistake in God's creation? Tharu farmers' perception and knowledge of insects: A case study of Gobardiha Village Development Committee, Dang-Deukhuri, Nepal. *Agriculture and Human Values*, 20(4), 337–370.

Haddad, J. V., Amorim, P. C. H. D., Junior, H., Teixera, W., & Cardoso, J. L. C. (2015). Venomous and poisonous arthropods: Identification, clinical manifestations of envenomation, and treatments used in human injuries. *Revistda Sociedade Brasileira de Medicina Tropical*, 6, 650–657.

Hall, D. W., Minno, M., & Butler, J. F. (2007). *Harvester butterfly, Feniseca tarquinius (Fabricius) (Insecta: Lepidoptera: Lycaenidae: Melitinae)* (IFAS extension No. EENY-04). Retrieved 10 September 2019 from University of Florida: http://creatures.ifas.ufl.edu

Hall, A. P., Elcombe, C. R., Foster, J. R., Harada, T., Kaufmann, W., Knippel, A., & Nolte, T. (2012). Liver hypertrophy: A review of adaptive (adverse and non-adverse) changes conclusions from the 3rd International ESTP Expert Workshop. *Toxicologic Pathology*, *40*(7), 971-994.

Herms, C. P., Cullough, D. G., Miller, D. L., Bauer, L. S., & Haack, R. A. (1996). Laboratory rearing of Lycaeides melissa samuelis (Lepidoptera: Lycaenidae), an endangered butterfly in Michigan. *The Great Lakes Entomologist,* 29 (2), 63–75.

Herren, H. R., & Neuenschwander, P. (1991). Biological control of cassava pests in Africa. *Annual Review of Entomology*, 36(1), 257–283. Retrieved from https://doi.org/10.1146/annurev.en.36.010191.001353

Hintenou, M. (2015). *Host plant Suitability of the papaya mealybug Paracoccus Marginatus Williams and Granara De Willink (Hemiptera: Pseudococcidae) and the host specificity of its natural enemy acerophagus papayae noyes (Hymenoptera: Encyrtidae) introduced into Africa* (Dissertation). University of Ghana. Retrieved from http://197.255.68.203/handle/123456789/8664

Hinton, H. E. (1974). Lycaenid pupae that mimic anthropoid heads. *Jounral of Entomology*, 49, 65–69.

Horrigan, L., Lawrence, R., & Walker, P. (2002). How sustainable agriculture can address the environmental and human health harms of industrial agriculture. *Environmental Health Perspectives*, 110 (5), 445 - 456.

Hsu, Y., Wang, L., Huang, H., & Lu, C. (2005). Notes on immature biology and distribution of Rapala takasagonis Matsumura (Lepidoptera: Lycaenidae: Theclinae). *Bioformosa*, 40, 53–57.

ICAR-National Bureu of Agricultural Insect Resources. (2013). Fact sheets on Agriculturally Important Insects. Retrieved 14 February 2019 from https//www.nbair.res.in/ Featured_insects/index.php

IITA. (2015). Fast spreading and highly destructive pest reach Tanzania. Retrieved from https://search.proquest.com/openview/3a0a9c5cfa1381016cfb5356df742c92/1?pq-origsite=gscholarandcbl=25518

Inayoshi, Y. (2019). A check list of butterflies in Indo-China. Retrieved 12 February 2019, from http://yutaka.it-n.jp/

Intergrated Development of Artisanal Fisheries (IDAF). (1994). *Program for integrated development of artisanal fisheries in West-Africa* (Technical Report No. 60). Retrieved from ftp://ftp.fao.org/docrep/fao/006/AD342E/AD342E00.pdf

Iqbal, M. (2016). Vicia faba bioassay for environmental toxicity monitoring: A review. *Chemosphere*, 144, 785–802.

James, B. D., & Fofanah, M. (1992). Population growth patterns for Phenacoccus manihoti Mat-Ferr on cassava in Sierra Leone. *Tropical Pest Management*, 38(1), 89–92.

Jothi, B., Rahman, V., & Govindan, K. (2014). Seasonal occurrence and predatory efficiency of *Spalgis epius* (Westwood) on Mealy Bug Paracoccus marginatus (Williams and Granara De Willink) infesting Cotton. *Madras Agricultural Journal*, 10, 1142–1145.

Kaliszewska, Z. A., Lohman, D. J., Sommer, K., Adelson, G., Rand, D. B., Mathew, J., … Pierce, N. E. (2015). When caterpillars attack: Biogeography and life history evolution of the Miletinae (Lepidoptera: Lycaenidae). *Evolution*, 69(3), 571–588.

Kanagaraj, B., & Kathirvelu, C. (2018). The Bionomics of predatory butterfly, apefly (*Spalgis* epius) (Lepidoptera: Lycaenidae) on mealybug, Paracoccus marginatus (Hemiptera: Pseudococcidae) in cocoa. *Journal of Applied and Natural Science*, 10(4), 1243–1247.

Khan, I. (2013). Influence of intrinsic and extrinsic rewards on employee performance: The Banking Sector of Pakistan Ismail Khan1, Muhammad Shahid2, Samina Nawab, Syed Sikander Wali. *Academic Research International*, 4(1), 282-283.

Kielland, J. (1990). *Butterflies of Tanzania*. Retrieved from www.metamorphosis.org.za/ articlesPDF/1157/346 Genus *Spalgis* Moore.pdf

Koehler, H. H. (1999). Predatory mites (Gamasina, Mesostigmata). In M. G. Paoletti (Ed.), *Invertebrate Biodiversity as Bioindicators of Sustainable Landscapes* (pp. 395–410). Amsterdam: Elsevier. Retrieved from https://doi.org/10.1016/B978-0-444-50019-9. 50022-4

Krishnan, J., Meera, G., Ajesh, G., Jithine, J., Lekshmi, N., & Deepasree, M. (2016). A review on Paracoccus marginatus Williams papaya mealy bug (Hemiptera: pseudococcidae). *Journal of Entomolgy and Zoological Studies*, 4, 528–533.

Kristen, N., Scoble, M., & Karsholt, O. (2007). Lepidoptera phylogeny and systematics: The state of inventorying moth and butterfly diversity. *Zootaxa*, 1, 699–747.

Kumar, C. (2013). Status of the genus *Spalgis* moore with taxonomic notes on the type species, *Spalgis* epeus (Westwood) in the Indian Himalaya. *Halteres*, 4, 53–58.

Kumar, P. K. V., Vasudev, V., Seetharama, H. G., Irulandi, S., & Sreedharan, K. (2006). Biology and biometry of the Lycaenid predator *Spalgis* epius. *Journal of Coffee Research*, 34, 72–104.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, 35, 1547–1549.

Lamborn, W. A. (1914). On the relationship between certain West African insects, especially ants, Lycaenidae and homoptera, 61, 438–524.

Larsen, T. (2005). *Butterflies of West Africa: Text volume*. Stensrup Denmark: Apollo Books. Retrieved from https://www.cabdirect.org/cabdirect/abstract/20063028767

Larsen, T. B. (1991). *The butterflies of Kenya and their natural history*. Oxford: Oxford University Press.

Leuschner, K., & Nwanze, K. (1977). Preliminary observations of the mealybug (Hemiptera: Pseudococcidae) in Zaire. In T. Brekelbaum, A. Bellotti, & J. C. Lozano (Eds.), *Proceedings of the Cassava Protection Workshop* (pp. 195–198). Cali Colombia: Centro Internacional de Agricultura Tropical (CIAT).

Lohman, D. J., & Samarita, V. U. (2009). The biology of carnivorous butterfly larvae (Lepidoptera: Lycaenidae: Miletinae: Miletini) and their ant-tended hemipteran prey in Thailand and the Philippines. *Journal of Natural History*, 43, 569–581.

Longley, M., & Sotherton, N. W. (1997). Factors determining the effects of pesticides upon butterflies inhabiting arable farmland. *Agriculture, Ecosystems and Environment*, 61(1), 1–12.

Macharia, I., Kimani, E., Koome, F., Kosiom, T., Heya, H., Otipa, M., & Oronje, M. (2017). First report and distribution of the Papaya mealybug *Paracoccus marginatus* in Kenya. *Journal of Agricultural and Urban Entomology*, 33, 142–150.

Mani, M. (1995). Studies on the natural enemies of oriental mealybug, Planococcus lilacinus (CKll.) (Homoptera: Pseudococcidae) in India. *Journal of Entomology Research*, 19, 61–70.

Mani, M., Shivaraju, C., & Shylesha, A. (2012). Paracoccus marginatus an invasive mealybug of papaya and its biological control-an overview. *Journal of Biological Control*, 26, 201–216.

Mani, M., Thontadaya, T. S., & Sing, S. P. (1987). Record of natural enemies on the grape mealybug, Maconellicoccus hirsutus (Green). *Current Science*, 56, 624–625.

Mccomie, L. (2000). *Progress Report on the Papaya mealybug (Paracoccus marginatus) project in St. Kitts and Nevis*. Presented at the Procicaribe Cipmnet Meeting. Retrieved from https://www.cabi.org/isc/FullTextPDF/2016/20163311786.pdf

Meyerdirk, D., & Kauffman, W. (2001). Status on the development of a biological control program for Paracoccus marginatus Williams papaya mealybug in the Caribbean. Presented at the IV International Scientific Seminar of Plant Health, Veradero Cuba. Retrieved from http://www.isbca-2017.org/download/ISBCA-Proceedings.web.pdf#page=330

Meyerdirk, D., Muniappan, R., Warkentin, R., Bamba, J., & Reddy, G . (2004). Biological control of the papaya mealybug Paracoccus marginatus (Hemiptera: Pseudococcidae) in Guam. *Plant Protection Quartely*, 19, 110–114.

Miller, D. L., Williams, D., & Hamon, A. (1999). Notes on a new mealybug (Hemiptera: Coccoidea: Pseudococcidae) pest in Florida and the Caribbean: the papaya mealybug Paracoccus marginatus Williams and Granara de Willink. *Insecta Mundi*, 336. Retrieved from https://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1335&context=insectamundi

Miller, D., & Miller, G. (2002). Redescription of Paracoccus marginatus Williams and Granara de Willink (Hemiptera: Coccoidea: Pseudococcidae) including descriptions of the immature stages and adult male. *Proceedings of the Entomological Society of Washington*, 104, 1–23.

Mukherjee, P., Roychowdhury, R., & Roy, M. (2017). Phytoremediation potential of rhizobacterial isolates from Kans grass (Saccharum spontaneum) of fly ash ponds. *Clean Technologies and Environmental Policy*, 19(5), 1373–1385.

Muniappan, R., Shepard, B., Watson, G., Carner, G., Rauf, A., Sartiami, D., … Rahman, A. (2009). New records of invasive insects (Hemiptera: Sternorrhyncha) in Southeast Asia and West Africa. *Jounral of Agricultural and Urban Entomology*, 26, 167–174.

Myers, J. (1972). Pheromones and courtship behavior in butterflies. *American Zoologist*, 12(3), 545–551.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonesca, G. A. B., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, 403, 853–858.

Nébié, K., Nacro, S., Ouédraogo, I., Dakouo, D., & Otoidobiga, L. (2016). Inventory and distribution of mango mealybugs species in western Burkina Faso: relative abundance and population fluctuation. *Advances in Entomology*, 4(3), 1 - 9.

Nei, M., & Kumar, S. (2000). *Molecular Evolution and Phylogenetics*. New York: Oxford University Press.

NoYes, J., & Schauff, M. (2003). New Encyrtidae (Hymenoptera) from papaya mealybug (Paracoccus marginatus Williams and Granara De Willink) (Hemiptera: Sternorrhyncha: Pseudococcidae). *Proceedings of the Entomological Society of Washington*, 105, 180–185.

Olson, H. G., Betton, D., Robinson, D., Thomas, K., Monro, A., Kolaja, G., Heller, A. (2000). Concordance of the toxicity of pharmaceuticals in humans and animals, 32(1), 56–67.

Oranization for Economic Cooperation and Development (OECD). (2008). *Acute oral toxicity: Up-and-Down procedure* (Guidelines for testing of chemicals. Health effects No. 425) (pp. 1–27). Retrieved from https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecdtg425.pdf

Padhye, A., Shekle, S., & Dahanukar, N. (2012). Distribution and composition of butterfly species along the latitudinal and habitat gradients of the Western Ghats of India. *Check List*, 8, 1197–1215.

Palazzi, X., Burkhardt, J. E., Caplain, H., Dellarco, V., Fant, P., Foster, J. R., ... Harleman, J. (2016). Characterizing "adversity" of pathology findings in nonclinical toxicity studies: Results from the 4th ESTP international expert workshop. *Toxicologicpathology*, 44(6), 810-824.

Parsons, M. (1999). *The Butterflies of Papua New Guinea: Their Systematics and Biology*. London: Academic Press. Retrieved from https://lny9imonf06.storage.googleapis.com/MDEyNTQ1NTU1MA==06.pdf

Pennington, K., Kroon, D., & Dickson, C. G. C. (1978). *Pennington's butterflies of Southern Africa* (1st ed.). Johannesburg: Ad. Donker. Retrieved 26 November 2019 from http://agris.fao.org/agris-search/search.do? Record ID=US201300562595

Peterson, T. (2014). *Spalgis* lemolea (African Apefly or Lemolea Harvester). Retrieved 14 February 2020, from http://www.tanzaniabirds.net/butterfly/*Spalgis*_lemolea/sl.htm

Pickett, C. H., & Bugg, R. L. (1998). *Enhancing biological control: habitat management to promote natural enemies of agricultural pests*. Berkeley Los Angeles London: University of California Press. Retrieved from https://books.google.co.tz/books?id=fPi6eHMqdgMC&lpg=PA1&ots=lQcQ5RSv7v&dq=Bugg%20and%20Pickett%2C%201998%2B%20natural%20enemies&lr&pg=PA1#v=onepage&q=Bugg%20and%20Pickett,%201998+%20natural%20enemies&f=false

Pierce, N., Braby, M., Heath, A., Lohman, D., Rand, D., & Travassos, M. (2002). The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). *Annual Review of Entemology*, 47, 733–771.

Pierce, N. E. (1995). Predatory and parasitic Lepidoptera: carnivores living on plants, Journal of the Lepidopterists' Society, 49 (4), 412–453.

Prabakaran, S., Evangelin, G., & William, S. (2014). Diversity of butterflies (Lepidoptera: rhopalocera) in Tiruvallur district Tamil Nadu India. *Biolife*, 2(3), 769–778.

Rasheed, M., Bushra, S., & Tariq, M. (2014). Use and impact of insecticides in mealybug control. *International Journal of Advances in Biology*, 1, 1–11.

Raza, M., Al-Shabanah, O. A., El-Hadiyah, T. M., & Al Majeed, A. A. (2002). Effect of prolonged vigabatrin treatment on hematological and biochemical parameters in plasma, liver and kidney of Swiss albino mice. *Scientia Pharmaceutica*, 70 (2), 135–145.

Reddy, R. R., Lokanatha, O., Ratnam, K., Reddy, C. S., Raju, I. N., & Reddy, C. D. (2013). Acute and sub-acute toxicity of Moringa oleifera stem bark extract in Swiss albino mice. Reddy, Y. R. R., Lokanatha, O., Ratnam, K. S. V. P., Reddy, C. S., Raju, I. N., & Reddy, C. D. (2013). Acute and sub-acute toxicity of Moringa oleifera stem bark extract in Swiss albino mice. *International Journal of Life Sciences Biotechnology and Pharma Research*, *2* (4), 74–82.

Rich, M. S. (2008). *Invasive papaya pest discovered by Virginia Tech team in Asia*. Retrieved from Blacksburg: https://vtechworks.lib.vt.edu/bitstream/handle/10919/61411/2008-609.html?sequence=1

Robbins, R. K., & Opler, P. A. (1996). Butterfly diversity and a preliminary comparison with Bird and Mammal diversity. In E. O. Wilson, D. E. Wilson, & M. L. Reaka-Kudha (Eds.), *Biodiversity II: Understanding and Protecting our Biological Resources* (pp. 69–82). Washington DC: Joseph Henry Press.

Saengyot, S., & Burikam, I. (2012). Bionomics of the apefly *Spalgis epius* (Lepidoptera: Lycaenidae) predatory on the papaya mealybug Paracoccus marginatus (Hemiptera: Pseudococcidae) in Thailand. *Songklanakarin Journal of Science and Technology*, 34 (17), 1- 9.

Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution,* 4(4), 406–425.

Sakthivel, N., Kumaresan, P., Qadri, S., Ravikumar, J., & Balakrishna, R. (2012). Adoption of integrated pest management practices in sericulture-A case study in Tamil Nadu. *Journal of Biopesticides*, 5, 212-214.

Sanda, N. B., & Sunusi, M. (2014). Fundamentals of biological control of pests. The International Center for Chemical and Biological Sciences (ICCBS). *Review Paper*, 1(6), 1–11.

Santos, G. M. D., & Antonini, Y. (2008). The traditional knowledge on stingless bees (Apidae: Meliponina) used by the Enawene-Nawe tribe in western Brazil. *Journal of Ethnobiology and Ethnomedicine*, 4(1), 4–19. Retrieved from https://doi.org/10.1186/1746-4269-4-19

Savela, M. (2014). Lepidoptera Index. Retrieved 15 February 2016, from nic.funet.fi/pub/sci/bio/life/insecta/lepidoptera

Schneider, S., & Lapolla, J. (2011). Systematics of the mealybug tribe Xenococcini (Hemiptera: Coccoidea: Pseudococcidae) with a discussion of trophobiotic associations with Acropyga Roger ants. *Systematic Entomology*, 36, 57–82.

Schreiner, I. H., & Nafus, M. D. (1997). *Butterflies of Micronesia. Mangilao* (pp. 1–40). Retrieved from Agriculture Experiment Station, College of Agriculture and Life Sciences, University of Guam: https://cnas-re.uog.edu/wp-content/uploads/2016/03/ButterfliesOfMicronesia.pdf

Scott, J. (1974). Population biology and adult behavior of lycaena arota (Lepidoptera), Journal of the Lepidopterists' Society, 28, 64–75.

Shah, R., Parmar, S., Bhatt, P., & Chandra, S. (2011). Evaluation of hepatoprotective activity of ethyl acetate fraction of Tephrosia purpurea. *Pharmacology*, 3, 188–194.

Shajla, P., Vijayalakshmi, C., & Tintumol, K. (2014). Occurrence of the natural enemies of mealybugs in a small coffee farm. *The Journal of Zoology Studies*, 1, 26–28.

Sharma, A., & Pati, P. (2013). First record of Ashwagandha as a new host to the invasive mealybug (Phenacoccus solenopsis Tinsley) in India. *Entomological News*, 123(1), 59–62.

Sikkim, K. (2004). Protective devices of the carnivorous butterfly *Spalgis* epius (Westwood) (Lepidoptera: Lycaenidae). *Current Science*, 87 (5), 571-572.

Singh, S. P. (1994). *Technology for production of natural enemies* (Technical Bulletin No. 4) (p. 594). Bangalore: Project Directorate of Biological Control.

Stenberg, E. D., Lefevre, T., Li, J., Castillejo, C. L. F., Li, H., Hunter, M. D., & Roode, J. C. (2012). Food plant derived disease tolerance and resistance in a natural butterfly-plant-parasite interactions. Evolution. *International Journal of Organic Evolution*, 66(11), 3367–3376.

Suresh, S., Jothimani, R., Sivasurmanian, P., Karuppuchamy, P., Samiyappan, R., & Jonathan, E. (2010). Invasive mealybugs of Tamil Nadu and their management. *Agricultural Sciences*, 23, 6–9.

Swanson, J. L., Tokar, D. M., & Davis, L. E. (1994). Content and Construct Validity of the White Racial Identity Attitude Scale. *Journal of Vocational Behavior*, 44(2), 198–217.

Tanga, M. (2013). *Bio-ecology of the mango mealybug Rastrococcus iceryoides green (Hemiptera: Pseudococcidae) and its associated natural enemies in Kenya and Tanzania*. University of Pretoria, South Africa. Retrieved from http://hdl.handle.net/ 2263/24293

Tanwar, R. K., Jeyakumar, P., & Vennila, S. (2010). *Papaya mealybug and its management strategies* (Technical) (p. 26). Retrieved from New Delhi: National Centre for Integrated Pest Management: http://www.ncipm.res.in/ NCIPMPDFs/Publication/ Swarming_caterpillar.pdf

Teo, S. D., Stirling, S., Thomas, A., Kiorpes, A., & Vikram, K. (2002). A 90-day oral gavage toxicity study of D-methylphenidate and D, L methylphenidate in Sprague-dawley rats. *Toxicology*, 179(3), 183–196.

Thangamalar, A., Subramanian, S., & Mahalingam, C. (2010). Bionomics of papaya mealybug Paracoccus marginatus and its predator *Spalgis* epius in mulberry ecosystem. *Agricultural Sciences*, 23, 39–41.

Vaghasiya, Y. K., Shukla, V. J., & Chanda, S. V. (2011). Acute oral toxicity study of pluchea arguta boiss extract in mice. *Journal of Pharmacology and Toxicology*, 6, 113–123.

Venkatesan, T., Jalali, S., Ramya, S., & Prathibha, M. (2016). Insecticide resistance and its management in mealybugs. In *Mealybugs and their Mamagement in Agricultural and Horticultural Crops* (pp. 223–229). New Delhi: Springer. Retrieved from https://link.springer.com/chapter/10.1007/978-81-322-2677-2_17

Venkatesha, M. G., & Dinesh, A. (2011). Mass rearing of *Spalgis* epius (Lepidoptera: Lycaenidae), a potential predator of mealybugs (Hemiptera: Pseudococcidae). *Biocontrol Science and Technology*, 21, 929–940.

Vijayachander, A., & Arivudainambi, S. (2007). Biology of pulses blue butterfly, Lampides boeticus Linn. *Annals of Plant Protection Science*, 15, 53–56.

Wagler, R., & Wagler, A. (2012). External insect morphology: A negative factor in attitudes toward insects and likelihood of incorporation in future science education settings. *International Journal of Environmental and Science Education*, 7, 313–325.

Walker, A., Hoy, M., & Meyerdirk, D. (2006). *Papaya mealybug (Paracoccus marginatus Williams and Granara de Willink (Insecta: Hemiptera: Pseudococcidae)* (Entomology and Nematology Department Florida Cooperative Extension Service No. EENY-302). Retrieved from Gainesville, Florida: Institute of Food and Agricultural Sciences University of Florida: http://citeseerx.ist.psu.edu/viewdoc/ download?doi=10.1.1.608.9728&rep=rep1&type=pdf

Williams, M. (2008). Assessing diversity of diurnal Lepidoptera in habitat fragments: testing the efficiency of strip transects. *Environmental Entomology*, 37, 1313–1322.

Williams, M. (2015). Classification of the Afro-tropical butterflies to generic level. *Metamorphosis*, 26, 102–108.

Wood, S., & Tsui, V. (2008). *Advocacy, communication and social mobilization for TB control: A guide to developing knowledge, attitude and practice surveys. WHO Library. http://www.stoptb.org/resource_center/assets/documents/ ACSM_KAP%20 GUIDE. pdf* (WHO).

Wynhoff, I. (1998). Lessons from the reintroduction of Maculinea teleius and M. nausithous in the Netherlands. *Insect Conservation*, 2, 47–57.

Zhang, Z., Schwartz, S., Wagner, L., & Miller, W. (2000). A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology*, 7(1–2), 203–214.

APPENDICES

Appendix 1: The consensus sequences of the studied 10 *S. lemolea lemolea* strains

Splagis lemolea lemolea STR1

CGGCGTGCACTTCTCTCTTAGTAATGCATCGCGACCCGTTCGGGGTCGGTCTAAG
CGCCGTCCGGGAGTCCGACGGTGGACGCTTCACGGCGTCACGTCGTCGAACCGT
GACCGGTTGCCGGCCGGCCGTTGCGGACGGTAACAATGATAGAAATCGAATACG
CTTCGAAGCGAACCGCGCACGCGTTCAACGCGTCCGGCCCGACGCAAGCTGACC
GTCGGTGTCGATTTCCTGCCTATGTGCGGACGTCGGCTCGACGTCGCTGTCGTCT
AGCTGCCGTGCAGTCTCGGACTGTGCGCGTCTCTGTCTGCGATGATTCTGTTTCGG
GC

Splagis lemolea lemolea STR2

CGGCGTGCACTTCTCTCTTAGTAATGCATCGCGACCCGTTCGGGGTCGGTCTAAG
CGCCGTCCGGGAGTCCGACGGTGGACGCTTCACGGCGTCACGTCGTCGAACCGT
GACCGGTTGCCGGCCGGCCGTTGCGGACGGTAACAATGATAGAAATCGAATACG
CTTCGAAGCGAACCGCGCACGCGTTCAACGCGTCCGGCCCGACGCAAGCTGACC
GTCGGTGTCGATTTCCTGCCTATGTGCGGACGTCGGCTCGACGTCGCTGTCGTCT
AGCTGCCGTGCAGTCTCGGACTGTGCGCGTCTCTGTCTGCGATGATTCGGTTTCG
GG

Splagis lemolea lemolea STR3

CGGCGTGCACTCTCTCTTAGTAATGCATCGCGACCCGTTCGGGGTCGGTCTAAGC
GCCGTCCGGGAGTCCGACGGTGGACGCTTCACGGCGTCACGTCGTCGAACCGTG
ACCGGTTGCCGGCCGGCCGTTGCGGACGGTAACAATGATAGAAATCGAATACGC
TTCGAAGCGAACCGCGCACGCGTTCAACGCGTCCGGCCCGACGCAAGCTGACCG
TCGGTGTCGATTTCCTGCCTATGTGCGGACGTCGGCTCGACGTCGCTGTCGTCTA
GCTGCCGTGCAGTCTCGGACTGTGCGCGTCTCTGTCTGCGATGATTCGGTTTCGG
GC

Splagis lemolea lemolea STR4

CGGCGTGCACTTCTCTCTTAGTAATGCATCGCGACCCGTTCGGGGTCGGTCTAAG
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Splagis lemolea lemolea STR5

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Splagis lemolea lemolea STR6

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Splagis lemolea lemolea STR7

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Splagis lemolea lemolea STR8

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Splagis lemolea lemolea STR9

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Splagis lemolea lemolea STR10

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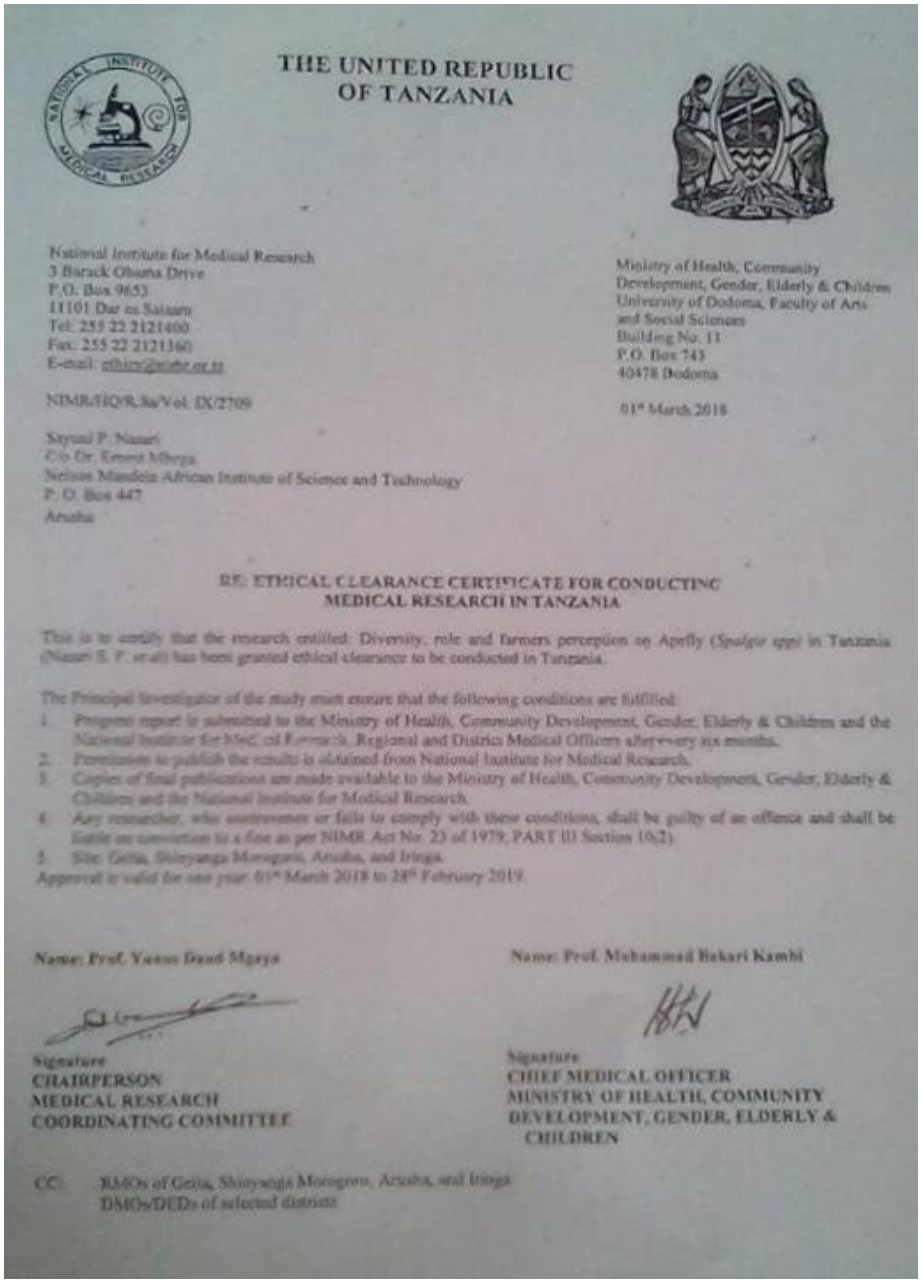
GC

Appendix 2: Ethical clearance



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NIMR/HQ/R.8a/Vol. IX/2709

01st March 2018

Sayuni P. Nassri
C/o Dr. Ernest Mbega
Nelson Mandela African Institute of Science and Technology
P. O. Box 447
Arusha

RE: ETHICAL CLEARANCE CERTIFICATE FOR CONDUCTING
MEDICAL RESEARCH IN TANZANIA

This is to certify that the research entitled: Diversity, role and farmers perception on Apefly (*Spalgis spp*) in Tanzania (Nassri S. P. *et al*) has been granted ethical clearance to be conducted in Tanzania.

The Principal Investigator of the study must ensure that the following conditions are fulfilled:

1. Progress report is submitted to the Ministry of Health, Community Development, Gender, Elderly & Children and the National Institute for Medical Research, Regional and District Medical Officers after every six months.
2. Permission to publish the results is obtained from National Institute for Medical Research.
3. Copies of final publications are made available to the Ministry of Health, Community Development, Gender, Elderly & Children and the National Institute for Medical Research.
4. Any researcher, who contravenes or fails to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine as per NIMR Act No. 23 of 1979, PART III Section 10(2).
5. Site: Geita, Shinyanga Mwanguru, Arusha, and Iringa.

Approval is valid for one year: 01st March 2018 to 28th February 2019.

Name: Prof. Yunus Daud Mgaya

Signature
CHAIRPERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE

Name: Prof. Muhammad Bakari Kambi

Signature
CHIEF MEDICAL OFFICER
MINISTRY OF HEALTH, COMMUNITY
DEVELOPMENT, GENDER, ELDERLY &
CHILDREN

CC: RMOs of Geita, Shinyanga Mwanguru, Arusha, and Iringa
DMOs/DEDs of selected districts

Appendix 3: Animal clearance

Kibong'oto Infectious Diseases Hospital- Nelson Mandela African Institution of Science and Technology- Centre for Educational Development in Health, Arusha (KIDH-NM-AIST-CEDHA)

RESEARCH ETHICAL CLEARANCE CERTIFICATE

Research Proposal No: KNCHREC0006 **9TH October 2018**

Study Title: **Diversity, Role, and Farmers' Perception on Apefly (*Spalgisspp*) in Tanzania**

Study Area: **THE NELSON MANDELA AFRICAN INSTITUTION OF SCIENCE AND TECHNOLOGY**

PI Name: Sayuni Philipo Nasari

Co-Invigilator:

Institutions: School of Life Science and Bio-Engineering (LiSBE) of the Nelson Mandela African Institution of Science and Technology

The Proposal has been approved by KNCHREC on 5th October 2018

1. **Subject to this approval you will be required to submit your progress report to the KNCHREC, National Institute of Research and Ministry of Health Community Development Gender Elderly and Children**
2. **Publication of your findings is subject to presentation to the KNCREC and NIMR Approval.**
3. **Copies of final publication should be made available to KNCHREC, National Institute of Research and Ministry of Health Community Development Gender Elderly and Children**

Duration of Study Renewal: Subject to Renewal within ONE YEAR

Span From: 5TH October 2018 to 4TH October 2019

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Mr. Simon Njeya
Secretary
KNCHREC

Prof. Raymond Mosha
Chairperson
KNCHREC

Appendix 4: Interview guide

INTERVIEW GUIDE FOR FARMERS AND OTHER MEMBERS OF THE COMMUNITY

Assessment of the existing knowledge, attitude and practices regarding Apefly by the local Communities in five Agro-Ecological zones

SECTION A- Background Information

S/N	Demographic Information	Fill/Tick where appropriate
1	Region	
2	District	
3	Ward	
4	Village/Street	
5	Age	20-30 ( ) 31-40 ( ) 41-50 ( ) 51-60 ( ) 60 > ( )
6	Sex	
7	Highest level of Education attained	Never went to school ( ) Class seven ( ) Form four ( ) Form six ( ) College (non-degree) ( ) Bachelor degree and above ( )
8	Occupation	
SECTION B-Knowledge, Attitude and Practices towards Apefly		
10	Are you familiar with Apefly?	Yes ( ) No ( )
11	How did you came to know the Apefly	I have seen it ( ) I heard from other people/media ( )
11	When did you hear/see it for the first time	Year-
12	How often do you see/hear about Apefly	Frequently ( ) Rarely ( )
13	If frequently, please specify how often	All year round ( ) Twice a year ( ) Other (please specify)
14	If rarely please approximate the time interval	Once in five years ( ) Once in ten years ( ) Unpredictable time interval ( )
15	Have you ever seen one in your farm?	Yes ( ) No ( )
16	In which category of crops do you/people find it most?	Cereals ( ) Roots and tubers ( ) Fruits and vegetables ( )
17	Please specify the crop types	
18	Does the Apefly by any means affect the crops named above?	Yes ( ) No ( )

		I don't know ( )
19	If Yes please explain how	
20	Do you find/hear that Apefly live closely with other insects?	Yes ( ) No ( ) I don't know ( )
21	If Yes please name/describe the insects living closely with Apefly	
22	What methods do you/other people use in managing/controlling Apefly?	Chemicals ( ) Mechanical/Cultural ( ) Biological ( ) None ( ) Other (please specify)
23	If chemicals or cultural/mechanical or biological control, please specify the types of chemicals or cultural methods or biological control agents in use.	
24	In which season of the year do you hear/see them most	All year round ( ) Rain season ( ) Dry season ( ) I don't know ( )
25	If you selected rainy or dry season, please specify the months	January ( )February( ) March ( )April( )May ( )June ( ) July ( ) August ( ) September ( ) October ( )November ( ) December ( )
26	How was your first reaction when you saw /heard of Apefly for the first time?	Normal ( ) Surprised ( ) Terrified ( ) Other (please specify)
27	Please explain the reason for the above answer	
28	Have you ever heard that Apefly is dangerous to human health?	Yes ( ) No ( )
29	When did you hear such information	Year-
29	From which source of information?	Health experts ( ) Media( ) Government leaders ( ) Farmers( ) Other members of the society ( )
30	Do you know anybody who fall sick/died because of Apefly?	Yes ( ) No ( )
31	What happened?	Consumed Apefly contained in vegetables ( ) Consumed only a plant/vegetable which hosted Apelfy ( ) Touched Apefly ( ) I don't know ( )
29	What is your opinion, do you also think that Apefly can be harmful to human health?	Yes ( ) No ( ) I am not sure ( )

30	Please clarify your answer above	
31	Did the information about Apefly affected farmers anyhow?	Yes ( ) No ( )
32	If yes please explain how?	
33	Has there been any explanation from experts as to what Apefly is and if it is harmful to human health?	Yes ( ) No ( ) I don't know ( )
34	If Yes please explain	
35	Do you know if Apefly is important /useful in agriculture?	Yes ( ) No ( )
36	If Yes please specify how	