ENHANCING SUSTAINABLE PRODUCTION AND HARVESTING OF THE EDIBLE GRASSHOPPER (*Ruspolia differens)*

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DECLARATION AND APPROVAL

Declaration

I declare that this thesis is my original work and has not been submitted wholly or in part for any award in this or any other institution of learning.

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DEDICATION

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ABSTRACT

The global increase in food insecurity due to growing human population and rising demand for animal proteins presents an urgent need to explore alternative sources of proteins. Edible insects such as *Ruspolia differens* form a suitable alternative due to their high nutrient content. In addition, it has a high economic value that can supplement existing animal protein sources. However, its production has remained low due to seasonality and lack of mass rearing strategies. The grasshopper is collected from the wild using locally designed trapping techniques that are associated with major health and safety concerns. This study determined the optimal conditions for sustainable production and harvesting of *R. differens*. The study assessed effects of diets and cage designs on production of *R. differens*; efficacy of a novel trapping technology for mass harvesting of *R. differens* and influence of geographical location of collection on nutritional composition of the grasshopper. The study was conducted in Kenya and Uganda using a completely randomized design. Effect of diets and cage type on weight gain, development, growth rate, survival, cannibalism, reproductive performance, longevity, nutritional composition and quantities of *R. differens* was determined. All data were analysed using Analysis of variance (ANOVA) and Generalized Linear Model (GLM) using R statistical software. Diet type influenced development ($P < 0.001$), survival ($P < 0.001$), longevity ($P = 0.015$) and reproductive performance of *R. differen*. Faster development (57 ± 2.2 days), higher survival (87 ± 2.5%), longevity (88.9 ± 11.2) and fecundity (248 ± 20.3 eggs) occurred in *R. differens* reared on Diet 3 which was contained equal proportions of maize bran, wheat bran, dried *Moringa oleifera* leaves, lake shrimps and soybean meal. Cage type influenced weight gain ($P = 0.022$) and survival rate ($P < 0.001$); higher weight (0.43 ± 0.01g) and higher survival (63.3 ± 8.8 %) occurred in the wooden and netted cages respectively. However, it had no effects on development and growth rate of *R. differens* with better performance observed in wooden cage. The local trapping technology demonstrated a higher efficiency in mass trapping of *R. differens* ($P = 0.002$) and collected fewer non target invertebrate species ($P = 0.014$) compared to the novel collapsible trap. Proximate composition, fatty acid, amino acid, mineral, vitamin and flavonoid content of *R. differens* varied among collection sites. These findings demonstrated that Diet 3 can be optimized for domestication of *R. differens*, the diet substrates and affordable and can be easily accessed by farmers. The wooden cage showed better growth and development of *R. differens*, however, it requires further improvement to enhance survival of the grasshopper. The novel trapping technology can be modified to enhance mass harvesting of *R. differens*. *Rusplia differens* should be used to complement existing protein sources, improve health nutritional outcomes and livelihoods especially in sub-Saharan Africa where food insecurity and malnutrition remain a challenge to socio-economic development.

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ACRONYMS/ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
ANOVA	Analysis of Variance
GLM	Generalized linear model
JOOUST	Jaramogi Oginga Odinga University of Science and Technology
ICIPE	International Center of Insect Physiology and Ecology
MOLP	*Moringa oleifera* leaves powder
UN	United Nations
UNDP	United Nations Development Program
WHO	World Health Organization
SDG	Sustainable Development Goals
NACOSTI	National Commission For Science, Technology & Innovation
SE	Standard Error

GLOSSARY

Colony	individuals of the same species living together
Host plants	Plants that an organism lives/feeds on
Artificial diet	Feed formulated using non-host plants
Oviposition	Laying of eggs
Preoviposition time	Number of days taken from adult moult until the first egg is laid
Oviposition Duration	Number of days between when the first and last eggs are laid
Post oviposition time	Duration of time between laying of the last egg and death of female
Fecundity	Total number of eggs laid by a female
Incubation period	Amount of time taken from onset of egg laying to onset of hatching
Eclosion	Emergence of nymphs from eggs
Eclosion period	Duration of time between the emergence of the first and last nymphs
Gravid female	Female that is carrying fully developed eggs
Zoophytophagous	Feed on both arthropods and plants
Circular agriculture	Use of agro-byproducts and food processing waste as reusable resource in food system

CHAPTER ONE: INTRODUCTION

1.1. Background Information

The rapid increase in food insecurity, demand for animal proteins and declining arable land calls for the need of alternative sources of proteins globally (Fanzo, 2012; van Huis, 2015). Edible insects provide a sustainable alternative source of proteins to animal proteins because they emit low quantities of greenhouse gases and ammonia (Oonincx et al., 2010). Edible insects are rich in proteins, carbohydrates, fibres and micro nutrients (Kinyuru et al., 2010; Rumpold & Schlüter, 2013a; Dobermann et al., 2017; Govorushko, 2019). They require less space to produce, have high fecundity, high feed conversion efficiency compared to other animals, and can form a source of livelihood for households (Yen, 2009; Rumpold & Schlüter, 2013a; Mmari et al., 2017; Okia et al., 2017).

The edible long horned grasshopper, *Ruspolia differens* Serville, (Orthoptera: Tettigoniidae) is an insect delicacy that is widely consumed in Eastern, Western and Southern Africa (Kinyuru et al., 2010; van Huis, 2013, 2022). It is rich in proteins (37-54%) and fats (33-49%), and has a high profile of essential amino acids and polyunsaturated fatty acids that are critical for human health (Kinyuru et al., 2010; Fombong et al., 2017; Kababu et al., 2023). It has high mineral and vitamin contents that can supplement other diets hence reduce mineral deficiencies and malnutrition that are common especially among children and women of childbearing age in Sub-Saharan Africa (Mwangi et al., 2018; Ssepuuya et al., 2019a). Furthermore, it is rich in anti-oxidants such as flavonoids and phenols, which provide anti-inflammatory, antimicrobial, anticancer and antiviral properties in humans (Cheseto et al., 2020; Ssepuuya et al., 2021). This grasshopper has a high economic value and forms a source of livelihood for many households in eastern Africa. A kilogram of the fried grasshopper sells at $1.85 and $2.8 in Tanzania and Uganda respectively (Mmari et al., 2017; Okia et al., 2017). In spite of this, the adoption and utilization of *R. differens* as food or source of livelihood is limited by its seasonal availability when it is harvested from the wild (Mmari et al., 2017; Okia et al., 2017; Sengendo et al., 2021a). Thus, efficient use of *R. differens* requires development and adoption of viable domestication techniques that can be utilised for mass-rearing and sustainable production at household and industrial level.

Domestication of *R. differens* requires optimization of appropriate rearing conditions such as establishment of suitable environmental conditions, quantity and quality feed, and rearing space for maximum growth, development, survival and reproductive performance (Pastor et al., 2011; Ghosh et al., 2014; Shrivastava et al., 2019). Although environmental conditions such as optimal temperature for production of *R. differens* has been determined (Lehtovaara et al., 2018; Ssepuuya et al., 2018), limited data exists on suitable diets and rearing cages that can be utilized to enhance production of *R. differens*.

In the laboratory, *R. differens* has been successfully reared on a wide range of artificial feed. However, limited data exists on an optimal diet that can be utilized for domestication of *R. differens*. Rearing has been done using processed animal feed such as starter chicken feed, chicken super feed egg booster and dog biscuits, which are expensive and inaccessible to many potential producers (Malinga et al., 2018b). Some diets comprise traditional food crops such as finger millet, rice, sorghum, germinated finger millet, groundnuts and millet that are consumed by humans. It therefore means that using them as feed may create competition for food resources (Malinga et al., 2018b, 2022). Agricultural by-products on the other hand present a cheap and suitable alternative feed source for production of orthopterans (Miech et al., 2016; Sorjonen et al., 2020). *R. differens* has been reared on mixtures of plant based by-products such as turnip rape, barley mash and barley feed that are highly perishable and not readily available in Eastern Africa region (Sorjonen et al., 2020). Thus, there is need for formulation of nutrient dense composite diet from cheap and readily available feed sources that have a long shelf life for rearing *R. differens.* Furthermore, there is a need to analyse the nutrient profile of the formulated diet its effects on the growth, survival and reproductive performance of *R. differens* assessed.

Although different types of rearing containers such as wooden boxes, glass cages, concrete blocks, cylinder blocks and plastic containers have been recommended for rearing *R. differens,* there is limited evidence on the evaluation of the effects of such rearing cages on its growth performance (Kinyuru et al., 2018). This is in spite of research evidence that suggests that rearing cages form a critical part of insect rearing systems and can contribute to significant changes in their growth, development, survival and reproduction (Huang et al., 2014; Jose et al., 2014; Cohen, 2018). Colonies

of *R. differens* have been established in wooden cages, Perspex cages and assorted plastic containers under laboratory conditions (Egonyu et al., 2021; Ssepuuya et al., 2018). In spite of the use of different cages and containers for rearing *R. differens*, there is paucity of knowldege on the suitability of different types of rearing cages for mass production of *R. differens*, thus the need to design, develop and evaluate the efficacy of diverse types of rearing cages that can be utilized for domestication and mass production of *R. differens* (Kinyuru et al., 2018).

Currently, *R. differens* is mainly collected from the wild through handpicking and use of local traps for household use and commercialization respectively (Agea et al., 2008; Mmari et al., 2017). Hand picking of *R. differens* is inefficient while the local trapping technique is associated with contamination of *R. differens*, negative health outcomes among collectors due to exposure to mercury light and allergic reactions from contact with narrow bee fly which is a by catch of *R. differens* harvesting (Labu et al., 2021; Sengendo et al., 2021a). There is scarcity of evidence on alternative trapping mechanisms for harvesting of *R. differens*. Recently, Sengendo et al., (2021a, b) developed and tested the efficacy of a modified trapping system for harvesting of *R. differens*. This trap was found to be comparable to the use of local trapping technology commonly used for mass harvesting of the grasshopper in Uganda. In spite of this evidence, modified trapping system has not been rolled out among commercial harvesters. There is therefore need for design, development and evaluation of the efficacy of safer and efficient alternative trapping techniques that can be adopted to enhance collection of *R. differens* when they are in season.

Swarming of *R. differens* in Uganda occur in different geographical areas where wild harvesting of the grasshopper is concentrated. However, information on variability of nutritional composition of *R. differens* collected from different geographical locations in Uganda remain limited. Ssepuuya and others analysed the nutritional profile of *R. differens* from Kampala, Masaka and Fort Portal in Uganda, however, the authors did not report on quantities of flavonoids and vitamins (except Vit B 12) (Ssepuuya, Smets, et al., 2019). Although Fombong et al., analysed nutrient profile of grasshoppers from Uganda, the samples analysed were a mixture of grasshoppers collected from both Kenya and Uganda; additionally, the authors focused on the effects of processing methods on nutritional profile of *R. differens* (Fombong et al., 2017). Kinyuru et al., and Siulapwa et al., on the other hand report on the nutrient composition of *R. differens*

collected from Kenya and Zambia respectively (Kinyuru et al., 2010; Siulapwa et al., 2012). Thus, nutritional analysis of *R. differens* from more geographical locations in Uganda will provide additional information on nutritional variability of grasshoppers from these regions. This information can be harnessed to enhance the use of *R. differens* as food or food ingredient regardless of source.

1.2. Statement of the Problem

In spite of the economical, nutritional and health benefits associated with supplementation of human diet with *R. differens*, the use of the grasshopper as a food source or market commodity remains limited (Rumpold & Schlüter, 2013a; van Huis, 2013; Dobermann et al., 2017). Availability of *R. differens* is limited to two biannual swarming seasons when the grasshopper is harvested from the wild using a local trapping technology that has been linked with safety concerns attributed to the use of mercury lights and rusted drums that are coated with used oil (Mmari et al., 2017; Sengendo, Subramanian, Chemurot, et al., 2021). The wild harvesting of *R. differens* is uncontrolled whereas there are inadequate sustainable environmental conservation and rearing practices among communities consuming the insect which has significantly reduced its availability and supply on the market (Okia et al., 2017). Whereas mass rearing for sustainable production and domestication of *R. differens* would be an alternative solution, this is limited by lack of standardized diets and suitable rearing cage designs among others.

Although different studies have been conducted to assess suitable diets for rearing *R. differens* (Malinga et al., 2018b, 2022; Sorjonen et al., 2020), there is limited information on optimal diet (nutrient dense, inexpensive, readily available and has a long shelf life) that enhance growth, development, survival and reproductive performance of *R. differens*. Similarly, there is inadequate data on suitable type cages that can be used to rear *R. differens*. Information on efficacy of different rearing cage types on the growth, development and survival of *R. differens* is limited.

The local trapping technique used for harvesting of *R. differens* is associated with microbial contamination and carcinogenic risk, thereby raising safety concerns (Okia et al., 2017; Labu et al., 2021; Sengendo et al., 2021a). In spite of this, alternative trapping systems remain limited, thus calling for development and testing of additional novel trapping systems that can enhance collection and safety of harvested *R. differens*.

Additionally, information on variability of nutritional profile of *R. differens* collected from predominant harvesting areas remains suboptimal. This study was aimed at optimizing mass rearing conditions and harvesting for sustainable production of *R. differens* to provide alternative protein source and enhance food security and livelihoods.

1.3. Study objectives

1.3.1. General objectives

To advance understanding of optimal rearing and harvesting techniques for *Ruspolia differens* in semi-field environment, thereby enhancing knowledge on sustainable management practices.

1.3.2. Specific objectives

1. To determine effects of diet on growth, survival and reproductive performance of *R. differens.*
2. To assess effects of rearing cage designs on production of *R. differens*.
3. To evaluate efficacy of a novel trapping technology for mass harvesting of *R. differens.*
4. To determine the nutritional composition of *R. differens* harvested from various geographical sites in Uganda.

1.4. Hypotheses

1. Diet has no significant effects on growth, survival and reproductive performance of *R. differens*
2. Rearing cage designs have no significant effects on production of *R. differens*
3. The efficacy of the novel trapping technology did not differ from the existing system for mass trapping of *R. differens*
4. There is no significant difference in nutritional composition of *R. differens* collected from different geographical sites in Uganda

1.5. Justification

Optimizing conditions for sustainable production of *R, differens* will provide a year round production of the grasshopper. This will reduce the dependence on erratic and dwindling seasonal swarms and provide an alternative and sustainable protein source to supplement existing protein sources such as milk, eggs and meat (Kinyuru et al., 2010; Mmari et al., 2017). It will also provide an alternative source of macro and

micronutrients that are important for human health and nutrition (Cheseto et al., 2020b; Ochieng et al., 2022; Ssepuuya, Smets, et al., 2019). Furthermore, continuous supply of *R. differens* will provide a sustainable source of livelihood for many households and contribute to the achievement of the UN Sustainable development goal (SDG) 1 and 2 on no poverty and zero hunger respectively (Agea et al., 2008; van Huis, 2022). An alternative mass harvesting technology with limited risks will alleviate the safety concerns associated with the use of the local trapping technology (Okia et al., 2017; Sengendo, Subramanian, Chemurot, et al., 2021).

1.6. Significance of the study

This study formulated nutrient dense artificial diets based on agro-byproducts and demonstrated their efficacy in enhancing the growth, development, survival and reproductive performance of *R. differens.* An efficient diet that enhanced the performance of *R. differens* was determined. This evidence provides information that can be utilised for the development of an optimal diet that will be utilized for domestication and mass rearing *R. differens*. Additionally, the use of agro-byproducts as feed substrate will enhance circular agriculture and limit the inclusion of food products in insect feed (Sorjonen et al., 2020).

The evidence generated on the effects of different cage types on the growth, development and survival of *R. differens* provides information that can be used for the design and development of a suitable cage type that will optimise the performance of *R. differens.* This is essential in the development of a mass rearing protocol for domestication and sustainable year round production of the grasshopper. Sustainable year round production of *R. differens* will enhance its utilization as source of food and livelihood ultimately leading to increased food security and improved livelihoods.

Usability of the novel trapping technology for harvesting *R. differens* provides evidence on efficacy of alternative trapping technologies for mass collection *R. differens*. The local trapping techniques currently used for wild harvesting of *R. differens* are associated with carcinogenic risks, high microbial load and contamination of *R. differens* harvest (Labu et al., 2021; Sengendo et al., 2021). Improvement, modification and adoption of the novel trapping technology for mass harvesting of *R. differens* will therefore reduce safety concerns associated with consumption of wild collected grasshoppers and limit negative health outcomes linked with the local trapping

technology. Evaluation of nutritional composition of *R. differens* from different locations provide additional knowledge on the nutritional profile of the grasshopper and highlight avenues through which this can be exploited for maximum nutritional and health gains. Consumption of *R. differens* can therefore be important in contributing to the reduction of the rising malnutrition and micronutrient deficiency especially in Sub Saharan Africa (Mwangi et al., 2018; Govorushko, 2019).

1.7. Scope and Limitations of the study

Formulation of experimental diets whose effects on growth, survival and reproductive performance of *R. differens* were analysed was based on the acceptance and preferential selection of diet substrates by the grasshopper. *Ruspolia differens* were reared on formulated diets and effects of diets on weight gain, duration of nymphal development to adult, rate of survival of nymphs to adult moult and cannibalism among nymphs assessed. The study did not encompass any morphological or anatomical changes that may have occurred in the process of growth and development. Effects of diet on nymphal survival to adulthood was limited to the number of nymphs that survived to adults. Effects of diet on reproductive performance was limited to parameter associated with female such preoviposition time, duration of oviposition, post oviposition time, fecundity, egg incubation time, hatchability of eggs and duration of eclosion. However, mating behaviour of the insects or reproductive parameters associated with male grasshoppers which may have influenced female fecundity were not incorporated within the scope of the study (Zhu et al., 2013).

Assessments of the effects of cage design on *R. differens* entailed the identification, design and development of different cages types assessed in the experiment. Effects of cage types on production of *R. differens* was limited to wet weight, duration of nymphal development and growth rate, and survival of *R. differens* nymphs to adult stage. Thus other factors that may contributed to the outcome of the study may have been overlooked. Additionally, effects of cage types on other biological parameters of the insect were not captured, this would have provided more insight on the results observed in the study.

Evaluation of the effects of a novel trapping technology for mass harvesting of *R. differens* comprised the design and development of a novel trap, which was tested against the traditional trap for mass harvesting of *R. differens*. The assessment was

limited to testing efficacy of the novel trap compared to the local trap based on quantities of daily catches collected. Stability of the novel trap under actual field conditions were not document with would have accounted for its low efficiency. Comparison of the safety of grasshoppers collected from the novel and local trap was not conducted. This would have provided knowledge on the safety implication of using either traps given the use of rusted drums coated with flour and used oil in the local trapping technology.

Determination of the nutritional composition of *R. differens* from different geographical locations was limited to raw samples collected from five districts in Uganda. Nutritional analysis was limited to proximate composition, amino acids profile, fatty acid content, minerals, vitamins and flavonoids. The study did not explore the presence of other bioactive compounds or sterols in *R. differens* which have numerous pharmaceutical benefits. Hence, the need for future exploration of these substances to provide a comprehensive account of the benefits associated with the grasshopper.

CHAPTER TWO: LITERATURE REVIEW

2.1. Background to Entomophagy

Humans are entomophagous beings based on existing archaeological evidence (Dobermann et al., 2017). Entomophagy is the consumption of insects and occurs as a staple, emergency food or delicacy (Durst et al., 2010). Insects are consumed at different life stages either as larvae, pupae, or adults (Govorushko, 2019). They can be consumed raw, fried, ground, boiled or roasted. They can be presented as snack food embedded in products such as chocolate, candy, ice cream, biscuits, bread and cakes (Ayieko et al., 2010; Dobermann et al., 2017). In many societies, however, entomophagy has declined in the modern era and has been shunned as old fashioned, dirty or unhealthy (Govorushko, 2019). In the recent past, the rising food insecurity, rising demand for animal proteins, volatility in food prices, climate change and land degradation have led to serious consideration on the potential of using edible insects to mitigate the emerging gaps (Durst et al., 2010).

There are more than 2000 species of insects consumed in 113 countries across the globe, out of which 470 species occur in Africa (Kelemu et al., 2015; Dobermann et al., 2017). In East Africa, edible insects include grasshoppers, crickets, ants, larvae of numerous beetles, termites and adult lake flies (Ayieko et al., 2010; van Huis et al., 2013; Okia et al., 2017). Edible insects in Kenya include grasshoppers, termites, black ants, lake flies, palm weevils and crickets. However, consumption of insects is majorly concentrated in western Kenya (Ayieko et al., 2010; Kinyuru et al., 2010; Rumpold & Schlüter, 2013a).

Edible insects consumed in the tropics are mainly harvested from nature including forests, waterways or agricultural fields (Melgar-Lalanne et al., 2019). This is however threatened by pollution, overexploitation and land degradation, which raises biosafety concerns and hamper future harvests due to population decline and species extinction (Durst et al., 2010; Okia et al., 2017; van Huis & Oonincx, 2017). Water pollution has led to a decline in aquatic insects across the world. In Africa, species of edible caterpillars are dwindling due to overexploitation and logging (van Huis & Oonincx, 2017). In eastern Africa, overharvesting, felling of trees and destruction of insect habitats is leading to a reduction in the population of edible insects. This is further aggravated by the seasonality of the insects, which limits their supply (Okia et al., 2017).

2.2. Benefits of entomophagy

Insects have a wide range of feed couples with a high efficiency of feed conversion compared to conventional livestock (Govorushko, 2019). Insect rearing requires less space, has low emission of greenhouse gases and limited water consumption (Durst et al., 2010; Rumpold & Schlüter, 2013b). They have the ability to convert low value organic waste into high value protein products (Rumpold & Schlüter, 2013b; van Huis & Oonincx, 2017).

Insects are highly nutritious and form a good source of fat, energy, proteins, vitamins and minerals (Govorushko, 2019). The energy content of 100g of insects is similar to 100g of fresh meat (Orkusz, 2021). Some insects also provide better protein sources by weight compared to some animal proteins (Bbosa et al., 2019; Orkusz, 2021). The average protein content of insects amount to 50-82% (dry weight) whereas fat content ranges from 10-30% based on fresh weight (Schabel, 2010; Sirimungkararat et al., 2010). Insects also show a high amount of micronutrients including potassium, calcium, iron and magnesium (Schabel, 2010; Sirimungkararat et al., 2010).

Edible insects can provide a source of livelihood by providing income and jobs for people who capture, rear, process and market them as food or feed (Mmari et al., 2017). Farming of edible insects in rural areas facilitates employment, provision of food especially against seasonal shortages and provides additional cash for basic expenditure (Gahukar, 2016). The estimated value of insects as food and feed for combined market in US, France, Belgium, France, The Netherlands, China, Vietnam, Mexico, Brazil and the UK was 25.1 million GBP for 2015 (Global Market Insights Inc, 2016). In Namibia, a kilogram of Mopane caterpillars sells on average at 1.4 USD whereas in Uganda a kilogram of *R. differens* sells at 2.8 USD (Agea et al., 2008; Thomas, 2013).

2.3. Grasshoppers as a source of food

Grasshoppers belong to the order Orthoptera where edible species are found in the families Acrididae, Tettigonidae, Pyrgomorphidae, Romaleidae, Tetrigidae and Catantopidae (Hanboonsong et al., 2013; Dobermann et al., 2017; Jongema, 2017).

Consumption of grasshoppers is an ancient tradition that dates back to biblical times and as stated in the book of Mathew 3:4 in the Holy Bible about the food John the Baptist ate in the wilderness (van Huis, 2013). The practice is widespread in urban and rural areas in South America, Asia, North American and Africa (Melgar-Lalanne et al.,

2019; Selaledi et al., 2021). There are more than three hundred species of grasshopper consumed globally (Melgar-Lalanne et al., 2019).

Grasshoppers are rich in proteins, carbohydrates, fats, minerals, vitamins and bioactive compound that are critical for human health and nutrition (Kinyuru et al., 2010; Melo-Ruiz et al., 2015; Di Mattia et al., 2019). As such they have the potential of complementing dietary needs in the developed countries and alleviating malnutrition in the developing countries (Das & Mandal, 2013; Zielińska et al., 2015). Commercialization of grasshoppers presents an opportunity for income generation globally and can provide a source of livelihood for households (Paul et al., 2016). Although grasshoppers are among the most sold insects in insect food businesses, their use is limited to seasonal availability when they are collected from the wild (Hanboonsong et al., 2013; Paul et al., 2016).

2.4. The Edible Long Horned Grasshopper (*Ruspolia differens*)

2.4.1. Distribution and Taxonomy of *R. differens*

Ruspolia differens (Serville 1838) (order Orthoptera, family Tettigonidae, sub family Copiphorinae) is the African cone headed long horned grasshopper commonly known in Kiswahili as *senene* (Matojo & Njau, 2010). The grasshopper is widespread throughout Africa and in some island of the Indian Ocean. It is found in parts of Eastern, Western and Central Africa including Kenya, Uganda, Tanzania, Angola, Cameroon and Ghana (Bailey & Mccrae, 1978; Matojo & Njau, 2010; Massa, 2015; Meutchieye et al., 2016).

Ruspolia differens is recognized by several synonyms by the Code of Zoological Nomenclature. These include *Conocephalus albidonervis* (Redtenbacher 1891), *C. melanostictus* (Karny 1907), *Conocephalus exiguus* (Stal 1876), *Conocephalus lemur* (Redtenbacher 1891), *Conocephalus differens* (Serville 1838), *Conocephalus longipennis* (Redtenbacher 1891), *C. mediotessellantus* (Karny 1915), *Homorocoryphus nitidulus* subsp *vicnus* (Walker, F. 1869). The multiple names imply a deficiency in taxonomic evidence used in classification of the insect (Bailey & Mccrae, 1978; Matojo & Njau, 2010; Matojo, 2017). Several authors have erroneously named *Ruspolia nitidula* for *R. differens* in the East African region (Agea et al., 2008; Ssepuuya et al., 2017). However, Matojo (2017) and Leonard et al. (2020) established that swarming long horned grasshopper found in the region is *R. differens*.

2.4.2. Biology and ecology of *Ruspolia differens*

Description of *R. differens*

Ruspolia differens is a slender insect measuring about 4-6.5 cm in length and has a thread-like antennae of the same length. It is polymorphic with six colour forms ranging from green to brown, the commonest colours being bright green (predominant in female) and straw brown (more common in males). Other colours include purple suffused brown, purple suffused green, purple stripped brown and purple stripped green (Mccrae, 1982; Matojo & Yarro, 2013; Matojo, 2017). It displays sex dimorphism where male adults are smaller in size and have 1.5 times longer antennae compared to female (Matojo & Yarro, 2013). The males have a pair of tongue like flaps found on the last segment of the thorax located on the hind wings while the females only have paired budlike nodules that appear like underdeveloped equivalent of the male flaps (Matojo & Yarro, 2013). Females possess a long slender ovipositor while the male have dorsoventrally bi-lobed cerci (Matojo & Yarro, 2013; Matojo, 2017).

Habitat and feeding of *R. differens*

Ruspolia differens is nocturnal in nature, attracted to light and occurs in grasslands and bushvelds (Bailey & Mccrae, 1978; Opoke et al., 2019a). It is described as an opportunistic feeder feeding proportionally on the most abundant grasses (Opoke et al., 2019a; Opoke et al., 2019b). In the wild, they mainly feed on flowers and seeds of grasses. The grasses on which *R. differens* have been observed include: *Panicum maximum, Brachiaria ruziziensis, Chloris gayana, Cynodon dactylon, Hyparrhenia rufa, Pennisteum purpureum* and *Sporobolus pyramidalis* (Opoke et al., 2019a; Opoke et al., 2019b). Other grasses that the grasshopper feed on were identified through molecular analysis of gut contents of wild collected *R. differens*. These include *Ageratum conyzoides* (L.), *Citrus depressa* Hayata, *Digitaria gayana* (Kunth), *Eragrostis mexicana* Hornem, *Eucalyptus saligna* SM., *Indigofera arrecta* Hochst. ex *A. Rich*., *Persicaria nepalensis* (L.), and *Sorghum halepense* (L.) (Leonard et al., 2020). They have also been reported as pests of cereals, as they feed on flowers, grains and leaves of maize, millet, sorghum, rice and wheat where their damage is inconspicuous (Matojo & Njau, 2010; Matojo & Hosea, 2013; Malinga et al., 2018b; Valtonen et al., 2018a).

Laboratory experiments have shown that *R. differens* is a polyphagous species that can feed on different feeds in the absence of their preferred host. They have been reared

successfully on mixtures of artificial foods. These included sunflower seed, linseed, oat meal, sesame seed, sugar beet fibre, coconut flour, wheat flour, maize starch, Tetra Min fish food, Casein, wheat germ, pea protein, milk whey, dog feed and chicken feed egg booster (Lehtovaara et al., 2017; Malinga et al., 2018a; Malinga et al., 2018b). The grasshopper has also been reared successfully on mixed diets based on inflorescences of different grasses (Rutaro et al., 2018a).

Ruspolia differens is a zoophytophagous insects that feeds on plants and other insects therefore may be cannibalistic when starved (Mccrae, 1982). Cannibalism accounted for almost 50% mortality of lab-reared *R. differens* out of which 83% were adults and 53% were female (Egonyu et al., 2021). Cannibalism is influenced by factors such as life stage, feed nutrient and lighting. However, high protein diet comprising live prey did not eliminate cannibalism, which could imply that this is an innate behavioural pattern key for the survival of the species (Egonyu et al., 2021; Fombong, 2022). They feed on other insects especially the narrow bee fly usually flying among the swarming individuals (Mmari et al., 2017). The grasshopper was shown to consume both live and dead prey of different insects under laboratory conditions (Fombong, 2022).

Life cycle of *R. differens*

Ruspolia differens has a life cycle of approximately 147 days with adult longevity of 50-90 days (Bailey & Mccrae, 1978; Brits & Throrton, 1981; Mccrae, 1982) depending on the climatic conditions. Mating is initiated when males produce a continuous song for about 5 minutes or more to attract females, after which mating proceeds with tactile stimulation once the pair are within antennal range. In mating pairs, copulation continues for a considerable period of time (Bailey & Mccrae, 1978; Mccrae, 1982; Opoke et al., 2019a).

The females have a pre-oviposition and oviposition period of approximately 16 and 32 days, respectively (Brits & Throrton, 1981). Oviposition occurs in batches in grass haulms in the wild whereas in laboratory conditions eggs are laid on grass stalk, maize stalk, soaked cotton wool and folded plastic cloth (Malinga et al., 2019). Malinga et al. (2019) demonstrated that the grasshopper laid more eggs on a folded plastic cloth compared to natural substrate while Egonyu et al. (2021) found that *R. differens* preferred laying on *P. maximum* and maize stalk over cotton.

Approximately 50 eggs are laid in ribbons cemented by a secretion produced from the accessory glands (Figure 1). The eggs are slender, slightly conical and curved

measuring 6.0 ± 0.1mm (Bailey & Mccrae, 1978; Matojo & Yarro, 2013). Under dry conditions, the eggs undergo diapause and remain resistant to desiccation until favourable conditions return (Mccrae, 1982). Egg development period range from 11 to 45 days at high (30°C) and low temperatures respectively (20°C) (Mccrae, 1982; Leonard et al., 2021). According to Ssepuuya et al. (2018) egg development period (14 days) and hatchability (89%) were optimised at a temperature of 30℃ when eggs were undetached from leaf sheath. Findings by Egonyu et al. (2021) demonstrated that hatchability of eggs increased with opening of leaf sheaths and moistening of eggs.

Egg hatching occurs at dawn. Newly hatched nymphs are green in colour and very active (Bailey & Mccrae, 1978). The grasshopper has 6-7 (6 for male and 7 for female) nymphal instars that develop in about 4-8 weeks in an insectary maintained at 30℃ and 50% R.H. (Mccrae, 1982; Ssepuuya et al., 2018; Leonard et al., 2021).

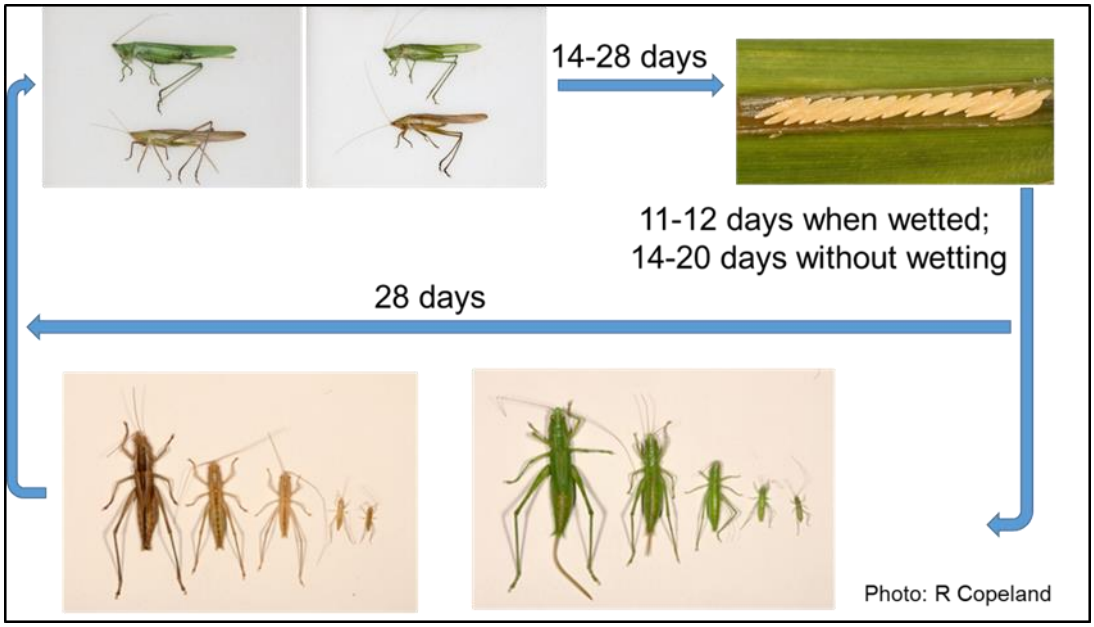

Figure 1: Life cycle of R. differens at 30°C & 50% RH (Photo: R. Copeland)

Swarming and non-swarming form of *R. differens*

The long horned grasshopper has swarming and non-swarming forms that occur in wet and dry seasons respectively (Matojo, 2017). The swarming phase has more female compared to non-swarming phase with the highest and lowest fecundity occurring in middle of the swarming season and at the end of non-swarming season respectively (Matojo & Njau, 2010; Matojo & Yarro, 2010). Swarms have more female compared to male (Mccrae, 1982). Swarming is seasonal occurring biannually during the long and short rainy seasons in eastern Africa (Mmari et al., 2017; Opoke et al., 2019a).

However, in Cameroon swarming occurs in the dry season (Meutchieye et al., 2016). Swarms reportedly arise from non-swarming populations that are recruited from local reproductive sites (Matojo & Njau, 2010; Opoke et al., 2019a), which is contrary to widespread traditional beliefs that swarms emerge from heaven, Lake Victoria, pine trees or dense clouds (Mmari et al., 2017). According to Bailey & Mccrae (1978), swarming is influenced by population density and the readiness of individuals to interact (Bailey & Mccrae, 1978). The swarming forms contain more fats indicating that swarming may be dependent on a high nutrient diet. Dense aggregations of *R differens* arrive overnight indicating a higher level of flight/migration over long distances (Mccrae, 1982). However, current research support the idea that swarming populations are recruited from local suitable reproductive habitats (Opoke et al., 2019a). After swarming, the grasshoppers hide in available bushes, which become their feeding and breeding habitat (Matojo & Njau, 2010). The swarming forms are static during the day and only fly short distances when disturbed.

Seasonal variations occur in the population densities of non-swarming forms with high and low densities respectively reported after the rainy seasons and in dry season (Matojo & Njau, 2010; Matojo & Yarro, 2013). The high densities after the rainy season results from increased rate of reproduction and development that is influenced by increased availability of quality food (Matojo & Njau, 2010; Matojo & Yarro, 2010). The low densities in dry season could be due to recruitment of large number of individuals to swarms (Matojo & Yarro, 2010; Opoke et al., 2019a). The non-swarming forms spend the day low among grasses, and if disturbed they fly very little and generally fall to the ground (Mccrae, 1982). The non-swarming forms usually have more female compared to male (Opoke et al., 2019a). Nymphs of all stages and adults of non-swarming forms are found in fields during dry and rainy seasons (Opoke et al., 2019a).

2.4.3. Rearing and mass production of *R. differens*

There is limited data on farming or rearing of *R. differens* at a small scale or commercial level across the world. Similarly, limited data exist on the farming of edible grasshopper species globally. There is, however, evidence on production of some grasshopper species in large quantities for use as pets or for the Zoo in the temperate regions and small scale production of some migratory locusts for human consumption in Netherlands (van Huis et al., 2013).

Colonies of *R. differens* have, however, been established under laboratory conditions for research purposes. *Ruspolia differens* has been reared successfully to the 10th generation at the ICIPE Duduville center in Nairobi, Kenya. Several experiments that involve laboratory rearing of *R. differens* have also been successfully conducted. However, in most of the experiments, the grasshoppers were reared individually or in few numbers (Lehtovaara et al., 2017, 2018; Rutaro et al., 2018a; Ssepuuya et al., 2018b).

The optimal temperature for rearing *R. differens* under laboratory conditions has been established. Laboratory rearing at 28 ℃ – 30 ℃ yielded the shortest development time, highest weight and highest survival (Lehtovaara et al., 2018; Ssepuuya et al., 2018; Leonard et al., 2021). The shortest egg development time and highest hatchability of eggs occurred at 30℃ (Ssepuuya et al., 2018). Most laboratory experiments dealing with *R. differens* have been established at a relative humidity range of 40-78% and a photoperiod of 12hours light: 12hours dark (Lehtovaara et al., 2018; Malinga et al., 2018b, 2022; Leonard et al., 2021). Fombong (2022) established that rearing of *R. differens* in a photoperiod of 1hour light: 23hours dark yielded a shorter development time (58 days), fewer adult moults (7), higher fecundity (27 eggs) and higher adult longevity (39 days) compared to those reared in a photoperiod of 12hours light: 12hours dark. However, other studies where *R. differens* was reared at a photoperiod of 12hours day: 12hours night recorded higher fecundity, adult longevity and shorter development time when the grasshoppers were reared in a photoperiod of 12hours light: 12hours dark (Malinga et al., 2018b; Leonard et al., 2022). These differences could be attributed to the diverse types of diets that were utilised in the studies.

2.4.4. Rearing diets and their effects on production of *R. differens*

Diet affects density, growth, size, development, reproduction and survival of insects including grasshoppers (Ghosh et al., 2014; X. Huang et al., 2017; Opoke, Malinga, et al., 2019). In *Oedaleus asiaticus* grasshopper, diet affected size, growth, development and survival while unfavourable diets led to stress among the insects, this was reflected by lower performance on growth, size, development and survival compared to those reared on favourable diets (Huang et al., 2017). A similar observation was made in *Oxya hyla hyla* where diet influenced food utilization, growth and survival (Ghosh et al., 2014). In the wild, increased production of new grass foliage and flowering of grasses in rainy seasons is associated with increased density of *R. differens*. This is

attributed to increased quantity and quality of food for *R. differens* resulting in enhanced egg production and faster development of nymphs (Opoke et al., 2019a).

Diverse diets based on single or multiple host plants, single and mixtures of artificial diets have been tested on the production of *R. differens* with varied effects on growth, development and reproductive performance. When reared on different grasses, Ssepuuya et al. (2018) reported the highest survival (53%) on *P. maximum* and *C. dactylon* and highest average weight of 0.59g when *R. differens* were reared on their host plants. Malinga et al., (2020) on the other hand recorded the highest survival (65%) on mixtures of *P. purpureum, B. ruziziensis, C. gayana, S. sphacelata, S. pyramidalis* with *P. maximum* and *C. datcylon.* The highest weight recorded was 0.38g and average development time of 63 days (Malinga et al., 2020).

Individual rearing of *R. differens* on single artificial diets (germinated finger millet, fresh maize comb, finger millet and sorghum seed heads) resulted in 87-100% survival, adult weight and development time of 0.6g and 60 days respectively; and average fecundity of 100 eggs (Malinga et al., 2022). Individual rearing of *R. differens* on artificial diet mixtures comprising rice seed head, finger millet seed head, germinated finger millet, wheat bran, chicken fed egg booster and sorghum seed head yielded 38% survival, adult longevity and development time of 88 and 100 days respectively; and average fecundity of 257 eggs (Malinga et al., 2018b). However, some of these diets were based on food crops that are used directly for human consumption.

An artificial diet mixture comprising ground black soldier fly (*Hermetia illucens*) larvae, ground bones, soybean flour, maize flour, vitamin premix and host plants (*D. gayana, C. dactylon, Megathyrsus maximus and Ageratum conyzoides*) yielded 53.7% survival; average development time and adult weight of approximately 89 days and 0.51g respectively; average fecundity of 46 eggs and adult longevity of less than 50 days (Leonard et al., 2022).

Ruspolia differens reared on plant based byproducts (barley feed, barley mash, and potato protein diets) recorded 84% survival and average weight of 0.51g (Sorjonen et al., 2020). The use of byproducts as feed is crucial in enhancing circular agriculture although the byproducts were derived from plants that are not readily available in Eastern Africa region where *R. differens* occurs (Sorjonen et al., 2020). The findings of these authors demonstrate that *R. differens* is indeed an oligophagous species that can

feed on diverse types of diet substrates (Valtonen et al., 2018). However, variation in diet substrates significantly influence their growth, development, survival and reproduction (Leonard et al., 2022; Malinga et al., 2018b; Sorjonen et al., 2020).

Emphasis has been laid on the need for development of locally available, inexpensive diets based on natural ingredients to support small scale rearing of *R. differens* in rural settings (Rutaro et al., 2018a). However most of the artificial diets tested are either based on food crops or expensive processed animal feed that may not be accessible for local small holder farmers (Malinga et al., 2018b; Leonard et al., 2022). Byproduct based diets tested for rearing of *R. differens* are based on highly perishable crops that are not available in East African region where *R. differens* is predominant (Sorjonen et al., 2020). This calls for the need to explore and formulate nutrient dense compound feed from readily available diets with a long shelf life for rearing of *R. differens*. Impact on desirable characteristics of the edible grasshoppers should be determined to inform domestication and mass production (Ssepuuya et al., 2018). Thus, this study provides evidence on the efficacy of novel agro byproduct-based artificial diets in the production of *R. differens*.

Ruspolia differens has been reared using feed sources such as wheat bran (*Triticum aestivum* Linnaeus) (Poales: Poacea), soy bean meal (*Glycine max* (L.) Merr) (Fabales: Fabaceae) and maize bran (*Zea mays* Linnaeus) (Poales: Poacea) (Malinga et al., 2018b, 2020). Maize and wheat bran are agro-industrial byproducts of maize and wheat processing respectively with high crude protein content and are used for formulation of animal feed (de Groote et al., 2010; Mutayoba et al., 2011; Mwesigwa et al., 2012). Soybean on the other hand is produced for human and animal consumption and is increasingly used as a protein source for animal feed globally owing to its high protein content (Dei, 2011; Mukherjee et al., 2016). Limited data, however, exists on the use of shrimps, *Caridina nilotica* P. Roux (Decapoda: Atyidae) and *Moringa oleifera* Lam (Brassicales: Moringaceae) in formulation of *R. differens* feed. *Moringa oleifera* is highly nutritious and rich in vitamins, minerals and essential amino acids. It has high digestibility and improves feed efficiency in animals (Moyo et al., 2016; Abbas et al., 2018; El-hack et al., 2018). *Caridina nilotica* (locally known as "ochonga" around Lake Victoria) is a by catch of the silver cyprinid, *Rastrineobola argentea*, fishery in Lake Victoria; it has limited use in Kenya where it is largely incorporated in animal feed due to its high protein content (Mugo-Bundi et al., 2015; Kubiriza et al., 2018).

2.4.5. Rearing containers/Cages and their effects on production of *R. differens*

Rearing container is one of the most significant components of insect rearing system. Selection of suitable containers is done based on size and behaviour of insect (Jose et al., 2014). A suitable rearing container must serve the insect's need including thermal features, gaseous exchange, humidity accommodation, development and reproductive needs and other micro habitat factors essential for the insect's biology (Cohen, 2018). The containers should be affordable, accessible, easy to store and clean, and allow for ease in access to the insects (Cohen, 2018).

Successful mass production of *R. differens* is dependent on suitable rearing containers for all life stages of the insect (Malinga et al., 2019). Recommended rearing containers for production of *R. differens* include glass cages, plastic containers, and wooden or concrete boxes (Kinyuru et al., 2018). However, limited data exists on efficacy of different rearing container types on growth, development and survival of *R. differens*.

In the laboratory, *R. differens* are reared in Perspex cages, wooden cages and assorted plastic containers of diverse shapes and sizes (Rutaro et al., 2018a; Ssepuuya et al., 2018; Egonyu et al., 2021). Lehtovaara et al. (2019) demonstrated that rearing environments (spikes, net and oat sprouts) within containers had minimal effects on survival of nymphs. The authors further recommended a rearing density of ≤ 36 nymphs per cubic meter (litre). However, there is limited data on appropriate designs of rearing cages/containers for domestication of *R. differens*. Information on effects of different rearing cages on the production of *R. differens* is also limited. This highlights the need for testing feasibility of using different inexpensive and readily available containers in rearing *R. differens*. This study provides evidence on the suitability of using different rearing containers on the production of *R. differens* and this will influence future production practices by small scale farmers.

2.4.6. Effects of rearing diets and rearing containers on production of other insects

Effects of diets on growth and development of insects

Diet provides nutrients that are crucial for development of organisms which determines the way organisms can maximise their performance. Availability of diverse nutrients during development affect growth rate, survival, adult body size and reproductive success of animals (Nash & Chapman, 2014; Clissold & Simpson, 2015). Slight

alterations in diets can translate into changes in survival rates, larval and pupal weight, development time and fecundity rates (Du et al., 2015). Limiting of nutrients in larval diets results in reduced adult size, delayed eclosion and reduced fecundity in insects that undergo incomplete metamorphosis (Adler et al., 2013). The growth, development, reproduction and survival of insects are influenced by quantities of protein and carbohydrate in diet (Fanson & Taylor, 2012a; Harvey et al., 2012; Rho & Lee, 2014). Proteins supply essential amino acids required for viability while carbohydrates provide energy that fuel development and allows for storage of energy for future use. Protein deficiency results into delayed development, low survival to adulthood, reduced body size and low reproductive performance of the male (Nash & Chapman, 2014). However, most organisms require a species specific blend of protein and carbohydrates for optimal performance (Roeder & Behmer, 2014). High ratio of protein: carbohydrates has toxic effects on insects (Adler et al., 2013), while decreasing protein: carbohydrate ration may increase adult lifespan in some insects (Fanson & Taylor, 2012a; Lee, 2015).

The chemical constituents of host plants such as carbohydrates, proteins, amino acids, nitrogen and lipid among others are essential parameters that determine the amount of food ingested by insect herbivores (Shobana et al., 2010). Faster growth rate results from increased feeding rate, assimilation of food and efficiency of feed conversion (Fielding & Defoliart, 2007). Diet consumption of Queensland fruit fly increased when other amino acids were combined with other nutrient classes (Fanson & Taylor, 2012a). However, feed consumption in grasshoppers from Sub-arctic and Temperate regions increased with low nutritional quality of diet to allow for compensation for low levels of nutrients (Fielding & Defoliart, 2007). Consumption of low and high quality diets by the grasshoppers differed by a factor of two. Grasshoppers reared on low quality diet yielded less weight gain and slower growth, implying the nutrient levels in the diet was too low to be compensated by increased food consumption (Fielding & Defoliart, 2007). Higher growth rate of *Diacrisia casignetum* occurred in diets that contained higher levels of carbohydrates, proteins, amino acids and lipids (Roy & Barik, 2012a, 2012b). Higher weight gain and faster growth was recorded in the common Mormon, *Papilio polytes* reared on nitrogen dense diets (Shobana et al., 2010). Unsuitable diets decreased survival, size and growth rate of the grasshopper, *Calliptamus abbreviatus* (Huang et al., 2020). Similarly, variations occurred in the weights of the lady bird, *Harmonia axyridis* when reared on different diets (Li et al., 2020). The weight of

grasshopper *Ageneottettix deorum* was higher in diets with intermediate levels of nitrogen but increased with an increase in the quantity of carbohydrates in diets (Joern & Behmer, 1997). There was, however, no significant variations in weights of the lepidopteran, *Conogethes punctiferalis* reared on diverse diets (Du et al., 2015)

Low nutrient content of diets retards growth and development rate of insects. High carbohydrate: protein ratio leads to decreased development rate and increased body mass (Clissold & Simpson, 2015). Longer development time occurred among phytophagous insects that grew on strongly carbohydrate biased diets while smaller body mass occurred in diets with extreme protein: carbohydrate imbalances (Roeder & Behmer, 2014). Development of neonate of the yellow peach moth differed based on diets (Li et al., 2015). A longer development time was recorded in medflies reared on protein diets compared to starch diet while carbohydrate treatment did not affect development (Nash & Chapman, 2014).

Effects of diet on survival of insects

Insect diets have diverse effects on their survival which is mainly influenced by constituents of diets. The yellow peach moth, *Conogethes punctiferalis* recorded lower survival when reared on unsuitable diets (Li et al., 2015). Reduction of protein quantity in diet significantly increased mortality of immature forms of the medfly, *Ceratitis capitata.* Proportion of medflies surviving to adults were also lower on the protein based diet compared to starch diets (Nash & Chapman, 2014). Similarly the reduction of protein: carbohydrate diet to a ratio of 1:16 significantly increased the mortality, delayed development and reduced body mass of *Drosophila melanogaster* (Kim et al., 2020). Survival of Queensland fruit fly decreased with the addition of amino acids to sucrose diet but increased with combination of amino acids to other nutrient classes (Fanson & Taylor, 2012a). Differences in survival were recorded in the lady bird, *Adalia bipunctata*; predatory anthaocorids (*Orius thripoborus* and *Orius naivashae*) and the generalist red velvet mite predator when reared on diverse diets (Bonte et al., 2012; Bonte et al., 2010; Muñoz-Cárdenas et al., 2014). These variations were attributed to differences in the constituents and nutrient composition of diets where more diversified diets yielded higher survival.

Effects of diet on reproductive performance of insects

Reproductive performance of insects and other arthropods is influenced by diet. Preoviposition time of *H. axyridis, Co. punctiferalis, A. bipubctata* , generalist red velvet mite predator differed by diet types while poor nutrient quality prolonged preoviposition in the grasshopper, *Choroedocus illustris* (Bonte et al., 2010; Ahmad & Nabi, 2012; Muñoz-Cárdenas et al., 2014; Li et al., 2015; Li et al., 2020;). Oocyte development is triggered by the correct levels of nutrients in diet, thus poor diets delay oocyte development leading to prolonged pre-oviposition time (Ahmad & Nabi, 2012). Similarly, ovarian development and egg production insects is influenced by nutritional quality of diets which in turn affect their fecundity (Nash & Chapman, 2014). Fecundity, egg incubation and hatchability of *H. axyridis, E. kuehniella and Paederus fuscipes* differed by diet types (Bonte et al., 2010; Bong et al., 2014; Li et al., 2020).

Effects of rearing containers on growth, development and survival of insects

Coupled with optimal rearing diets, rearing containers form an integral part of insect rearing systems (Cohen, 2018). Rearing containers should provide suitable thermal features, gas exchange, humidity; and support the biological features of target insects (Bueno et al., 2006; Cohen, 2018). There was limited literature on the effects of rearing containers types on growth and survival of *R. differens*. However, there was evidence on the effects of rearing containers on other insect species which may provide insights on the findings of this study.

Rearing containers can induce significant changes in the growth and survival of insects in an artificial rearing system (Jose et al., 2014). The shape, size and material used for making rearing cages can influence the performance of insects (Huang et al., 2014). According to Ngonga et al., (2021), an improvised cage system that was made with plywood was more appropriate for rearing of crickets compared to plastic buckets. This was attributed to the higher temperature and lower humidity recorded in the improvised cage system compared to the plastic bucket. The evaluation of three rearing containers (petri dishes, plastic bags and glass jar) revealed that glass jar was the most suitable for production of *Orius insidiosus.* The glass jar yielded the highest percentage of adults and highest female fecundity compared to the others (Bueno et al., 2006). This was attributed to better control of humidity inside the glass jar, greater visibility of the inner part of jar which allowed for monitoring of the development of the insects and ease of cleaning.

Evaluation of cage designs obtained from international research groups for production of honey bees showed that cage design has a significant effect on survival of honey bees with higher rate of mortalities recorded in some cage types compared to others (Huang et al., 2014). Similarly, a higher rate of survival was recorded *Hyblaea puera* reared in a combination of Petri plate and rearing tubes compared to other rearing containers (plastic tube, plastic wire mesh, petri plate and glass bottles) (Jose et al., 2014). The Petri plates provided a higher surface area for diet that was preferred by early instars while rearing tubes provided adequate space that provided space for movement by later instars larvae. Contrary to this, a comparison between use of Petri dishes and cubic containers for rearing *Helicoverpa armigera* did not yield significant differences on larval development and survival (Allahyari et al., 2022). Similarly, assessment of a rearing container for the production of *Lissorhoptrus aryzophilus* (Rice water weevil) yielded a survival rate that was not significantly different than survival of neonates reared in mud (Zhang et al., 2004).

There is inadequate data on the effects of rearing containers on growth and development of grasshoppers and other insects. However, research shows that environmental conditions in which animals including insects are reared have significant effects on their growth rate and body composition (Alemneh & Getabalew, 2019). Similarly, management factors such as house type for rearing animals influence feed conversion, growth and degree of fatness in animals (Suk & Washburn, 1995). There is therefore need for evaluation and documentation of efficacy of rearing containers on growth, development and survival of insects in order to optimize their production under mass rearing or small scale production.

2.5. Collection and harvesting of *R. differens*

Like other edible grasshopper species, *R. differens* is mainly harvested from the wild during swarming, however, this is limited to their seasonal availability. Swarms are attracted to light at night and usually concentrate around streetlights in urban areas, on shrubberies and grasses without causing obvious damage (Mmari et al., 2017). In Cameroon, the grasshoppers are harvested in the dry seasons of the year (January-February and October-December) when they are available (Meutchieye et al., 2016) while in Eastern Africa grasshoppers are harvested in the rainy seasons (April-July and November-December) (Meutchieye et al., 2016; Okia et al., 2017).

Collection of *R. differens* occurs in different ways. In Rwanda and Burundi collection is done traditionally by hand beating of flying grasshoppers with tree branches. This method is laborious with limited catches (Okia et al., 2017). In Tanzania and Uganda, the grasshoppers for home consumption are traditionally collected by hand picking at night under bright lights at home or early in the morning when the grasshoppers are inactive or sluggish. This is generally done by women and school going children (Agea et al., 2008; Mmari et al., 2017).

Commercial harvesting of the grasshopper is more elaborate using locally made traps (*Figure 2*) comprising drums, raised platform for placing drums, iron sheets, high voltage electric wires, electric bulbs (400-1000W or 6000-15000lm), and capacitators for power stabilization (Mmari et al., 2017; Okia et al., 2017). Open drums are arranged in a row on a platform, then one iron sheet placed slanting at the top of each drum at an angle of 45° and 75° with the upper part resting on a wooden pole for support (Ssepuuya, 2019). The high voltage bulbs are placed above the iron sheets and the light switched on by 7 pm to attract the swarming grasshoppers. The traps are set up against the direction of the wind to trap more grasshoppers (Sengendo et al., 2021). The drums and iron sheets are coated with moisten cassava flour and/cooking oil to make them slippery for trapped grasshoppers to climb out. These substances used in coating of the drums have, however, been implicated in contamination of the grasshopper harvest making processing for markets difficult (Sengendo et al., 2021). The waste oil utilized is associated with heavy metals which are carcinogenic to humans (Ganesan et al., 2017).

Swarming grasshoppers are attracted by the bright light, hit on the slanted iron sheets then fall into the coated drums (Sengendo et al., 2021a; Ssepuuya, 2019). Communities apply smoke from herbaceous plants in trapping sites to increase their catches (Figure 2). The smoke is believed to induce docility in *R. differens* through intoxication (Okia et al., 2017). Harvested *R. differens* are collected from the drums in the morning, packaged in aerated polythene, nylon, sisal or cloth mesh bags then delivered to markets (Ssepuuya, 2019).

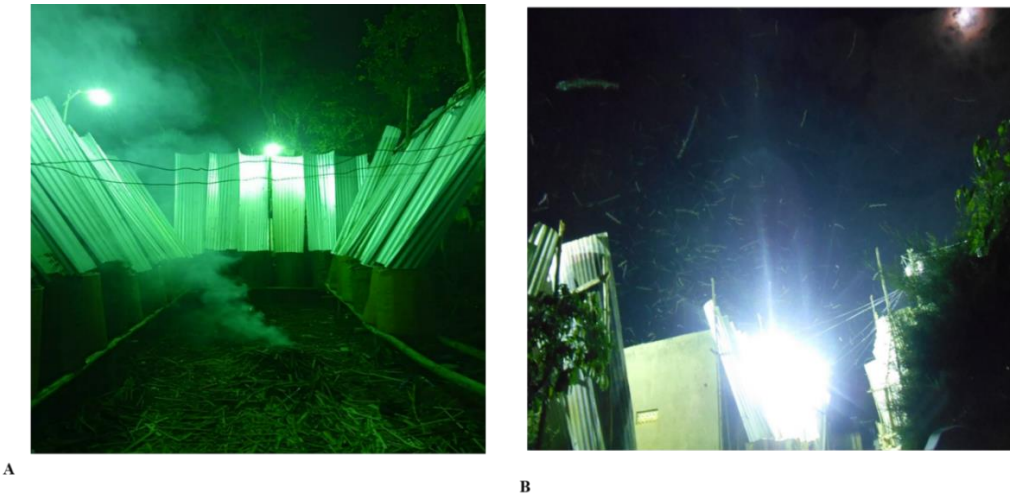

Figure 2: Set up of commercial harvesting of R. differens (A) mounted traps (B) R. differens swarms flying above mercury lights (Source: Kababu)

Limited data exist on alternative harvesting techniques for collection of *R. differens* (Mmari et al., 2017; Okia et al., 2017; van Huis, 2022). An improved trapping system for harvesting of *R. differens* was recently developed and evaluated against the local trapping system (Sengendo et al., 2021). It comprised collection drums fitted with a funnel to limit the escape of *R. differens* catches, wire mesh to strain non-target insects and LED bulbs to replace the potentially hazardous mercury bulbs. The modified trapping system was found to be safer and more energy efficient. The authors established that catches in the modified and traditional drums were comparable while non-target insects were filtered out more effectively from the modified drum compared to the local ones. The performance of the LED bulb and mercury bulbs were also comparable with low power consumption on the former. Sengendo et al. (2021b) established that the local commercial trap and the modified trapping techniques were profitable ventures. However, the modified trap was more profitable compared to the local one. In spite of this, there is no evidence on the adoption of the modified trapping technology for harvesting *R. differens*. Thus, the need for the design and development of alternative harvesting techniques with limited safety concerns such as use of old and rusted drums, mercury lights and sprinkling of drums with used cooking oil and flour which present several health risks (Sengendo, Subramanian, Chemurot, et al., 2021). The efficacy of the alternative techniques should be evaluated to optimize wild collection of *R. differens.*

2.6. Variability in nutritional profile of *R. differens*

Analysis of nutritional profile of *R. differens* by different authors reveal considerable variations based on geographic area of collection, colour morph, processing methods and diet in cases where *R. differens* is reared under laboratory conditions.

Proximate composition of raw and processed *R. differens* on dry matter basis range between 39-54% crude protein, 20-48% crude fat, 4-13% crude fibre, 3-22% carbohydrate, 10-15.9% chitin, 2-5% ash and 1-77% moisture content (Kinyuru et al., 2010; Siulapwa et al., 2012; Fombong et al., 2017; Ssepuuya et al., 2017; Bbosa et al., 2019; Nyangena et al., 2020; Ochieng et al., 2022). Variations in proximate composition of *R. differens* was observed based on region and season of collection, colour morph and processing method (Kinyuru et al., 2010; Fombong et al., 2017; Ssepuuya et al., 2019; Nyangena et al., 2020). The nutritional composition of *R. differens* is directly influenced by diet consumed (Rutaro, Malinga, Opoke, et al., 2018). In the wild, this insect consumes plants materials whose chemical composition is influenced by soil types that vary across geographical regions that is influenced by the type of feed they consume; this may account for the variations observed in the proximate composition of *R. differens* obtained from different geographical locations and swarming seasons (Ssepuuya, Smets, et al., 2019). Thermal processing methods such as toasting, boiling, blanching and oven drying altered the proximate composition of *R. differens* probably due to leaching or loss of nutrients (Meyer-Rochow et al., 2021; Nyangena et al., 2020; Ochieng et al., 2022).

R. differens contains variable quantities of all essential and non-essential amino acids. The essential amino acids include valine (16-67 mg/g), leucine (27-95mg/g), isoleucine (26-49 mg/g), phenylalanine (26-39mg/g), threonine (29-43 mg/g), lysine (54-64 mg/g), histidine (24-44 mg/g), methionine (1.4-7 mg/g) and tryptophan (0.3-9 mg/g) (Siulapwa et al., 2012; Fombong et al., 2017; Ssepuuya et al., 2019a). Thus, *R. differens* presents a more superior source of proteins compared to some cereal proteins that lack methionine and lysine amino acids (Zielińska et al., 2015; Elhassan et al., 2019).

Ruspolia differens is rich in fatty acids. They contain high levels of unsaturated fatty acids including oleic (25-44%) and linoleic (19-31%) acids and saturated fatty acid such as palmitic acid (27-32%) (Kinyuru et al., 2010; Ssepuuya et al., 2019a). Some of the unsaturated fatty acids such as linoleic acid is a precursor for production of long chain

polyunsaturated fatty acids such as arachidonic and docosahexaenoic acid, which are important in maintaining the nervous system and cell membrane (da Silva Lucas et al., 2020). The polyunsaturated fatty acids are also associated with prophylaxis of cancer and cardio vascular diseases (Govorushko, 2019).

Ruspolia differens is rich in minerals that are essential for human health and nutrition. They are rich in minerals including phosphorous (121-694 mg/100g), potassium (259-834mg/100g), calcium (9-1124 mg/100g), iron (2-259 mg/100g) and zinc (8-18 mg/100g) (Kinyuru et al., 2010; Siulapwa et al., 2012; Fombong et al., 2017; Ssepuuya et al., 2017, 2019). Addition of *R. differens* in diets can reduce zinc and iron deficiency that affect women of reproductive age and children in developing countries (Kinyuru et al., 2010; Mwangi et al., 2018). The quantities of minerals varied by geographical location and processing methods. Higher quantities of calcium, magnesium and zinc were recorded in boiled, blanched and toasted grasshoppers while levels on iron were lower in toasted grasshoppers (Ochieng et al., 2022).

Ruspolia differens contain vitamins including retinol, α-tocopherol, niacin, riboflavin, folic acid, ascorbic acid, pyridoxine and vitamin B12 (Kinyuru et al., 2010; Ssepuuya et al., 2019). The quantities of α-tocopherol and retinal are adequate to meet the RDI for humans, therefore consumption of the grasshoppers can curb vitamin deficiency (Kinyuru et al., 2010). Heat processing was, however, shown to reduce the levels of niacin, retinol and riboflavin, pointing to the need for optimization of processing methods to ensure nutritional benefit to users (Kinyuru et al., 2009).

Although most studies that have analysed the nutritional profile of *R. differens*, there was limited documentation of flavonoid content of grasshoppers collected from different regions. A few authors, however, extracted, analysed and documented different types of flavonoids from *R. differens* samples. Flavonoids documented in lab reared *R. differens* include: Quercetin, Luteolin, Kaempferol and Orientin (Cheseto et al., 2020). The quantities of flavonoid in wild collected *R. differens* is higher than values recorded for most fruits. *R. differens* has high antioxidant activity similar to that of many vegetables such as cabbages and egg plants that are consumed in Africa (Ssepuuya et al., 2021). Other major antioxidants extracted from *R. differens* include glutathione, glutathione peroxidase and catalase (Kasozi et al., 2019). The nutritional profile of the grasshoppers highlights their significance for human health and nutrition.

Nutritional analysis of *R. differens* in this study provided more information on nutritional profile of *R. differens* from major commercial harvesting sites in Uganda compared to the number of previously reported sites (Ssepuuya, Smets, et al., 2019). A higher number of vitamins, minerals and fatty acids were also isolated which will add to the existing knowledge base on the suitability of *R. differens* as an ingredient in formulation of processed products or supplementary food that can contribute to improving human health and nutrition

2.7. Conceptual framework

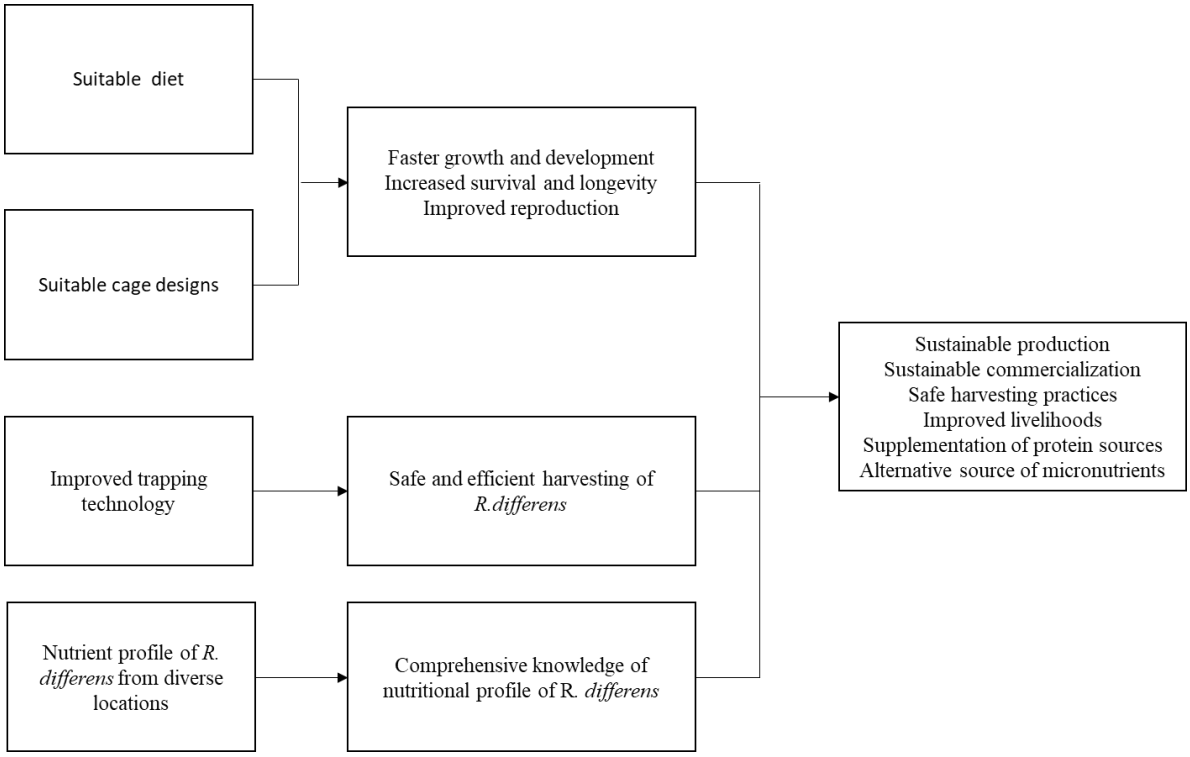

Figure 3: Conceptual framework

CHAPTER THREE: MATERIALS AND METHODS

3.1. Introduction

This chapter presents the materials and methods that were employed in the study. It gives a comprehensive account of the study area, research design, experimental design, data collection procedure and methods of data analysis employed for each of the study objectives.

3.2. Experiment 1: To evaluate effects of diet on growth, survival and reproductive performance of *R. differens.*

3.2.1. Study area

This study was conducted in Kenya and Uganda (Figure 4). Experiment to evaluate effects of diet on growth, survival and reproductive performance of *R. differens* was carried out at the International Centre of Insect Physiology and Ecology (ICIPE), Duduville Campus, Kasarani Nairobi County in Kenya. Duduville is located at an altitude of 1798 metres above the sea level, latitude of 1° 18' S and longitude of 36° 49' E. This experiment was conducted between August 2020 and June 2021.

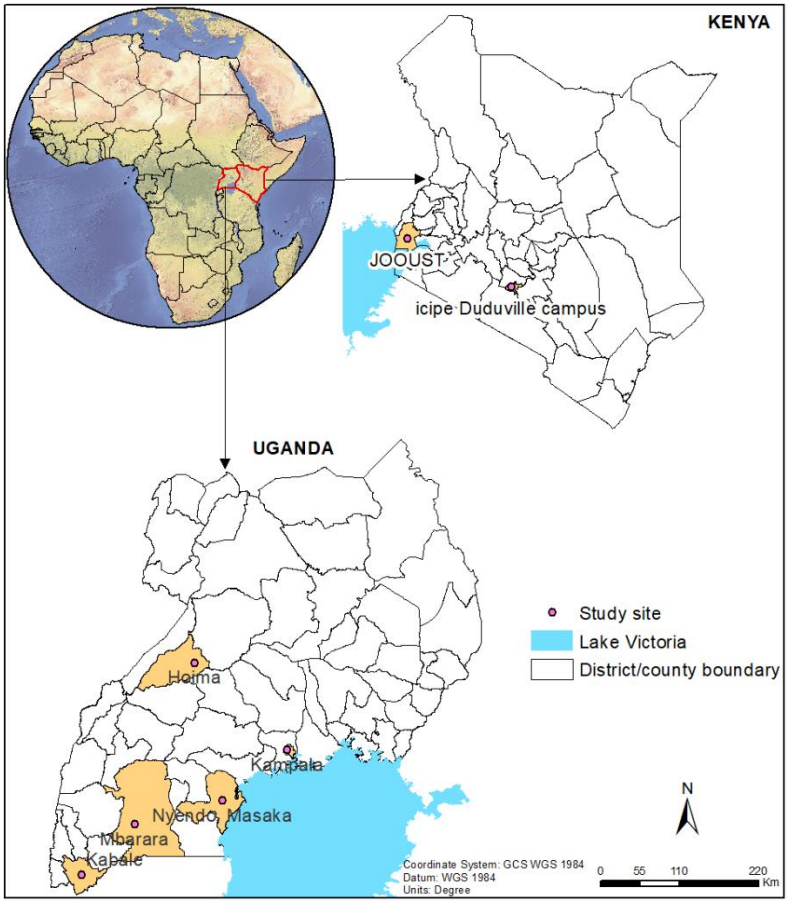

Figure 4: Map of the study area (Image: R. Mongare)

3.2.2. Study design

A completely randomized design was used with four diet treatments namely Diet 1, Diet 2, Diet 3 and Diet 4 (Table 1), with three replicates.

Table 1: Constituents of experimental diets

Diet 1	Maize bran, wheat bran, *Moringa oleifera* leaves
Diet 2	Maize bran, wheat bran, *M. oleifera* leaves, Lake shrimps
Diet 3	Maize bran, wheat bran, *M. oleifera* leaves, Lake shrimps and soybean meal
Diet 4 (control)	Bergafat, maize flour, lysine amino acid, vitamin and mineral premix, Dicalcium Phosphate (DCP), lime, silver cyprinid (local name: omena), salt, wheat pollard, rice polishing, soy bean meal and methionine

Rearing of *Ruspolia differens* colony for experiments

The *R. differens* used in this study were obtained ICIPE Duduville Campus in Nairobi. This was the eighth generation of the grasshopper colony that was established in November 2017 using grasshoppers collected from commercial traps in Masaka, Uganda. The grasshoppers were reared in Perspex cages measuring 50 × 50 × 50 cm placed in a rearing room maintained at 27-30℃, 50-65% RH and a photoperiod of 12:12hours of day: night. The *R. differens* were fed mainly on a powdered artificial diet used for rearing the grasshopper at ICIPE; this diet was formulated by Treasure Feeds Limited (Thika, Kenya) (Egonyu et al., 2021). The diet was supplemented with fresh shoots of *Panicum maximum* Jacq (Poales: Poacea) and *Brachiaria ruziziensis* Germ & C.M Evrard (Poales: Poacea). The artificial diet was presented in Petri dishes whereas fresh shoots from both plant materials were presented as bouquets in clean plastic containers (height 11 cm and diameter 5 cm) half filled with water and secured at the surface with cotton wool, respectively. This prevented the plants from withering and the insects from drowning in water while feeding (Egonyu et al., 2021). Three Petri dishes containing diet and three bottles of plant materials were presented in each cage. The plant materials were replenished after every three days. Water was provided through soaked cotton balls, which were also used as oviposition substrates. The eggs were collected by opening the cotton balls to remove eggs and unsheathing the plant materials that contained eggs. The collected eggs were placed on moist cotton wool in two-litre rectangular plastic containers measuring 220 mm long × 156 mm wide × 82 mm high (Rectangle Food Mate No.2, Kenpoly Manufacturers Limited, Nairobi,

Kenya) ventilated at the top for aeration. Water was sprinkled on the eggs daily until hatching to prevent desiccation. The containers were placed on a table within the rearing room and monitored regularly for nymphal hatching.

Experimental diets

Source and processing of experimental diets

Five diet substrates namely maize bran, wheat bran, Lake shrimps (*C. nilotica*; local name *ochong'a*), *M. oleifera* leaves and soybean meal were selected for formulation of experimental diets. *Moringa oleifera* leaves were acquired in dried and powder form from a farmer in Bondo County. Maize bran, wheat bran, soy bean meal (powdered) and *ochong'a* were purchased from a vendor at a local market (Gikomba market, Nairobi) and ground into fine powder (0.01-0.02 mm) using an electronic grinder (Preethi TRIO, 500w, MG182/00, Preethi Kitchen Appliances Pvt, Ltd. Chennai, India).

Assessment of the acceptance and preference of the diet components

The selected diet substrates were subjected to acceptance test to determine if the grasshopper would feed on them. Based on acceptance of feed substrates, preference test was conducted to determine preferential consumption of the substrates by *R. differens*. Study diets were then formulated based on preferential selection by *R. differens.*

Acceptability and preference of five diet substrates by *R. differens* were tested using no-choice and choice bioassays respectively. In the no choice test, a total of ten adult grasshoppers were presented with 2 grams of each of the feed substrate for a period of 48 hours. Each grasshopper was held in a ventilated four-litre transparent plastic container where they had ad libitum access to feed and water. The diets were presented on a piece of aluminium foil and water was provided in a soaked cotton ball. The feed residue was weighed after 48 hours. The quantity of feed substrate consumed was determined by subtracting the amount of feed residue from the feed offered (Orinda et al. 2017).

In the choice experiment, each of the ten grasshoppers used in the no choice test were each presented with 2 grams each of the five diets placed side by side for a period of 48 hours. Each grasshopper was placed in a ventilated four-litre transparent plastic container. The diets were presented on a piece of aluminium foil and water provided in

a soaked cotton ball. The quantity of each of the diets left was weighed after 48 hours. The quantity of feed substrate consumed was determined by subtracting the amount of feed residue from the initial feed provided (Orinda et al., 2017).

Formulation of experimental diets

Three diet mixtures were constituted using an equal proportion (100 grams) of the five ingredients in an increasing gradient of three to five based on their preferential selection by *R. differens* (Table 2). The diet mixtures were stored in clean transparent plastic containers and placed in a cupboard. The artificial diet mixture used for the rearing of *R. differens* at ICIPE was used the fourth diet (control) (Egonyu et al., 2021). The control diet comprised bergafat, maize flour, lysine amino acid, vitamin mineral premix, Dicalcium Phosphate (DCP), lime, silver cyprinid (local name: *omena*), salt, wheat pollard, rice polish, soya meal and methionine (sourced from Treasure Feeds Ltd, Thika, Kenya).

Table 2: Composition of experimental diets used to assess development, survivorship, longevity and reproductive performance of *Ruspolia differens*

Diet	Composition in percentages				
	Maize bran	Wheat bran	MOLP	Lake shrimp *(Ochong'a)*	Soybean meal
Diet 1	33.3	33.3	33.3	0.0	0.0
Diet 2	25.0	25.0	25.0	25.0	0.0
Diet 3	20.0	20.0	20.0	20.0	20.0
Diet 4 (Control)	Bergafat, maize flour, lysine amino acid, vitamin and mineral premix, Dicalcium Phosphate (DCP), lime, silver cyprinid (local name: *omena*), salt, wheat pollard, rice polishing, soy bean meal and methionine				

Nutritional analysis of the experimental diets

Nutritional analysis of the diets was conducted at ILRI laboratory. Dry matter, crude fat, crude protein, crude fibre, ash, ADF (Acid detergent fiber) and NDF(Neutral detergent fiber) contents of the diets were determined using the official methods of Association of Official Analytic Chemists (AOAC, 2012). Sugar and starch content of the diets were computed using NIR technology (Lebot et al., 2009). Protein digestibility of the diets was determined using modified method described by Mertz et al. (1984) using pepsin enzyme. Inductively Coupled Plasma-Optical Emission Spectrometer

(ICP-OES) was used to determine the mineral composition of study diets (Campbell & Plank, 1991; Horwitz, 2000).

Experimental design

The experiment was set up in a laboratory maintained at 27-29℃, 50-60% RH and a photoperiod of 12: 12hours day: night. Thirty newly emerged nymphs of *R. differens* were reared in ventilated Perspex cages measuring 40 cm × 40 cm × 40 cm. Each diet treatment was assigned three cages (three replicates). The cages were placed on shelves elevated at 1m above the ground. All the cages were placed 15 cm away from the wall and 10 cm away from each other.

Effect of diets on wet weight, development and survival of R. differens

A total of 30 newly hatched (1-2 days) nymphs were released into each of the twelve experimental cages to assess the effects of diet on growth, development and survivorship of *R. differens*. The nymphs were released by placing uncovered incubation containers into a clean cage at the onset of egg hatching. The process was repeated until the desired number of nymphs was attained for each of the experimental cages. The nymphs had *ad libitum* access to food and water. Five grams of each of the diets were presented in a Petri dish and replaced every week. The quantity of diet was deemed adequate to feed the nymphs with minimal wastage since the left over diet was replaced on weekly basis. Water was provided through soaked cotton balls replaced after every three days. The cages were cleaned at three-day intervals. The cages were monitored for mortality on daily basis. Ten randomly selected nymphs were weighed weekly while adults were weighed within 24 hours of emergence using an electronic weighing scale (Model kern PCB 350-3) and their sex was determined. The sexes were differentiated by the presence of the ovipositor in female (Matojo & Yarro, 2013). The nymphs were placed in a transparent plastic container (12 cm height × 6 cm diameter) and weight measurement readings on the scale were taken once there was no movement of the grasshoppers. The weight of the grasshoppers was logged as the total weight minus the container weight (Magara et al., 2019). Development time was determined as the days between hatching and adult moult (Malinga et al., 2020). Survival was estimated by calculating the number of individuals alive at the end of the experiment (Sorjonen et al., 2019).

Reproductive performance and longevity of R. differens

In order to determine the effects of diets on reproductive performance and longevity, ten pairs (male and female) of newly moulted adult *R. differens* were used for each of the diets and monitored till death. The paired insects were placed in transparent four-litre plastic (Figure 5A) containers measuring 220 mm long × 156 mm wide × 160 mm high (Rectangle Food Mate No.3, Kenpoly Manufacturers Limited), placed randomly on shelves in the laboratory. The insects were maintained under similar conditions and feeding regimes described above. When cleaning and changing feed the position of containers were randomized to avoid potential shelf effects (Lehtovaara et al., 2018). Soaked cotton balls used as a water source also doubled as an oviposition substrate for laying of eggs (Figure 5B). Eggs collected from each female were incubated in one-litre cylindrical plastic containers (Thermopak TPL 2033, Thermopak Limited, Nairobi, Kenya) which were ventilated with plastic mesh at the top (Figure 5C, D). The eggs were spread on wet cotton wool and sprinkled with water every morning to moisten the cotton wool and prevent desiccation. The containers were monitored daily for freshly hatched nymphs and the numbers were recorded until no more hatching occurred.

Preoviposition time was determined as the duration from the day of emergence of female to the day when the first egg was laid (Magara et al., 2019). Fecundity was determined as the total number of eggs that were laid per female (Malinga et al., 2018b). Egg incubation was calculated from the day of egg oviposition to hatching. Hatchability was calculated as a percentage of eggs hatched/total eggs laid per female (Roy & Barik, 2012b). Duration of egg eclosion was determined from the onset of hatching to the last day when hatching occurred. Post oviposition time was calculated as the time between the laying of the last egg and the death of female adult. Adult longevity was calculated from the day of adult emergence and its death.

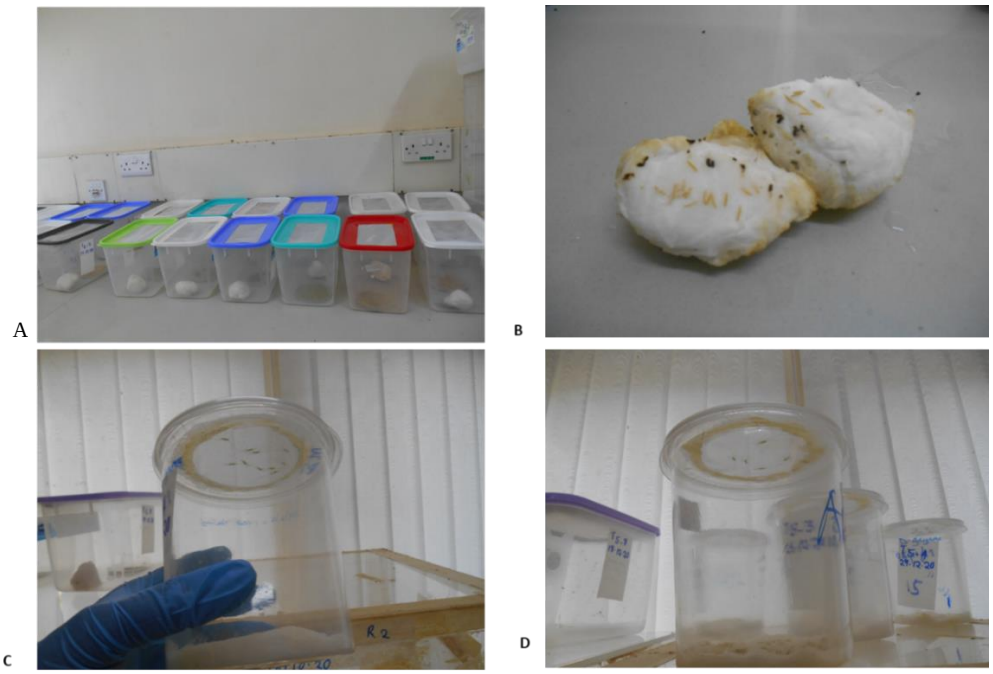

Figure 5: (A) Rearing containers containing paired male and female R. differens (B) cotton balls containing eggs (C_D) egg incubation containers showing incubated eggs and newly hatched nymphs (Source: Kababu)

3.2.3. Data collection

Wet adult weight was determined as the wet weight of newly moulted adult within 24 hours of emergence. Duration of development was determined by the number of days taken from egg hatching to adult moult (Lehtovaara et al., 2018). Rate of survival was determined by the percentage of individuals that survived to adult stage (Lehtovaara et al., 2018) while cannibalism was determined as the proportion of insects whose body parts had been eaten out of the number used at the onset of the experiment (Egonyu et al., 2021).

Preoviposition period was defined as the number of days between the date of pairing of adult male and female and the date when the first egg was recorded. The duration of oviposition was determined by the number of days between the date when the first egg was laid and the date when the last egg was recorded. Post oviposition time was determined as the number of days between the date when the last egg was laid and the date when the female died. Fecundity was determined by summing up the total number of eggs laid by each female. Pre-eclosion time was determined by the number of days between the date when the first egg was laid and the date of onset of hatching. Duration

of eclosion was determined as the number of days between the date when the first nymph hatched and the date when the last nymph hatched. Hatchability was determined as the percentage of eggs that hatched out of the total number of eggs laid per female. Adult longevity was determined as the number of days between the date of adult emergence and the date of death of grasshopper.

3.2.4. Data analysis

All data were subjected normality and homogeneity tests using Shapiro Wilk's and Bartlett tests respectively prior to analysis. Kruskal-Wallis test and Friedman's ANOVA were used analyze the difference among means on food acceptance and preference respectively. Pairwise Wilcoxon test with Bonferroni correction was used to separate the means. The differences in mean survival rate of *R. differens* nymphs to adult moult and percentage hatchability of eggs between diets were analyzed using logit linked binomial generalized linear model (GLM). Differences in preoviposition time, oviposition duration, post oviposition time, fecundity, duration of egg incubation and egg eclosion time among diets were determined using negative binomial GLM, which was used to correct over dispersion that occurred when the data were subjected to a log-linked Poisson distribution. One-way analysis of variance (ANOVA) was used to analyze the difference in means of weight, development time and longevity. Differences between means were separated using Tukey's Honest Significant Difference (HSD), post hoc test. All effects were considered significant at $P < 0.05$. All analyses were performed using R version 4.1.2 (R Core Team, 2020), statistical software.

3.3. Experiment 2: To determine effects of rearing cage designs on production of *R. differens*

3.3.1. Study site

Effects of rearing cage designs on production of *R. differens* were assessed at the JOOUST insect farm in Siaya county, Bondo Sub County in Kenya (Figure 4). JOOUST is located at an altitude of 1250 metres above the sea level, latitude of 0° 14 ' N and longitude of 34° 16' E (Achonga et al., 2011; Nyakeri, 2018).

3.3.2. Study design

A completely randomized experimental design with three treatments namely wooden cage, plastic cage and netted cage (control) (Figure 6) were used and replicated three times.

Figure 6: A) wooden, B) Plastic and C) netted cages used to assess the effects of cage design on production of R. differens.(Source: Kababu)

Preparation of experimental cage types

Three cage types (Figure 6) were identified, designed and developed. The cages were selected based on recommended container types and previous use of cages made from similar materials in laboratory experiments (Kinyuru et al., 2018). The cages were affordable, easy to clean and store and allowed for ease of access to the grasshoppers (Cohen, 2018). The first cage was made of wooden material with a wire mesh at the top to allow aeration and ventilation (Wooden Cage). The cage measured 42 cm × 42 cm × 42 cm; had a lower compartment (42 cm × 42 cm × 5 cm) partitioned by a wire mesh to allow passage of insect frass to the lower compartment for easy cleaning. However, the lower compartment was sealed off using a fine plastic mesh after piloting to prevent nymphs from escaping through the wire mesh. The cage was opened from the top side.

The second cage was a modified 60L rectangular plastic container covered with a lid (H330 × W410 × L540 mm, Storage Box No.3, Kenpoly Manufacturers Limited, Nairobi, Kenya) (Plastic cage). One side of the container and lid were cut out and replaced with plastic mesh for aeration while the opposite end of the container was also cut out replaced with a sleeve made of netted plastic material for easy access. The top part of the cage was sealed with netted plastic and the lid was replaced to prevent grasshoppers from escaping. The third cage (control) was made of Plastic net all around with a metallic frame (42 cm × 42 cm × 42 cm) for support (Netted cage). A zipper was placed on one side of the cage for ease of access. This cage was selected as control since it was previously utilized for rearing *R. differens* at the JOOUST insect farm.

Ruspolia differens colony

A colony of *R. differens* was established at the insect farm of JOOUST in a prefabricated rearing house (5m ×3m×2.5m) at 25-30 ^{0}C, 60-75% RH and a photoperiod of 12:12hours (D: N). Temperature was regulated using a heater with a thermostat

(ARMCO Fan heater, Model No. AFH-1000 A) while humidity was regulated by pouring water on the floor. Water was poured on pieces of mattress to reduce the rate of evaporation. A thermohygrometer (Thermo Pro indoor humidity and temperature monitor, Model No. TP-50) was used to record temperature and humidity of the room on a daily basis. Eggs of *R. differens* were obtained from the 8th generation of the exiting colony reared at ICIPE Duduville Campus, Nairobi, Kenya. This colony was established from *R. differens* originally collected from commercial trappers in Uganda in November 2017. The eggs were incubated in two-litre rectangular plastic containers (L220mm×W156×H82mm, Kenpoly) which were ventilated at the top. The incubation containers were placed on a table in the rearing room and regularly sprinkled with water to avoid desiccation. At the onset of hatching of nymphs, incubation containers were transferred to rearing cages (42 cm × 42 cm × 42 cm) which were placed on tables that were elevated at one metre above the ground. The nymphs had *ad libitum* access to food and water. They were fed on fresh cutting of *Panicum maximum* and formulated artificial diet. The grass cuttings were presented in cylindrical plastic containers (9 cm high and 5 cm diameter) that were half filled with water and the top part sealed with cotton wool to prevent the nymphs from drowning. The diet mixture was presented in a petri dish while water was provided through water-soaked cotton wool placed in a Petri dish. The soaked cotton wool also served as a site for oviposition. Eggs from adult females were collected weekly and incubated in two litre plastic containers (220 mm long × 156 mm wide × 82 mm high: Rectangle Food Mate No.2, Kenpoly Manufacturers Limited, Nairobi, Kenya) that were lined with wet cotton wool and ventilated at the top. The eggs were regularly sprinkled with water to prevent desiccation while cages were cleaned weekly.

Experimental design

The experiment was set up in a prefabricated room (measuring 3.6 m × 2.9 m × 2.1 m) at 25-30°C, 60-75% RH, and a photoperiod of 12:12 hours (D: N). Fifteen newly emerged nymphs (1-3 days old) were introduced into each of the experimental cage designs, the number of nymphs in each cage was based on the total number of hatchlings obtained from the parent colony at the start of the experiment. At the onset of hatching the incubation containers were transferred to clean experimental cages where fifteen nymphs were released. The cages were placed on tables elevated at 1 meter above the ground. The cages were placed 20 cm away from the wall and 30 cm

away from each other. The nymphs were fed the novel artificial diet which was presented in petri dishes. The nymphs of *R. differens* in each of the experimental cages were provided with 5 grams of feed which was replaced on weekly basis. The quantity of feed residue was weighed before replacement. The feed was weighed using an electronic weighing scale (BEL Engineering, Serial No. IT 1500 073). The nymphs had ad libitum access to food and water. Water was provided in soaked cotton wool that was placed in three Petri dishes per cage. Soaked cotton wool was replaced after every three days.

3.3.3. Data collection

To determine the effects of cage types of wet adult weight, a total of five randomly selected *R. differens* from each treatment were weighed at the start of the experiment using an electronic weighing scale (BEL Engineering, Serial No. IT 1500 073). The nymphs were placed in a transparent plastic container (9 cm height × 6 cm diameter) and readings on the scale taken once there was no movement of *R. differens*. The weight of the nymphs was determined by subtracting weight of empty plastic container from the weight of container and the nymphs. Newly moulted adults were weighed individually within 24 hours of emergence using the same procedure. Duration of development was determined by number of days taken from egg hatching to adult moult (Lehtovaara et al., 2018). Daily growth rate was determined as increase in body weight (mg)/development time (days) (Huang et al., 2017). Survival rate was determined by percentage of individuals that survived to adult stage (Lehtovaara et al., 2018).

3.3.4. Data analysis

All data were subjected to Shapiro Wilk's and Bartlett test to assess for normality and homogeneity respectively prior to analysis. Data was analyzed using One way-ANOVA. Differences between means was separated using Tukey's Honest Significant Difference (HSD), post hoc test. All effects were considered significant at $P < 0.05$. All analyses were performed using R version 4.1.2 (R Core Team, 2020), statistical software.

3.4. Experiment 3: To assess efficacy of a novel trapping technology for mass harvesting of *R. differens*

3.4.1. Study site

Efficacy of a novel trapping technology for mass harvesting of *R. differens*_was evaluated in Nyendo, Masaka district in Uganda during the April to June 2021 swarming season (Figure 4). Masaka occurs at an altitude of 1215 metres above the sea level, latitude of 0° 18' S and longitude of 31° 45' E. It has a bimodal rainfall pattern with mean annual rainfall ranging between 1100-1200 mm and a temperature range of 18-28℃ (Labu et al., 2021).

3.4.2. Study design

A completely randomized design was used with two treatments namely collapsible trap and local trap (**Error! Reference source not found.**) by three replicates.

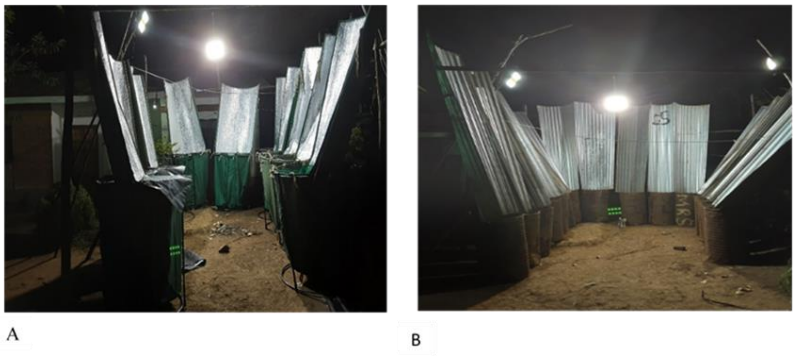

Figure 7: Photos of A) collapsible trap and B) local trapping technology (Photos: Kababu)

Design and development of a novel collapsible trap mass trap for harvesting of R. differens

A collapsible novel trap was designed and developed at ICIPE Duduville campus then produced with assistance of a local Jua Kali artisan. A sample design borrowed from the local trap was developed. This was modified severally until the desired design was achieved. The final sample was developed and several pieces produced. The local trap comprises of a collection drum (collects insect) and folded corrugated iron sheet (intercept insects in flight). The novel trap was made of collapsible parts that can be dismantled at the end of a swarming season. The novel trap comprised a collection bag which played the role of a collection drum; trapping surface for interception of flying

grasshoppers; upper and lower metal rings and metal rods to prop the trap as illustrated below (**Error! Reference source not found.**):

A

B

C

D

Figure 8: A) Structure and B-D) components of the novel trap (Photo: Muchichu H.)

The collection bag was made of canvas and shade net on the lower end of one side to allow for aeration of trapped grasshoppers. The lower part of the trapping surface was made of canvas while the upper part was made from a reflective PVC tarpaulin material. The canvas collection bag substituted the use of rusted metallic drums while the reflective PVC tarpaulin material replaced the rusted corrugate iron sheets that form

part of the local trapping technique. These materials were selected to reduce the safety concerns that are associated with the use of the rusted drums and corrugated iron sheets.

Piloting and modification of the trap

The novel trapped was piloted in a commercial *R. differens* harvesting stall in Nyendo, Masaka for two consecutive nights. Afterwards the angle of inclination of the trapping surface was adjusted to ensure that the traps fit into the commercial harvesting stalls. This also ensured that *R. differens* that fell on the trapping surface slid down into the collection bag soon after landing on it.

Experimental set up

The experiment was set up in three purposively selected commercial harvesting sites situated 250-300 metres apart to ensure homogeneity in environmental conditions (Silva et al., 2016). Two harvesting stalls spaced 50 m apart were used to set up the novel and local traps (control) respectively in each of the commercial harvesting sites.

The traps were arranged in a U-shape to allow access from the open end (Figure 9). Three LED lights bulbs (Tronic/AJ2 400 model No SL 2079-40-DL; MAX CE MP5440 0S 400W 6500K model no. HT 3003L; HiFi lite IP 66 400W) were fitted above the traps on Eucalyptus poles that were elevated at 6 m high and connected to a power source. The bulbs were placed at equidistance to provide uniform light for all traps. Trapping of *R. differens* was done each night from 1900 to 0500 h, East African Time for a period of thirty days. The traps were mounted every night and dismounted every morning.

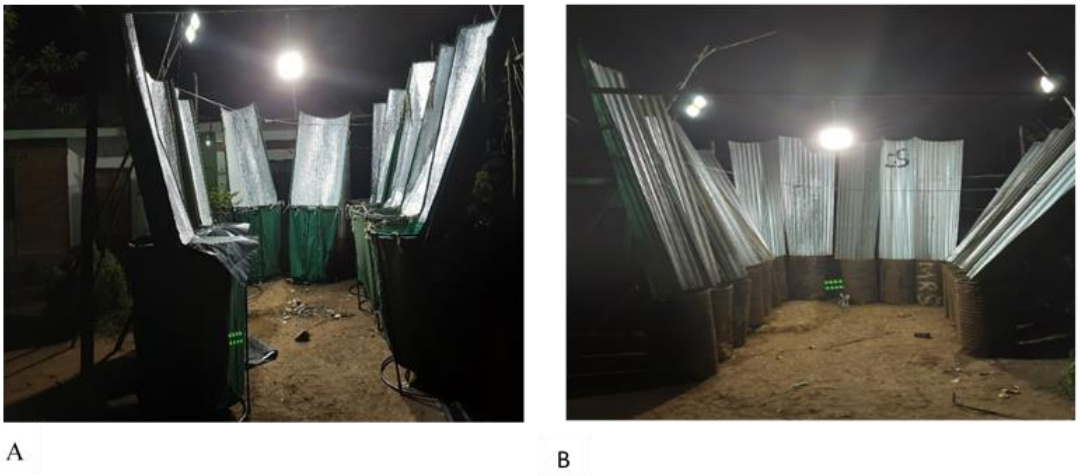

Figure 9: Set up of (A)novel and (B) local traps in commercial harvesting sites (Source: Kababu)

3.4.3. Data collection

After each trapping night starting from 0500 h to 0700 h, *R. differens* catches were collected from ten randomly selected drums/collection bags, separated into male and female and their numbers recorded. The numbers of non-targeted invertebrate species collected from the randomly selected drums/collection bags were also recorded.

The samples were then packed in Ziploc bags and stored under cold storage after which they were transported in cooler boxes filled with dry ice to ICIPE Nairobi where assessment of the gonotrophic status of female *R. differens* was determined. The gonotrophic status of female *R. differens* was determined by dissection of a third of females that were randomly selected from collected samples from each trapping night and the number of gravid female was recorded.

The collection and transportation of the grasshopper samples across the Kenya-Uganda border was facilitated by permits provided by Uganda's Ministry of Agriculture, Animal Industries and Fisheries Plant Quarantine and Inspection Services (License No: UQIS4471/93/PC (E)) and Ministry of Agriculture, Kenya Plant Health Inspectorate Services (KEPHIS), (Permit No: BIP/PS/22176/2021).

Effects of trapping technology on quantity of *R. differens* collected was determined by counting the number of grasshoppers collected from each of the selected drums/collection bag. The grasshoppers were separated into male and female based on the presence of the ovipositor. The proportion of male and female *R. differens* collected was determined by percentage of male and female respectively out of the total number of *R. differens* collected from each of the selected drums/collection bag. Proportion of gravid female was determined by percentage of gravid female collected out of total number of female collected from each of the selected drums/collection bags. Quantity of non-target invertebrate species was determined by counting the number of all the invertebrate collected from the selected drums/collection bags.

3.4.4. Data analysis

Total counts of *R. differens* and non-target species trapped were subjected to generalized linear models (GLM) with Poisson distribution error and logit link to determine the effects of trap on *R. differens* catches. Over dispersion (where the ratios of residual deviances to degrees of freedom were more than 1) was corrected by fitting

a negative binomial GLM. Data on proportion of male and female and gravid female were analysed using logit linked binomial GLM.

3.5. Experiment 4: To determine the nutritional composition of *R. differens* harvested from various geographical sites in Uganda

3.5.1. Study site

Raw *R. differens* were obtained from five different districts in Uganda: Kabale, Hoima, Mbarara, Kampala (Nakasero) and Masaka districts during the April to June 2021 swarming season (Figure 4). Nutritional analysis of *R. differens* samples was done at icipe Duduville campus, Nairobi, Kenya.

Collection of R. differens

Raw *R. differens* sample were purchased directly on-site from randomly selected commercial harvester from each of the five districts. The samples were processed by plucking the wings, legs and ovipositors. The samples had a mixture of the major morphotypes (brown, green, pink or brown infused with green coloured morphs.

Ruspolia differens samples were packed in polyethylene sterile Ziploc bags, labelled accordingly, packed with dry ice in cooler boxes and sealed hermetically then transported by road to the laboratory in icipe Duduville campus in Nairobi. *R. differens* samples were transferred into a freezer (Model: RZ41FARAEWW, Samsung, China) at *icipe* Duduville campus where they were stored at −20℃ until further analysis.

Sample preparation

A kilogram of frozen *R. differens* from each of the respective collection sites were allowed to thaw overnight under normal refrigeration at 5℃ and rinsed with water to remove dirt. The samples were spread out evenly on aluminium foil then oven dried (Model: SDO-225-CLAD-F-200 HYD, Wagtech Projects Ltd, Thatcham, UK) at 60 ℃ for 24 hours (Fombong et al., 2017; Ochieng et al., 2022). The dried samples were ground using an electronic blender (Preethi TRIO, 500w, MG182/00), packed into Ziploc bags and stored in a freezer at −20℃ prior to analysis at *icipe's* Behavioral and Chemical Ecology Unit (BCEU).

3.5.2. Data collection

Determination of Proximate composition of R. differens

Proximate parameters (Ash, crude protein, crude fat, moisture content) was determined using the official methods of Association of Official Analytic Chemists (AOAC, 2012).

The content of carbohydrates in each of the samples was estimated by subtracting the ash, moisture, fat and protein content from 100%. Total energy (kJ/100g) was computed using the formula: total energy = 4 × carbohydrate (%) + 4 × protein (%) + 9 × fat (%) (FAO, 2003).

Determination of fatty acids content of R. differens

Fat extraction from the samples was conducted by adding 1g of sample into 15ml falcon tube. The sample was mixed with 10ml DCM: MeOH (2:1) in a hood. The mixture was agitated in a vortex and a sonicator for 10 seconds and 20 minutes respectively then allow to stand for 1 hour. The mixture was centrifuged for 10 minutes at 4200 rpm then filtered into clean falcon tubes and allowed to evaporate overnight in a hood until all solvents evaporated and crude oil extract remained.

Fatty acids methyl esters (FAMEs) were extracted as previously described by Cheseto et al. (2020). A quantity of 300 mg of oil extract was weighed into clean narrow neck vials. One and a half millilitres of sodium methoxide prepared by dissolving 2g of sodium methoxide into 20ml of dry methanol was added to the sample. The mixture was vortexed for 1 minute, sonicated for 10 minutes then incubated in a water bath at 70 ℃ for 1 hour. Distilled deionized water (100 µL) was added into the mixture to quench the reaction then vortexed for 1 minute. GC- grade hexane (1000 µL; Sigma-Aldrich, St. Louis, MO, USA) was used to extract resultant FAMEs. A millilitre of hexane was added to the mixture, vortexed for 20 seconds then transferred to Eppendorf tubes prior to centrifugation at 14,000 rpm from 20 min. A quantity of 100 µL of supernatant was filtered into clean vials and dried through anhydrous sodium sulphate on insert fitted tips followed by 900 µL hexane. The supernatant was analysed using gas chromatography and mass spectrometry (GC-MS) on a 7890A gas chromatograph linked to a 5975C mass selective detector (Agilent Technologies Inc, Santa Clara, CA, USA). Analysis for each sample was done in triplicates. The GC instrument conditions were as described by Cheseto et al. (2020).

Determination of amino acids composition of R. differens

The amino acid composition of *R. differens* collected from the diverse localities were analysed using a modified protocol previously described by Musundire et al. (2016). A quantity of 100mg of each sample was weighed into a 5ml vial followed by 1.5 ml of 6N HCL. The vial was capped following the introduction of nitrogen then vortexed for

one minute. The samples were then placed in a GC oven at 110℃ for 24 hours to allow for complete hydrolysis. The samples were evaporated to dryness in a vacuum then reconstituted in 1 ml 90:10 water: acetonitrile. The mixture was vortexed for 30 seconds, sonicated for 30 minutes then centrifuged at 14,000 rpm for 20 min. The supernatant was transferred into 1.5 ml vials and analysed using UPLC-MS/MS. An ACQUITY UPLC BEH C18 column (2.1 mm × 150 mm, 1.7 µm particle size; Waters Corp, Wexford, Ireland, oven temperature 45℃) was used to perform chromatographic separation of the samples. Each of the samples were analysed in triplicates. Instrument conditions are as described by Musundire et al. (2016).

Mineral analysis of R. differens samples

Mineral composition was determined using ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometer) analysis (Campbell & Plank, 1991; Horwitz, 2000). A quantity of 0.5g of ground and thoroughly homogenised grasshopper samples were mixed with 8.0 ml of concentrated nitric acid and 2 ml 30% hydrogen peroxide in a digestion tube then left to stand overnight in a fume hood. The digestion tube was placed into a block digester (Model: The BD50/BD28 Series Bock Digestion System, Seal Analytical limited) for serial digestion at 75, 120, 180 and 200 ℃ at an interval of 30, 20, 20, and 10 minutes respectively, until the solution was clear with no debris. The digestion tubes were removed from the block digester and allowed to cool. The digest was quantitatively transferred to 25 and 50ml falcon tubes then diluted to the mark with 2% Nitric acid then taken to the ICP-OES equipment (Model: Optima 2100DV, PerkinElmer) for mineral quantification. The operating conditions for ICP-OES equipment were as described by Ochieng et al. (2022). The samples were analysed in triplicates.

Determination of vitamin content of R. differens

Composition of water soluble vitamins was determined by Liquid Chromatography-Diode array detector (Thermo Fisher Scientific, 2010). Ground sample (100mg) was weighed in a 50ml falcon tube and mixed with 25ml of distilled water. The mixture was ultra-sonicated for 15 minutes then filtered into UPLC (Ultra Performance Liquid Chromatography) vials through 0.2 µm filters. The vials were capped and loaded into the UPLC autosampler for analysis. Stock solutions of 1.0mg/ml were prepared by dissolving individual water soluble vitamins in distilled water with the exception of Vit B2 and Vit B9 which were dissolved in 5mM potassium hydroxide and 20mM

potassium hydrogen carbonate respectively. Four calibration standards were prepared from the mix at a concentration of 2, 5, 10 and 15 µg/ml. Chromatography was performed using Nexera Liquid Chromatograph LC-30AC with Nexera column oven CTO-30A with the following specifications: Detector, Diode Array Detector; Column, Phenomenex Synergi 2.6um plar C18-100 mm × 3.0mm; Column Oven temperature, 30 ℃; LC program of 12 min Run time, Mobile Phase A: 25mM Phosphate buffer, Mobile Phase B: 7:3 v/v Acetonitrile-Mobile phase A; Flow Rate of 0.4ml/min and distilled water as Column Flushing Solution. Extraction, detection, identification and quantification of each sample was done in triplicate.

Composition of fat soluble vitamins (retinol and tocopherols) was determined by High Performance Liquid Chromatography (HPLC) (Hosotani and Kitagawa 2003; Bhatnagar-Panwar et al., 2013). A quantity of 0.5 g sample of ground grasshopper was weighed in a 25ml tube in triplicate, mixed with 6ml ethanol with 0.1% BHT (Butylated Hydroxytoluene) and homogenized for a minute. 120 µL of potassium hydroxide 80% (w/v) was added to the solution and mixed by vortexing. The mixture was incubated for 5 minutes at 85 ℃, removed from the water bath and immediately cooled in ice. 4 ml of deionized water was added to each tube then mixed in a vortex. 5 ml of hexane was added to the tubes then mixed in a vortex. The sample was centrifuged for 5 minutes at 3000rpm. The upper phase (hexane) was transferred to centrifuge tube using Pasteur pipette. This was extracted three more times with 4 × 3 × 3 ml hexane and the extract pooled into 25ml tube. Additional 5ml deionized water was added to the extract, vortexed for a minute at centrifuged at 3000rpm for 5 minutes. The hexane layer was recovered into clean test tubes then evaporated to complete dryness under nitrogen in the N-Evap. This was then reconstituted 1 ml of methanol: tetrahydrofuran (85:15 v/v), vortexed and sonicated for 30 seconds then transferred to 0.8ml HPLC vials. HPLC system used was Shimadzu Nexera UPLC system linked to SPD-M2A detector with Reverse phase gradient HPLC method with the following specifications: Oven temperature, off; YMC C30, carotenoid column (3 µm, 150 × 3.0mm, YMC Wilmington, NC); injection volume, 10 µl; Mobile phase A: Methanol/tert-butyl methyl ether/water (85:12:3, v/v/v, with 1.5% ammonium acetate in the water); Mobile phase B: methanol/tert-butyl methyl ether/water (8:90:2, v/v/v, with 1 % ammonium acetate in the water and a total flow rate of 0.4ml/min. Extraction, detection, identification and quantification of each sample was done in triplicate.

Determination of flavonoids

Flavonoid content of *R. differens* samples was determined using Aluminium Chloride calorimetry (Singleton & Rossi, 1965; Zhishen et al., 1999; Dewanto et al., 2002). Sample extraction was done by weighing 0.5g into a clean propylene tubes. This was followed by addition of 10ml of 80% methanol then shaking in a mechanical shaker at 25 ℃ for 24 hours. The mixture was centrifuged for 10 minutes at 4000rpm and the supernatant aliquot taken out for determination of total flavonoids. Approximately 20 µl aliquot of sample extract or standard solution of catechin (10, 20, 40, 60, 80 and 100 µg/ml) was pipetted into microtiter well then mixed with 80 µl of deionized distilled water. Ten microlitres of 5% $NaNO_2$ was added to the mixture and mixed by priming. After 5 minutes 10 µl of 10% $ALCL_3$ was added and mixed gently by priming. Five minutes later 80 µl of 2 M NaOH was added to the mixture and mixed gently by priming. The reaction was incubated at room temperature for 30 minutes. The absorbance of the samples and standards were read against the reagent blank using a UV-visible spectrophotometer at a wavelength setting of 510 nm. Standard calibration (0.01-0.02-0.04-0.06-0.08-0.1 mg/ml) curve of catechin was plotted in 80% methanol. Extraction, detection, identification and quantification of each sample was done in triplicate.

3.5.3. Data analysis

All analyses were performed using R version 4.1.2 (R Core Team 2020), statistical software. Shapiro Wilk's and Bartlett's tests were used to test for normality and homogeneity of data respectively. All normal and homogenous data were subjected to One-way analysis of variance (one-way ANOVA) to test for differences in nutritional composition of *R. differens* collected from different sites. Non normal data were subjected to log transformation prior to ANOVA. Mean separation was done using Tukey's Honest Significant Difference (HSD), post hoc test where the differences were significant. All effects were considered significant at $P < 0.05$.

CHAPTER FOUR: RESULTS

4.1. Introduction

This chapter presents the finding of the study. The findings are discussed based on the study objectives. Tables and figures are used to display some of the study findings.

4.2. Effects of diet on growth, survival and reproductive performance of *R. differens*

Acceptance and preference of diet substrates by R. differens

In no choice experiment, all the five diet substrates were accepted by *R. differens* in diverse quantities. The acceptance of the diets differed significantly ($\chi^2 = 34.85$, df = 4. $P < 0.001$). Maize and wheat bran were the most accepted while soybean was the least accepted feed substrate (Figure 10A). There were no differences in the acceptance of maize and wheat bran (P = 0.94); and MOLP and *ochong'a* (P = 0.78) by *R. differens.*

In the choice test, feed preference by *R. differens* differed significantly among substrates ($x^2 = 33.29$, df = 4, $P < 0.001$). Maize bran was the most preferred followed by wheat bran, Moringa, *ochong'a* then Soybean meal (Figure 10B). However, *R. differens* preference for MOLP and *ochong'a* (P = 1.00); Soy bean meal and MOLP (P = 1.00), Soy bean meal and *ochong'a* (P = 1.00) and soybean and wheat bran (P = 0.09) were not significantly different.

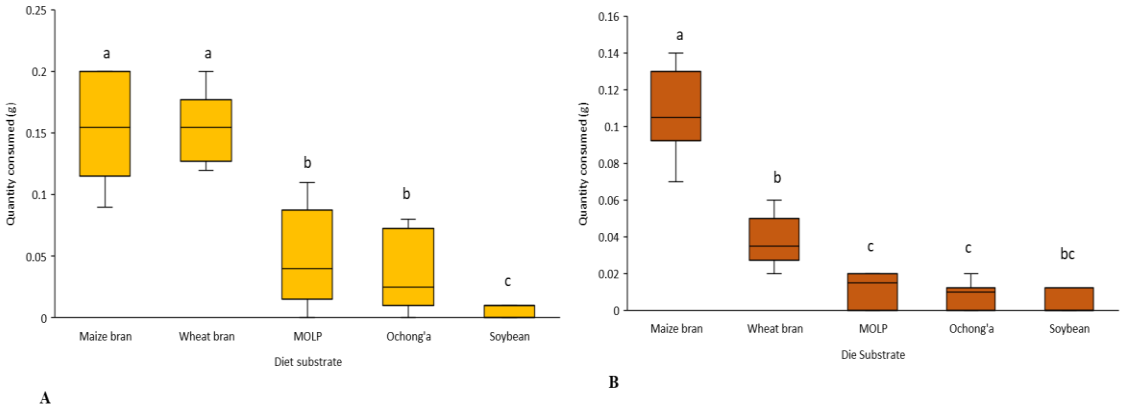

Figure 10: (A) Acceptance and (B) preference of different diet substrates by Ruspolia differens ranging from the most to the least preferred. Box plots capped with different letters differed significantly at P < 0.05

Nutritional composition of the diets used for rearing R. differens

Diet 3 contained significantly higher protein (27.5±0.4%) compared to the other diets ($P < 0.001$). Similarly, Diet 4 contained significantly higher quantities of carbohydrates ($P < 0.001$), crude fat ($P < 0.001$) and energy ($P = 0.04$) compared to the other experimental diets (Table 3). The digestibility of Diet 1 was significantly lower than other diets (67.2 ± 1.0%) ($P <0.001$). Diet 1 had significantly lower quantities of micro minerals including iron (185 ±2.9mg/kg), copper (6.73±0.3mg/kg), Zinc (50.4±0.3 mg/kg), Cobalt (0.08 mg/kg), Manganese (64.2± 0.6mg/kg) and Sodium (51.3±1.2 mg/kg). Diet 2 contained the highest quantity of Iron (882±4.16 mg/kg), copper (21.6±0.4 mg/kg), Manganese (92.9 ± 0.4 mg/kg) and Sodium (1203 ±8.8 mg/kg). The quantity of Sulphur, Magnesium, Potassium, Phosphorous and Calcium was less than 2% in all the experimental diets.

Table 3: Nutritional composition (% DM basis) of diets used for rearing *Ruspolia differens.*

Nutritional composition	**Diet/treatment types**				**P value**
	Diet 1	**Diet 2**	**Diet 3**	**Diet 4**	
Energy Kcal/kg	13.5 ± 0.3^{ab}	12.6 ± 0.1^{ab}	12.1 ± 0.62^{a}	14.9 ± 0.87^{b}	0.035
Carbohydrate%	16.8 ± 0.2^{a}	11.6 ± 0.3^{b}	11.6 ± 0.37^{b}	26.4 ± 0.68^{c}	0.001
Crude Fat (%)	5.3 ± 0.0^{a}	4.4 ± 0.2^{b}	7.09 ± 1.15^{c}	9.07 ± 0.07^{d}	0.001
Crude Protein (%)	16.7 ± 0.2^{a}	25.4 ± 0.4^{b}	27.5 ± 0.4^{c}	21.3 ± 0.6^{d}	0.001
Dry matter (%)	91.6 ± 0.4^{a}	91.5 ± 0.4^{a}	90.3 ± 0.7^{a}	91.5 ± 0.6^{a}	0.342
Ash (%)	6.3 ± 0.2^{a}	10 ± 0.6^{b}	9.1 ± 0.2^{b}	8.7 ± 0.3^{b}	0.001
Fibre (%)	11.4 ± 0.2^{a}	12.3 ± 0.2^{a}	15.7 ± 0.3^{b}	3.5 ± 0.3^{c}	0.001
ADF (%)	19.1 ± 0.1^{a}	16.4 ± 0.3^{b}	15.8 ± 0.4^{b}	19.7 ± 0.4^{a}	0.001
NDF (%)	31.6 ± 0.3^{a}	22.7 ± 0.2^{b}	21.2 ± 0.17^{b}	23.1 ± 1.4^{b}	0.001
Digestibility (%)	67.2 ± 1.0^{a}	72.8 ± 0.2^{b}	74.6 ± 0.4^{b}	74.4 ± 0.4^{b}	0.001
Boron (Mg/Kg)	8.3 ± 0.0^{a}	7.8 ± 0.1^{a}	10.9 ± 0.1^{b}	9.7 ± 0.2^{c}	0.001
Molybdenum (Mg/Kg)	1.4 ± 0.2^{a}	1.3 ± 0.3^{a}	1.4 ± 0.1^{ab}	1.6 ± 0.0^{b}	0.005
Iron (Mg/Kg)	185 ± 2.9^{a}	882 ± 4.2^{b}	685 ± 3.3^{c}	553 ± 3.4^{d}	0.001
Copper (Mg/Kg)	6.7 ± 0.3^{a}	21.6 ± 0.4^{b}	18.6 ± 0.4^{b}	13.7 ± 1.2^{c}	0.001
Zinc (Mg/Kg)	50.4 ± 0.3^{a}	74.8 ± 2.6^{b}	71.9 ± 1.0^{b}	95.3 ± 1.1^{c}	0.001
Cobalt (Mg/Kg)	0.1 ± 0.0^{a}	0.3 ± 0.0^{b}	0.4 ± 0.0^{c}	0.5 ± 0.0^{d}	0.001
Manganese (Mg/Kg)	64.2 ± 0.6^{a}	92.9 ± 0.4^{b}	88.1 ± 1.0^{c}	80.7 ± 0.7^{d}	0.001
Sodium (Mg/Kg)	51.3 ± 1.2^{a}	1203 ± 8.8^{b}	939 ± 4.6^{c}	758 ± 9.0^{d}	0.001
Sulphur (%)	0.4 ± 0.0^{a}	0.5 ± 0.0^{b}	0.4 ± 0.0^{ab}	0.3 ± 0.0^{c}	0.001
Magnesium (%)	0.4 ± 0.0^{a}	0.4 ± 0.0^{a}	0.3 ± 0.0^{ab}	0.3 ± 0.0^{b}	0.002
Potassium (%)	1.4 ± 0.0^{a}	1.5 ± 0.2^{a}	1.6 ± 0.2^{a}	0.9 ± 0.0^{a}	0.076

Phosphorus (%)	0.7 ± 0.0^{a}	0.8 ± 0.1^{ab}	0.7 ± 0.0^{a}	1.0 ± 0.0^{b}	0.011
Calcium (%)	0.5 ± 0.0^{a}	1.5 ± 0.1^{b}	1.4 ± 0.0^{b}	1.0 ± 0.0^{c}	0.001

ADF: Acid detergent fiber; NDF: Neutral detergent fiber. Mean values in the same row with different superscript letters differ at $P < 0.05$

Effect of diets on developmental duration of R. differens

Nymphal development period varied significantly among the diets ($F_{3, 8} = 9.52$, $P = 0.01$) (Table 4). The shortest mean development duration occurred grasshoppers fed in Diet 2 (57.2 ± 3.2) and Diet 3 (57 ± 2.18 days) while the longest duration occurred in grasshoppers reared on Diet 1 (75 ± 2.06). No variations were observed in development time of male grasshoppers raised on different diets ($F_{1, 8} = 3.92$, $P = 0.06$), however, females fed Diet 2 and Diet 3 matured faster than those raised on Diet 1 ($F_{1, 8} = 8.94$, $P = 0.01$).

Table 4: Mean (±SE) development duration of *Ruspolia differens* reared on the different diets

Diet	Overall developmental period (days)	Developmental period by sex (days)	
		Male	Female
Diet 1	75.4 ± 2.1^{a}	70.4 ± 1.9	78.7 ± 0.3^{a}
Diet 2	57.2 ± 3.2^{b}	54.7 ± 5.0	59.3 ± 3.9^{b}
Diet 3	57.2 ± 2.2^{b}	52.9 ± 1.0	61.3 ± 2.3^{b}
Diet 4	64.5 ± 2.9^{ab}	60.8 ± 4.0	68 ± 3.6^{ab}
P-value	<0.00l		0.01

Note: Mean (± SE) number of days with different superscript letter (s) in the same column are significantly different at $P < 0.05$.

Effect of diet on survival rate of R. differens nymphs to adult moult

A significant difference was observed in the rate of survival of nymphs to adults when *R. differens* was fed on the four diets ($\chi^2 = 62.184$, df = 3, $P < 0.001$). Survival was highest in grasshoppers fed on Diet 3 (87%) and lowest where Diet 1 was used (33%) (Figure 11A). Survival of female grasshoppers was higher compared to survival of males when they were fed Diet 1, 2 and 3. However, more male than female *R. differens* survived when fed on Diet 4 (Figure 11B).

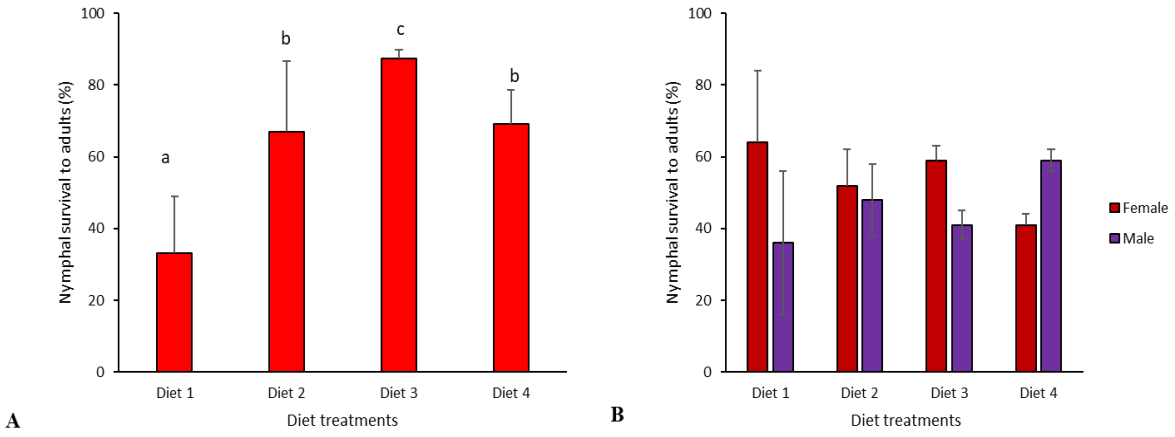

Figure 11: (A) Overall survival rate (%), (B) survival rate (%) by sex of Ruspolia differens nymphs to adults when reared on different diets. Error bars capped with different letters differed significantly (Tukey HSD, p <0.05)

Effects of diet on the rate of cannibalism among R. differens nymphs

There was significant variation in the rate of cannibalism of *R. differens* nymphs reared on diverse study diets (χ^2 = 54.27, df=3, P < 0.001). The rate of nymphal cannibalism was significantly higher in *R. differens* reared on diet 1 and 2 compared to diet 3 and 4. However, diet 1 and 2, and diet 3 and 4 were not statistically different. Nymphal cannibalism was highest in grasshoppers reared on Diet 1 (50 ± 18.9%) and least in Diet 3 (9.8 ± 3.5%) (Figure 12).

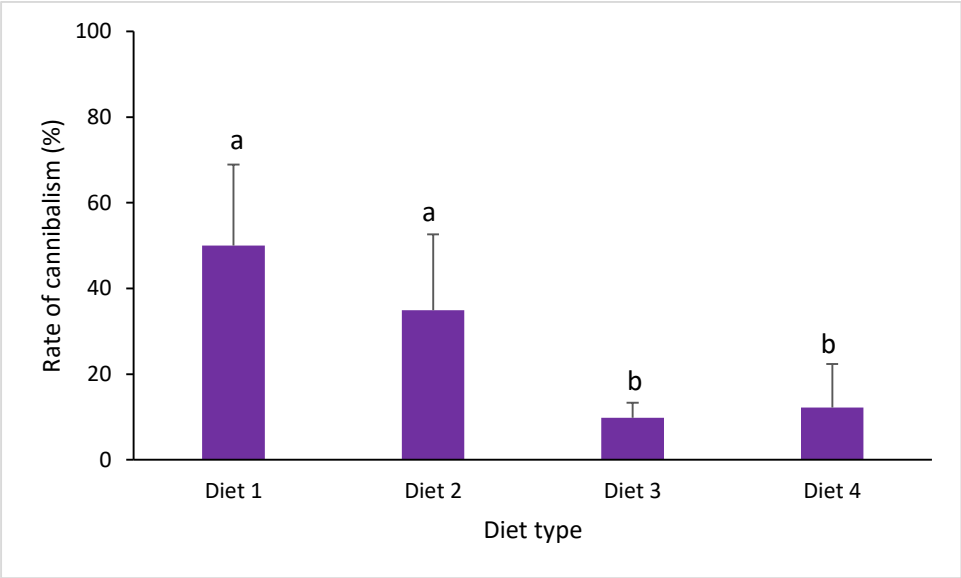

Figure 12: Rate of cannibalism among R. differens nymphs reared on different diet types. Error bars capped with different letters are significantly different (Tukey HSD, p <0.05)

Effect of diet on wet weights of R. differens

R. differens fed on Diet 4 maintained the highest average increase in weight throughout the nymphal development period (Figure 13:A) and recorded the highest average fresh adult weight (0.58g) while those fed on Diet 1 had the least weight (0.45g) (Figure 13:B). However, wet adult weights did not vary significantly among *R. differens* fed on different diets ($F_{3,8} = 0.95$, $P = 0.46$). The wet weight of female grasshoppers was higher than the weight of males ($F_{1, 18} = 34.81$, $P < 0.001$). Weight of the male grasshoppers differed among diets ($F_{3,8} = 6.66$, $P = 0.02$) while there was no difference among the female ($F_{3,8} = 2.56$, $P = 0.12$). The highest male (0.5g) and female (0.66g) weights were recorded in *R. differens* fed Diet 4.

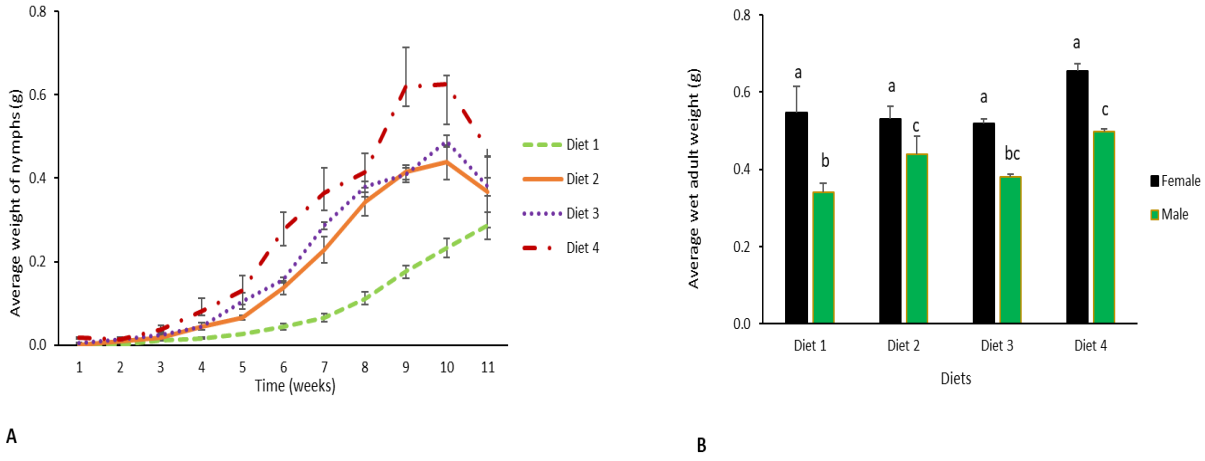

Figure 13: (A) mean weekly wet weights of nymphs over a period of 11 weeks and (B) mean wet weights by sex of adult R. differens raised on different diets. Error bars capped with different letters differed significantly (Tukey HSD, p <0.05)

Effect of diet on reproductive performance of R. differens

Effect of diet on pre-oviposition period of R. differens

Preoviposition time differed among *R. differens* raised on different diets ($\chi^2 = 11.11$, df = 3, P = 0.01). The shortest pre-oviposition time occurred in *R. differens* fed Diet 4 (13 days) while longest time was observed in Diet 1 (28 days).

Effect of diet on oviposition duration of R. differens

Duration of oviposition ($\chi^2 = 36.59$, df = 3, $P < 0.001$) differed significantly among *R. differens* fed the different diets. The longest oviposition duration occurred in *R. differens* fed Diet 3 (54 days) while those fed Diet 2 oviposited over the shortest period (36 days) (Table 5).

Effect of diet on post-oviposition period of R. differens

A significant difference was recorded in the post-oviposition duration of *R. differens* fed on various diets ($\chi^2 = 14.45$, df = 3, $P < 0.01$). The shortest post oviposition duration occurred in Diet 2 (< 1 day) while the longest duration was recorded in *R. differens* fed Diet 4 (16 days) (Table 5).

Table 5: Mean (±SE) preoviposition, oviposition and post oviposition duration of *Ruspolia differens* reared on different diets

Diet	Pre-oviposition period (days)	Duration of oviposition (days)	Post-oviposition duration (days)
Diet 1	27.9 ± 2.8^{a}	41.7 ± 9.6^{a}	7.4 ± 5.0^{a}
Diet 2	13.6 ± 0.4^{b}	36.4 ± 9.2^{b}	0.1 ± 0.1^{b}
Diet 3	16.5 ± 1.6^{ab}	53.5 ± 7.0^{a}	13.6 ± 7.0^{a}
Diet 4	13.1 ± 0.8^{b}	39 ± 8.1^{a}	15.6 ± 3.4^{a}
P-value	0.01	< 0.001	< 0.01

Note: Mean (± SE) number of days with different superscript letter (s) in the same column are significantly different at $P < 0.05$.

Effect of diet on fecundity and hatchability of eggs of R. differens

Average fecundity differed significant between treatments ($\chi^2 = 11.82$, df = 3, $P = 0.01$). The highest fecundity occurred in Diet 3 (248 eggs) while the lowest fecundity was recorded in Diet 4 (77 eggs) (Figure 14A). Percentage hatchability of eggs varied significantly among *R. differens* fed on different diets ($\chi^2 = 461.86$, df = 3, $P < 0.001$). The highest percentage hatchability of eggs was recorded in Diet 2 (52%) while the lowest hatchability was recorded in Diet 1 (15%) (Figure 14B).

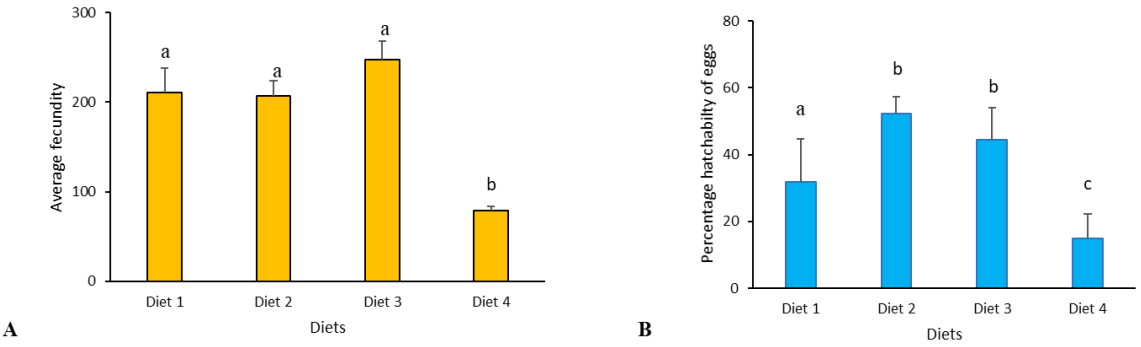

Figure 14:(A) Average female fecundity and (B) percentage hatchability of eggs of Ruspolia differens fed on the different diets. Error bars capped with different letters differed significantly (Tukey HSD, p <0.05)

Egg incubation duration did not differ among diets (χ^2 = 5.92, df = 3, P = 0.12). The shortest incubation duration occurred in *R. differens* fed Diet 1 (21 days) while the longest duration was observed in Diet 4 (29 days). Duration of egg eclosion varied significantly among diets (χ^2 = 129.38, df = 3, P < 0.001). The shortest duration of egg eclosion occurred in Diet 4 (20 days) while the longest duration was recorded in Diet 1 (59 days) (**Table 6**).

Table 6: Mean (±SE) incubation and egg eclosion duration of *Ruspolia differens* raised on different diets

Diet	Incubation duration (days)	Duration of egg eclosion (days)
Diet 1	20.7 ± 0.4^{a}	59.2 ± 5.7^{a}
Diet 2	24.2 ± 0.9^{a}	51.9 ± 5.5^{a}
Diet 3	23.4 ± 0.7^{a}	56.2 ± 8.8^{a}
Diet 4	28.8 ± 1.5^{a}	19.6 ± 6.1^{b}
P-value	0.12	<0.001

Note: Mean (± SE) number of days with different superscript letter (s) in the same column are significantly different at P < 0.05.

Effect of diet on adult longevity

The overall adult longevity varied significantly among diets types ($F_{3, 36}$ = 4.02, P = 0.02) (Table 7). The highest longevity occurred in grasshoppers fed on Diet 3 (89 days) while the lowest was in *R. differens* fed Diet 2 (51 days. Male longevity differed significantly among the diets ($F_{3, 36}$ = 4.19, P = 0.01) with the highest longevity

occurring in male *R. differens* fed Diet 3 (97 days). Longevity of female *R. differens* was not different among diets ($F_{3, 36} = 0.91$, P = 0.45) (Table 7).

Table 7: Mean (±SE) overall adult longevity and adult longevity by sex of *Ruspolia differens* reared on various diets

Diet	Mean (± SE) number of days		
	Overall longevity of adults	Male longevity	Female longevity
Diet 1	66.8 ± 10.1^{ab}	60.1 ± 11.6^{ab}	73.4 ± 16.9^{a}
Diet 2	50.5 ± 6.2^{a}	51 ± 9.2^{a}	50 ± 8.79^{a}
Diet 3	88.9 ± 11.2^{b}	96.8 ± 8.6^{b}	80.9 ± 21^{a}
Diet 4	84.7 ± 8.3^{b}	89.7 ± 13.4^{ab}	79.8 ± 10.3^{a}
P-values	0.02	0.01	0.45

Note: Mean (± SE) number of days with different superscript letter (s) in the same column are significantly different at $P < 0.05$**.**

4.3. Effects of rearing cage designs on production of *R. differens*.

Effects of cage type on wet adult weight of *Ruspolia differens*

The average adult wet weight of newly moulted *R. differens* varied significantly among the three cage types ($F_{2, 6} = 4.21$, P = 0.02) (Figure 15). The highest weight occurred in *R. differens* reared in wooden cage (0.43 ± 0.01g) while netted cage had the least weight (0.34 ± 0.02g). The weight of *R. differens* reared in wooden cage was higher compared to those reared in netted cage (P = 0.02). However, there was no difference between wet adult weight of *R. differens* reared in wooden cage and plastic cage (P = 0.33) as well as those reared in netted cage and plastic cage (P = 0.36).

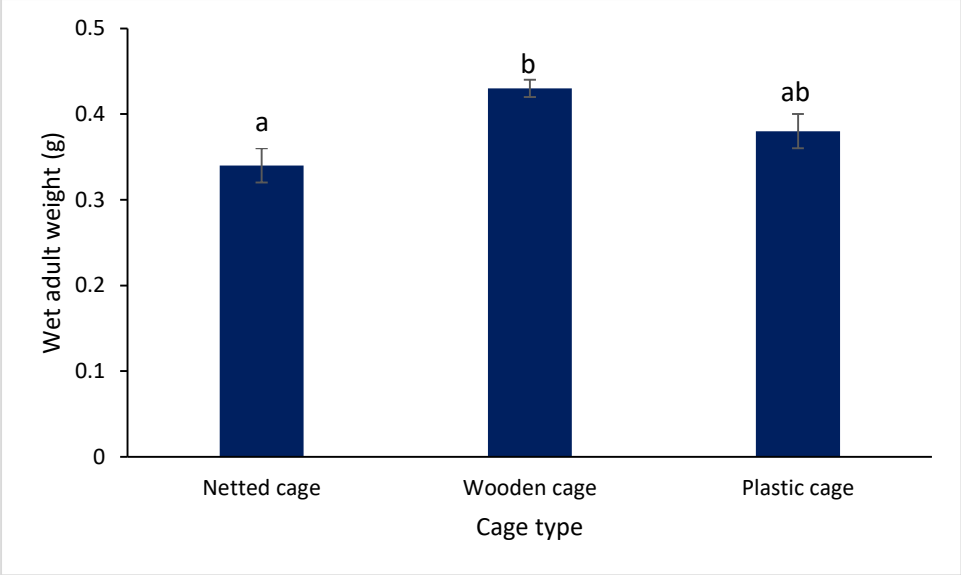

Figure 15: Mean (± SE) of adult wet weight of Ruspolia differens reared in different cage designs. Error bars with different letter (s) are significantly different (Tukey HSD test: P < 0.05).

Effect of Cage type on nymphal development time and growth rate of *Ruspolia differens*

Nymphal development time of *R. differens* was similar among different cage types ($F_{2, 6} = 2.60$, P = 0.24) (Figure 16A). Relatively faster development occurred in *R. differens* reared in wooden (51.6 ± 3.6 days) and plastic (51.8 ± 1.8 days) cages than in a netted cage (60.1 ± 4.1 days).

Similarly, daily growth rate of *R. differens* was not dependent on the type of cage used for rearing ($F_{2,6} = 3.26$, P = 0.11). The highest daily growth rate occurred among *R. differens* reared in a wooden cage (7.7 ± 0.2 mg/day) while the least growth rate was observed in the netted cage (5.4 ± 0.2 mg/day) (Figure 16B).

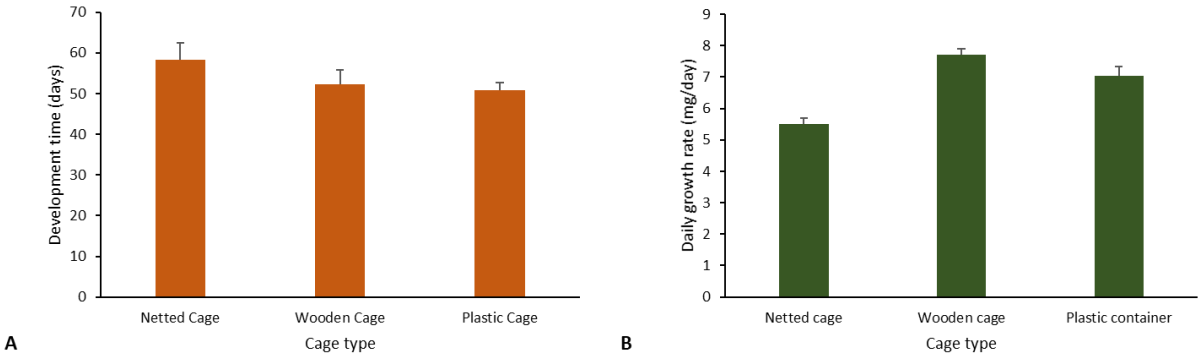

Figure 16:Mean (±) SE of (A) Nymphal development time (B) Daily growth rate of Ruspolia differens reared in netted, wooden and plastic cages

Effects of Cage design on survival rate of *Ruspolia differens* nymphs to adult moult

Cage type significantly influenced survival of *R. differens* nymphs to adulthood (χ^2 = 56.92, df =2, P < 0.001). *Ruspolia differens* reared in netted cage (63.3 ± 8.8 %) had the highest percentage of survival of nymphs to adult moult while the least survival was observed in *R. differens* reared in the wooden cage (33.3 ± 6.7 %) (Figure 17).

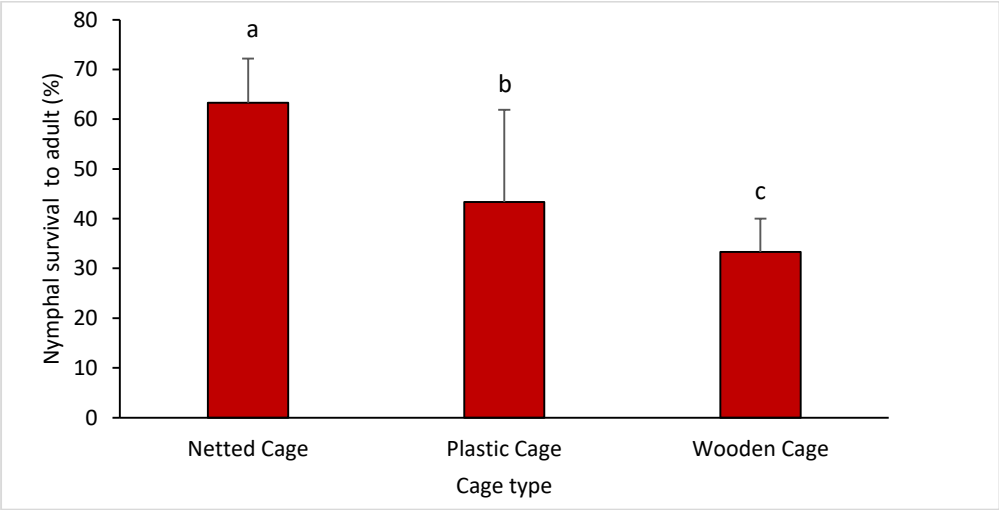

Figure 17: Mean (± SE) survival of R. differens nymphs to adult moult when reared in different cage types. Error bars followed by different letter (s) are significantly different (Tukey HSD test: P < 0.05).

4.4. Efficacy of the novel trapping technology for mass harvesting of *R. differens*

Effects of trapping technology on catches/collections of *R. differens*

A significantly lower number of *R. differens* was collected in the novel trap (7±1.8) compared to the local trap (20 ± 5.5) ($\chi^2 = 9.76$, df = 1, P < 0.01) (Figure 18).

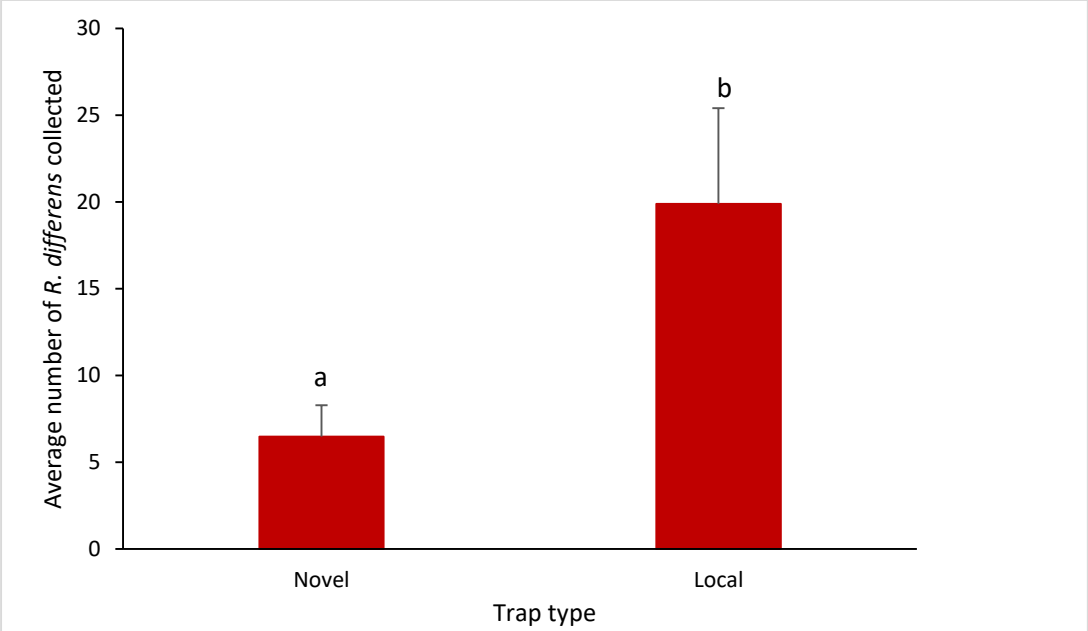

Figure 18: Mean (± SE) of number of R. differens collected using different trapping technologies. Error bars followed by different letter (s) are significantly different (Tukey HSD test: P < 0.05).

Effects of trapping technology on catches of male and female *R. differens* collected

Both novel and local traps collected more female *R. differens* compared to male. The novel trap (38 ± 4.0%) collected significantly fewer males compared to local trap (42 ± 2.8 %) ($\chi^2 = 7.44$, df = 1, P = 0.01). The local trap (57±2.8 %) collected significantly fewer females compared to the novel trap (60 ± 4.0 %) ($\chi^2 = 9.42$, df = 1, P < 0.01) (Figure 19).

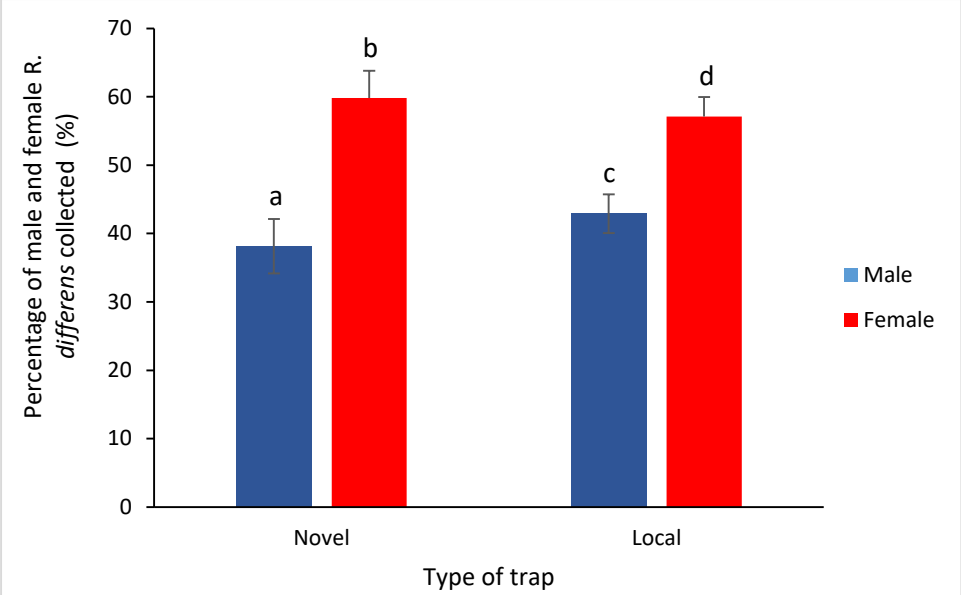

Figure 19: Mean (± SE) Percentage of male and female R. differens collected using a novel and local trap design. Error bars followed by different letter (s) are significantly different (Tukey HSD test: P < 0.05).

Effects of trapping type on collections of gravid *R. differens*

The proportions of gravid female *R. differens* collected were not dependent on the type of trap used ($\chi^2 = 1.76$, df = 1, P = 0.19) (Figure 20).

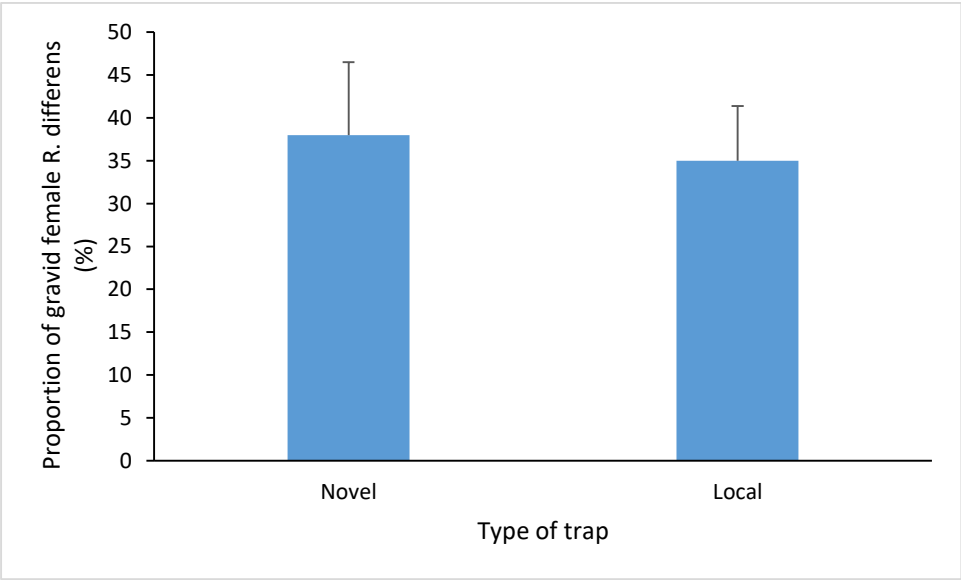

Figure 20: Mean (± SE) proportion of gravid R. differens collected using a novel and local trap design

Effects of trapping design collections of non-target invertebrate species

The novel trap collected significantly more non-target species compared to the local trap design (P = 0.01) (Figure 21). The non-target invertebrates collected alongside *R. differens* included different types of moths, mole crickets, ants, narrow bee fly, other grasshoppers' species, praying mantis, beetles and cockroach.

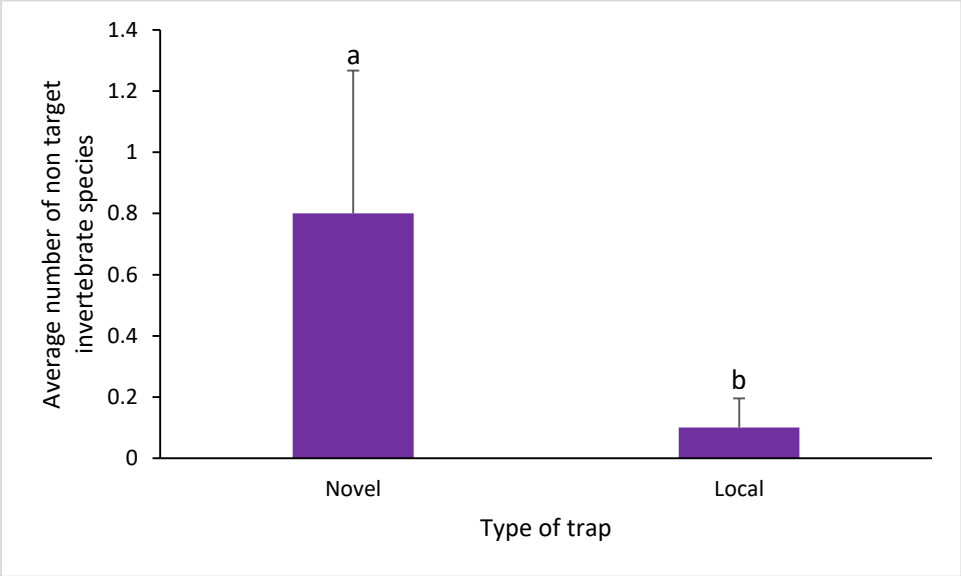

Figure 21: Mean (± SE) of non-target species collected using a novel and local trap design. Error bars followed by different letter (s) are significantly different (Tukey HSD test: P < 0.05).

4.5. Nutritional composition of *R. differens* harvested from various geographical sites in Uganda

Proximate composition of R. differens

Proximate composition of *R. differens* varied across the geographical locations of collection (Table 8). The highest crude protein, crude fibre and carbohydrates contents were recorded in *R. differens* obtained from Kabale (44.8 ± 0.3%), Mbarara (5.3 ± 0.1%) and Kampala (16.2 ± 1.1%) respectively. *R. differens* obtained from Masaka contained the highest energy (645 ±2.2 kj/100g) and moisture (5.3 ± 0.0%) content while those from Hoima recorded the highest ash (2.8 ± 0%) content.

Table 8: Proximate composition (% DM basis) of *R. differens* (mean ± SE) collected from different geographical locations

Component	Proximate composition (% DM)					p value
	R. differens collected from different locations					
	Hoima	Kabale	Masaka	Mbarara	Kampala	
Crude Protein	40.4 ± 0.4^{a}	44.8 ± 0.3^{b}	28.2 ± 1.7^{c}	40 ± 0.3^{a}	35.3 ± 0.3^{d}	< 0.001
Crude Fat	42.6 ± 0.8^{a}	41 ± 0.6^{a}	54.3 ± 0.4^{b}	46.7 ± 0.4^{c}	41.8 ± 0.7^{a}	< 0.001
Crude Fibre	5.0 ± 0.2^{ab}	4.7 ± 0.2^{ab}	4.6 ± 0.2^{a}	5.3 ± 0.1^{b}	4.5 ± 0.1^{a}	0.014
Carbohydrates	10.7 ± 1.2^{a}	8.6 ± 0.8^{a}	10.8 ± 1.4^{a}	7.5 ± 0.2^{a}	16.2 ± 1.1^{b}	0.001
Ash	2.8 ± 0^{a}	2.7 ± 0.0^{a}	1.4 ± 0.1^{c}	2.3 ± 0.0^{ab}	1.9 ± 0.1^{bc}	< 0.001
Moisture	3.5 ± 0.1^{a}	3.0 ± 0.1^{b}	5.3 ± 0.0^{c}	3.6 ± 0.1^{a}	4.7 ± 0.1^{d}	< 0.001
Energy (Kj/100g)	588 ± 3.6^{a}	583 ± 2.5^{a}	644 ± 2.2^{b}	610 ± 2.0^{c}	582 ± 3.3^{a}	< 0.001

Mean (± SE) in the same column followed by same letter (s) are not significantly different (Tukey HSD test: $P < 0.05$)

Fatty acid composition of R. differens

A total of 37 fatty acids were identified from oils extracted from *R. differens* samples analysed out of which 51%, 38% and 11% were saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) respectively (Table 9). The quantities of fatty acids varied significantly across the geographical areas of collection.

The most abundant SFA was Methyl 16-pentacosenoate (2558.34 ± 27.5 µg/g) which was detected in *R. differens* collected from Kabale ($F_{1,4} = 43266$, $p < 0.001$). Palmitic acid (methyl hexadecanoate) was the second most abundant SFA with quantities that differed significantly among *R. differens* collected from the different sites ($F_{4, 10} = 2024$, $p < 0.001$); higher quantities were recorded in *R. differens* collected from Masaka (1490.29 ± 68.4 µg/g) and Kampala (1053.22 ± 26.6 µg/g).

Oleic acid (Methyl 9Z-octadecenoate) was the most prevalent MUFA. A significantly higher amount of oleic acid was detected in *R. differens* collected from Masaka (1231.75 ± 47.7 µg/g) compared to the other locations ($F_{4, 10} = 1388$, $p < 0.001$). Linoleic acid was the most abundant PUFA with significantly high amounts ($F_{3, 8} = 2056$, $p < 0.001$) extracted from *R. differens* collected from Masaka (2322.43 ± 60.4 µg/g), Kabale (2228.11 ± 107.5 µg/g) and Mbarara (593.89 ± 40.7 µg/g).

Table 9: Mean (± SE) of Fatty acid composition (µg/g of oils) of *R. differens* collected from different geographical locations

Fatty acid methyl ester	Common name	Quantity of fatty acid methyl esters (µg/g) in *R. differens* collected from diverse location					P value
		Hoima	Kabale	Mbarara	Masaka	Kampala	
Saturated Fatty acids (SFA)							
Methyl docosanoiate	Behenic acid	402.5 ± 75.3^{a}	0.3 ± 0.0^{b}	2.4 ± 0.1^{c}	77.3 ± 32.8^{d}	0.1 ± 0.0e	<0.001
Methyl eicosanoate	Arachidic acid	32.0 ± 0.8^{a}	0.1 ± 0.0^{b}	27.8 ± 7.3^{a}	38.6 ± 6.7^{a}	ND	<0.001
Methyl heneicosanoate		797.0 ± 55.6^{a}	7.9 ± 0.6^{b}	9.3 ± 0.8^{b}	1.4 ± 0.0^{c}	1.0 ± 0.1^{c}	<0.001
3-methyl-heptacosanoiate		ND	6.7 ± 0.2^{a}	ND	ND	122.5 ± 3.5^{b}	<0.001
Heptadecanoic acid, 3-methyl-, methyl ester		514.9 ± 39.8^{a}	42.2 ± 3.2^{a}	ND	ND	ND	<0.001
Methyl 16-pentacosenoate		ND	2558.3 ± 27.5^{a}	1.9 ± 0.1^{b}	ND	ND	<0.001
Methyl 18-methylnonadecanoate		3.2 ± 0.1^{a}	8.2 ± 0.4^{a}	ND	ND	5.9 ± 0.1^{c}	<0.001
Methyl 2 4-methyl-hexacosanoate		ND	5.0 ± 1.0^{a}	1.1 ± 0.1^{b}	ND	0.2 ± 0.0^{c}	<0.001
Methyl dodecanoate	Lauric acid	5.1 ± 0.2^{a}	1892.2 ± 104.4^{b}	1141.5 ± 58.9^{c}	1.7 ± 0.1^{d}	0.6 ± 0.0^{e}	<0.001
Methyl hexadecanoate	Palmitic acid	4.1 ± 0.3^{a}	18.5 ±0.7^{b}	918.2 ± 91.6^{c}	1490.3 ± 68.4^{d}	1053.2 ± 26.6^{c}	<0.001
Methyl octadecanoate	Stearic acid	ND	ND	15.3 ± 0.8^{a}	8.5 ± 0.5^{b}	ND	0.001
Methyl tetradecanoate	Myristic acid	7.9 ± 0.6^{a}	9.6 ±1.4^{a}	7.7 ± 0.4^{a}	81.9 ± 4.4^{b}	3.8 ± 0.4^{c}	<0.001
Methyl nonadecanoate		3.7 ± 0.8^{a}	5.9 ±4.4^{a}	1323.3 ± 13.9^{b}	8.5 ± 0.4^{a}	6.9 ± 0.4^{a}	<0.001
Methyl octanoate		ND	74.2 ± 8.8^{a}	5.7 ± 0.3^{b}	0.2 ± 0.0^{c}	ND	<0.001
14-methyl-pentadecanoate		3.5 ± 0.8	ND	5.1 ± 0.1	ND	ND	0.14
Methyl pentadecanoate		6.5 ± 0.2^{a}	ND	10.2 ± 1.4^{a}	2.9 ± 0.4^{b}	27.0 ± 0.5^{c}	<0.001
Methyl tetracosanoate		4.5 ± 0.8^{a}	17.0 ± 1.1	1302.3 ± 37.7^{c}	4.3 ± 0.2^{a}	11.3 ± 1.8^{b}	<0.001
Methyl tricosanoate		3.1 ± 0.1^{a}	2.1 ± 0.1^{b}	4.2 ± 0.2^{c}	1.6 ± 0.1^{d}	2.7 ± 0.1^{a}	<0.001
Methyl tridecanoate	Tridecylic acid	ND	1.2 ± 0.0^{a}	3.0 ± 0.1^{c}	2.3 ± 0.1^{c}	1.9 ± 0.1^{d}	<0.001
∑SFA		1787.9 ± 175.4	4649.3 ± 153.8	4779 ± 213.8	1719.11 ± 114.1	1237.06 ± 33.6	

Monounsaturated Fatty acids (MUFA)							
Methyl hexadec-9-enoate	Palmitoleic acid	ND	61.5 ± 1.8^{a}	2.2 ± 0.1^{b}	175.4 ± 26.8^{c}	14.6 ± 0.7^{d}	<0.001
Methyl 9Z-hexadecenoate	elaidolinolenic acid	36.6 ± 2.7^{a}	ND	1.99 ± 0.1^{b}	ND	ND	<0.001
Methyl cis-13-eicosenoate	Puallinic acid	9.8 ± 1.7^{a}	ND	277.0 ± 95.0^{b}	ND	0.6 ± 0.0^{c}	<0.001
Methyl 11-octadecenoate	vaccenic acid	ND	0.03 ± 0.0^{a}	0.04 ± 0.0^{a}	5.0 ± 0.2^{b}	ND	<0.001
Methyl 13Z-docosenoate	Erucic acid	ND	26.1 ± 2.6^{a}	4.3 ± 1.2^{b}	3.9 ± 0.1^{b}	0.3 ± 0.0^{c}	<0.001
Methyl 6Z-octadecenoate	Petroselinic acid	0.2 ± 0.0^{a}	ND	0.4 ± 0.0^{b}	6.4 ± 0.2^{c}	ND	<0.001
Methyl 7-octadecenoate		ND	566.4 ± 22.3	ND	ND	ND	
Methyl 9Z-octadecenoate	Oleic acid	12.1 ± 0.6^{a}	35.7 ± 3.7^{b}	37.4 ± 1.8^{b}	1231.8 ± 47.7^{c}	2.6 ± 0.1^{d}	<0.001
Methyl cis-10-heptadecenoate		1.9 ± 0.1^{a}	3.9 ± 0.1^{b}	0.6 ± 0.0^{c}	10.4 ± 0.5^{d}	38.5 ± 1.5^{e}	<0.001
Methylcis-10-Nonadecenoate		0.9 ± 0.1^{a}	203.8 ± 8.7^{b}	223.2 ± 10.5^{b}	9.6 ± 0.4^{c}	0.8 ± 0.1^{a}	<0.001
Methyl cis-11-eicosenoate		15.3 ± 1.3^{a}	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}	22.1 ± 3.2^{a}	ND	<0.001
Methyl cis-13-octadecenoate		ND	1.6 ± 0.1^{a}	ND	13.6 ± 0.3^{b}	2.2 ± 0.1^{c}	<0.001
Methyl cis-9-tetradecenoate	Myristoleic acid	ND	233.2 ± 5.8^{a}	115.0 ± 4.1^{b}	ND	2.5 ± 0.0^{c}	<0.001
Methyl 11Z-tetradecenoate		ND	2.0 ± 0.2^{a}	ND	0.4 ± 0.1^{b}	ND	0.002
∑MUFA		76.7 ± 6.5	1134.2 ± 45.3	662 ±1 12.8	1478.4 ±79.5	62.03 ±2.4	
Polyunsaturated fatty acids (PUFA)							
3,7,11,15-tetramethyl- 6,10,14-Hexadecatrienoate [R-(E,E)]-	Phytanic acid methyl ester	2.4 ± 0.2^{a}	9.2 ± 0.6^{b}	ND	ND	ND	<0.001
Methyl (6Z,9Z,11E)-octadecatrienoate		ND	ND	ND	ND	1518.9 ± 177.6	
Methyl (9Z,11E,13E)-octadecatrienoate	α eleostearic acid	ND	12.7 ± 1.2^{a}	3.4 ± 0 .1^{b}	4.6 ± 0.1^{c}	3.7 ± 0.3^{bc}	<0.001
Methyl (9Z,12Z)-octadecadienoate	Linoleic acid	ND	2228.1 ± 107.5^{a}	593.9 ± 40.7 ^{b}	2322.4 ± 60.4^{a}	7.3 ± 0.2^{c}	<0.001
∑PUFA		2.41 ± 0.2	2250.0 ± 109.3	597.3 ± 40.8	2327.0 ± 60.5	1529.9 ± 178.1	

Mean (± SE) in the same row followed by the same letter (s) are not significantly different (Tukey HSD test: P < 0.05). ND: not detected

Amino acid composition of R. differens

Nine essential and eight non-essential amino acids were detected in *R. differens* samples analysed (Table 10). The quantities of methionine ($F_{4, 10} = 4.38$, $p = 0.03$) and lysine ($F_{4, 10} = 4.19$, $p = 0.03$) varied by area of collection while the quantities of the other essential amino acids did not differ. Quantities of proline ($F_{4, 10} = 3.52$, $p = 0.05$) and glycine ($F_{4, 10} = 3.66$, $p = 0.04$) varied by area of collection while the quantities of other non-essential amino acids were not different.

Table 10: Amino acid content of *R. differens* (Mean ± SE) collected from diverse geographical sites

Amino acid	Quantity of amino acids (mg/g)					
	R. differens collected from different locations					P value
	Hoima	Kabale	Mbarara	Masaka	Kampala	
Essential amino acids						
Leucine	99.4 ± 20.0	93.2 ± 25.7	52.8 ± 21.6	74.8 ± 23.2	92.4 ± 38.0	0.729
Arginine	4.3 ± 0.6	5.6 ± 0.1	2.9 ± 1.2	4.2 ± 0.7	5.3 ± 0.1	0.068
Lysine	4.6 ± 2.3^{a}	10.4 ± 0.3^{b}	4.0 ± 2.1^{a}	5.7 ± 1.5^{ab}	10.5 ± 0.1^{b}	0.030
Threonine	2.2 ± 0.3	4.2 ± 3.0	1.4 ± 0.4	1.5 ± 0.3	1.2 ± 0.0	0.532
Valine	4.1 ± 0.2	5.2 ± 0.3	3.0 ± 1.3	3.1 ± 0.4	5.5 ± 0.4	0.053
Histidine	2.6 ± 0.2	3.4 ± 0.1	1.7 ± 0.7	2.7 ± 0.5	3.3 ± 0.1	0.068
Isoleucine	9.9 ± 2.0	9.3 ± 2.6	5.3 ± 2.2	7.5 ± 2.4	9.2 ± 3.8	0.729
Methionine	1.5 ± 0.1^{ab}	2.3 ± 0.2^{ab}	1.2 ± 0.7^{ab}	1.0 ± 0.2^{a}	2.7 ± 0.3^{b}	0.026
Phenylalanine	3.5 ± 0.7	5.2 ± 0.6	3.3 ± 2.2	2.8 ± 1.1	8.7 ± 1.4	0.063
Non-essential amino acids						
Alanine	1.7 ± 0.6	0.8 ± 0.1	2.2 ± 1.2	1.7 ± 1.2	0.6 ± 0.1	0.655
Aspartic Acid	5.2 ± 0.6	3.2 ± 0.2	3.6 ± 0.9	3.5 ± 0.7	3.1 ± 0.1	0.171
Cysteine	0.4 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.0	0.842
Glutamic Acid	1.2 ± 0.2	1.8 ± 0.2	2.9 ± 1.1	1.6 ± 0.5	1.6 ± 0.1	0.327
Proline	4.2 ± 0.2^{abc}	5.7 ± 0.4^{a}	3.3 ± 1.0^{c}	3.8 ± 0.5^{bc}	5.4 ± 0.2^{ab}	0.048
Glycine	3.4 ± 0.2^{a}	2.1 ± 0.1^{bc}	2.2 ± 0.7^{bc}	3.1 ± 0.0^{ab}	2.0 ± 0.1^{c}	0.043
Tyrosine	3.4 ± 0.3	4.8 ± 1.0	2.5 ± 1.5	2.2 ± 0.5	5.9 ± 0.7	0.076
Serine	2.6 ± 0.2	1.6 ± 0.1	1.8 ± 0.6	2.3 ± 0.1	1.6 ± 0.1	0.095

Mean (± SE) in the same row followed by similar letter (s) are not significantly different (Tukey HSD test: $P < 0.05$)

Mineral composition of R. differens collected from different geographical locations

Several macro and micro minerals were obtained in varying quantities from *R. differens* collected from the five districts (Table 11). Potassium was the most abundant macro mineral (198-871 mg/100g) with significantly higher quantities obtained in *R. differens* collected from Kabale compared to the other locations ($F_{4, 10} = 177.9$, $p < 0.001$). Iron was the most prevalent micro mineral (7.2-155mg/100g), significantly higher quantities were recorded in *R. differens* collected from Hoima compared to other locations ($F_{4, 10} = 2435.5$, $p < 0.001$).

Table 11: Mineral content of *R. differens* (Mean ± SE) collected from different geographical locations

Mineral	Mineral content (mg/100g) *R. differens* from different locations					P value
	Hoima	Kabale	Masaka	Mbarara	Kampala	
Phosphorus	96.1 ± 4.7^{a}	99.4 ± 5.7^{a}	38.8 ± 0.4^{b}	99.2 ± 0.4^{a}	510 ± 18.4^{c}	< 0.001
Potassium	526 ± 12.9^{a}	871 ± 30.4^{b}	198 ± 6.6^{c}	641 ± 14.8^{d}	466 ± 18.8^{a}	< 0.001
Magnesium	54.6 ± 1.5^{a}	46.8 ± 0.8^{ac}	16.1 ± 1.1^{b}	47.1 ± 1.8^{ac}	44.5 ± 2.6^{c}	< 0.001
Calcium	50.1 ± 1.7^{a}	39.1 ± 0.4^{b}	19.3 ± 0.8^{c}	58.9 ± 2.6^{d}	33.2 ± 0.5^{b}	< 0.001
Sodium	65.7 ± 1.7^{a}	129 ± 6.6^{b}	27.4 ± 1.7^{c}	98.1 ± 5.1^{d}	56.7 ± 2.3^{a}	< 0.001
Aluminium	22.5 ± 1.1^{a}	15.9 ± 0.2^{b}	4.4 ± 0.2^{c}	17.4 ± 0.4^{b}	15.7 ± 0.8^{b}	< 0.001
Zinc	16.8 ± 0.5^{a}	11.1 ± 0.2^{b}	6.3 ± 1.9^{c}	12.3 ± 0.6^{b}	17.2 ± 0.5^{a}	< 0.001
Iron	155 ± 6.7^{a}	80.1 ± 1.7^{b}	7.2 ± 0.2^{c}	20.9 ± 1.1^{d}	30.5 ± 0.0^{e}	< 0.001
Copper	2.8 ± 0.2^{a}	2.7 ± 0.0^{a}	1.7 ± 0.1^{b}	2.3 ± 0.1^{c}	2.17 ± 0.1^{c}	< 0.001
Manganese	4.1 ± 0.1^{ab}	3.6 ± 0.1^{ac}	1.1 ± 0.1^{d}	4.7 ± 0.2^{b}	3.4 ± 0.1^{c}	< 0.001
Molybdenum	0.2 ± 0.0^{ab}	0.3 ± 0.0^{a}	0.2 ± 0.0^{b}	0.3 ± 0.0^{a}	0.1 ± 0.0^{c}	< 0.001

Mean (± SE) in the same row followed by letter (s) are not significantly different (Tukey HSD test: $P < 0.05$)

Vitamin content of R. differens collected from different geographical locations

Vitamin content of *R. differens* differed significantly by area of origin (Table 12). The most abundant vitamins obtained were pantothenic acid (165.2-248 mg/100g), folic acid (79-137.6 mg/100g) and pyridoxine (87.9-151.5 mg/100g). Significantly higher quantities of pantothenic acid were obtained in *R. differens* from Hoima ($F_{4, 10} = 25.2$, $P < 0.001$) while significantly higher amounts of folic acid ($F_{4, 10} = 285.6$, $p < 0.001$) and pyridoxine ($F_{4, 10} = 148.5$, $P < 0.001$) occurred in *R. differens* collected from Kabale.

Table 12: Vitamin content of *R. differens* (Mean ± SE) collected from different geographical locations

Vitamin	Vitamin content (mg/100g)					p value
	R. differens from different locations					
	Hoima	Kabale	Masaka	Mbarara	Kampala	
Vit A/Retinol	0.8 ± 0.0^{a}	0.3 ± 0.1^{b}	0.7 ± 0.0^{a}	1.0 ± 0.3^{c}	0.3 ± 0.1^{b}	< 0.001
Vit B2/Riboflavin	2.6 ± 0.2^{a}	1.3 ± 0.1^{b}	1.2 ± 0.1^{b}	6.3 ± 0.2^{c}	1.1 ± 0.0^{b}	< 0.001
Vit B3/niacin	26.4 ± 1.3^{a}	29.2 ± 1.1^{a}	ND	21.0 ± 0.5^{b}	ND	0.004
Vit B5/Pantothenic acid	239.9 ± 6.4^{a}	234.5 ± 12^{a}	174.5 ± 5.6^{b}	165.2 ± 1.9^{b}	248.1 ± 9.4^{a}	< 0.001
Vit B6 /Pyridoxine	101.7 ± 2.4^{a}	151.5 ± 2.8^{b}	93.4 ± 2.1^{ac}	87.9 ± 1.2^{c}	91.0 ± 2.0^{c}	< 0.001
Vit B9/folic acid	135.2 ± 2.4^{a}	137.6 ± 7.1^{a}	79.0 ± 0.8^{b}	134.3 ± 1.4^{a}	107.8 ± 1.4^{c}	< 0.001
Vit B12/cobamalin	3.2 ± 0.2^{a}	3.07 ± 0.1^{a}	2.2 ± 0.1^{b}	3.3 ± 0.2^{a}	2.0 ± 0.1^{b}	0.001
Gamma tocopherol	1.1 ± 0.0^{a}	1.1 ± 0.0^{a}	ND	ND	1.1 ± 0.0^{a}	0.288
α-tocopherol	0.5 ± 0.0^{a}	01 ± 0.0^{b}	0.1 ± 0.0^{b}	$0.1 \pm 0 .0^{b}$	0.5 ± 0.0^{a}	< 0.001

Mean (± SE) in the same row followed by letter (s) are not significantly different (Tukey HSD test: $P < 0.05$). ND: not detected.

Flavonoids content in R. differens obtained from different locations

The flavonoid content of *R. differens* varied by geographical location of collection ($F_{4, 10} = 11.84$, $P < 0.001$). Grasshoppers collected from Hoima (484 ± 11.4 mg/100g) contained the highest amount of flavonoids while those collected from Masaka (382 ± 11.8 mg/100g) contained the least quantity (Figure 22).

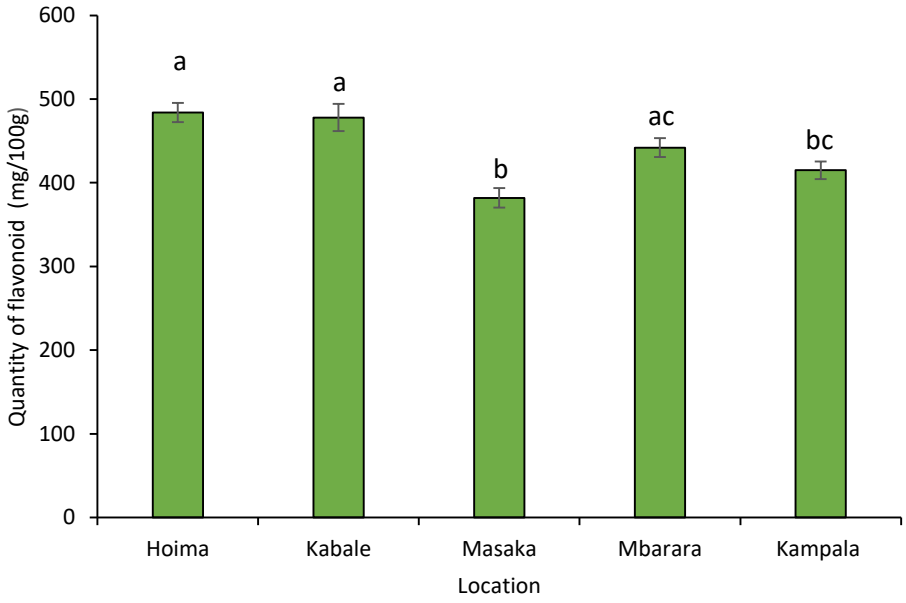

Figure 22: Mean (± SE) of flavonoid composition of R. differens collected from different geographical locations. Bars capped with different letters differed significantly (Tukey HSD test: $P < 0.05$)

CHAPTER FIVE: DISCUSSION

5.1 Effects of diet on growth, survival and reproductive performance of *R. differens*

The findings of this study demonstrate that variations in innovative feedstocks containing mixtures of agricultural byproducts, MOLP, *ochong'a* and soybean meal provided sufficient nutrients that influenced growth, survival and reproductive performance of *R. differens*. *R. differens* accepted all the diet substrates. However, agricultural byproducts (maize and wheat bran) were the most preferred. *Ruspolia differens* is known to accept diverse feed in the absence of its host plants with wheat bran reported as the most preferred feed (Malinga et al., 2018a; Valtonen et al., 2018). Diet 3 was the most optimal diet, which significantly reduced developmental time, improved survival above 87%, increased fecundity and extended longevity of *R. differens* probably due to its high nutritional value. Nutritional analysis of formulated diets showed that addition of MOLP, *ochong'a* and soybean meal increased the crude protein, crude fat and mineral content; and digestibility of Diet 3 compared to other diets. These diet substrates are rich in proteins and are increasingly used as alternative source of proteins for production of livestock feed (Mugo-Bundi et al., 2015; Mukherjee, et al., 2016; Ashour et al., 2020). High nutritional value of feed yields improved growth, development, survival and reproductive performance in insects (Lindroth et al., 1991).

A faster development occurred in *R. differens* raised on Diet 2 and 3 compared to Diet 1. Diet 2 and 3 had higher protein and minerals (sodium and iron) contents and higher digestibility compared to Diet 1 which could explain these variations. Proteins and carbohydrates are crucial in development of insects (Rho and Lee 2014; Roeder and Behmer 2014; Li et al., 2015). Diets with higher crude protein content yielded a shorter development time in crickets *Scapsipedus icipe* (Orthoptera: Gryllidae) and *Acheta domesticus* Linnaeus (Orthoptera: Gryllidae) (Magara et al., 2019; Kuo and Fisher 2022) while high protein and carbohydrate contents led to better growth and development rate in some grasshopper species (Malinga et al., 2018). t Poor diets on the other hand result in an increase in larval development time due to unmet nutritional demands in insects (Silva et al., 2009; Kekeunou et al.,2010; Li et al., 2020) which may have been the case in Diet 1. The poor performance of *R. differens* fed Diet 1 may also have been exacerbated by low sodium and high MOLP. Sodium deficiency is associated with decreased growth, survival and reproduction invertebrate herbivores while excessive use of MOLP is linked

with anti-nutritional factors that lower digestibility of diets in livestock (Chadare et al., 2018; El-hack et al., 2018; Peterson et al., 2021). Although diet 1 had a higher carbohydrate content compared to diet 2 and 3, the lower digestibility of the diet inhibited the release and uptake of this nutrient from the diet. The shorter development of *R. differens* observed in this study (57 days) compared to the development period of 100 days and 89 days reported by Malinga et al. (2018) and Leonard et al. (2022) respectively. However, these diets comprised of food crops among other ingredients which would lead to competition for limited food sources for human. Thus, Diet 3 which comprised of agro-byproducts among other non-food ingredients presents a suitable diet that can be optimized and utilized for domestication of the grasshopper.

Ruspolia differens raised on Diet 3 recorded the highest nymphal survival while the least survival occurred in Diet 1. These differences can be attributed to differences in nutritional value and diversity of diets. Diet 3 contained optimal quantity of nutrients such as proteins and minerals which may have increased the survival of *R. differens*. Feeding immature stages of insects on adequate food resources is associated with faster development and low mortality due to limited damage during development while substantial quantity and quality of proteins in diets results in lower mortality (Li et al., 2020; Nash & Chapman, 2014). In view of this, the faster development that occurred in nymphs fed on Diet 3 may have resulted from the high protein content of the diet which led to the low rate of mortality observed while the low protein content of Diet 1 could have led to the high mortality observed in grasshoppers fed this diet. Feeding of *R. differens* on diets with low protein content and inadequate nutrients results in cannibalism that has been observed in this species and other Tettigonids (Lehtovaara et al., 2017; Sorjonen et al., 2020). A higher rate of cannibalism occurred among nymphs of *R. differens* reared on Diet 1, which further highlights the inadequacy of this diet in meeting the nutritional needs of *R. differens*. Survival of insects require optimum amounts of sodium and iron where inappropriate amounts of these two minerals result in increased mortality (Hernández et al. 2012; Ferrero et al., 2017; Peterson et al., 2021). This was elucidated by lower survival in nymphs reared on Diet 1 that contained minimal levels of these minerals. Diet 3 seems to have had optimal quantity of nutrients for survival of *R. differens*. According to Sorjonen et al (2020), no further enhancement in survival was seen when *R. differens* were reared on plant based byproducts diets with a protein content beyond 17% (Sorjonen et al., 2020). However, this study demonstrated that survival of

R. differens was higher when fed on diets with a protein content beyond 20%. This difference could be attributed to differences in the byproducts used in formulation the diets. A higher rate of survival was observed in diet 3 compared to values recorded when *R. differens* were reared on single and mixtures of their natural host plants, mixed artificial diets and mixtures of host plants and artificial diet (Malinga et al., 2018b, 2020; Ssepuuya et al., 2018; Leonard et al., 2022). It was, however, lower than 96-100% survival recorded when *R. differens* were reared on food crops (germinated finger millet, fresh maize comb and sorghum seed head) (Malinga et al., 2022). The use of food crops in production of animal feed is, however, unsustainable due to increased competition, hence, Diet 3 that was based on non-food ingredients present an option that can be exploited to enhance domestication of *R. differens*.

The weight of *R. differens* was higher than their counterparts reared on natural host plants (0.41-0.45g), comparable to those fed on high protein and carbohydrate diets (0.56 and 0.55g) and lower than those reared on diets rich in fatty acids (0.64-0.95g) (Lehtovaara et al., 2017; Rutaro et al., 2018a). The wet adult weight of *R. differens* reared on Diet 3 was not different from the wet weight of grasshoppers reared on the other diets. However, higher weights were recorded in nymphs and adult grasshoppers reared on Diet 4 which could have been attributed to the high crude fat and carbohydrate contents of the diet. This was contrary to the findings of other studies where the weight of insects differed with variations in diet quality. The weight of *R. differens* and other grasshopper species increased with an increase in protein content of diets (Sorjonen et al., 2020), but variations of protein content in the study diets did not translate to differences in weight. This could be attributed to the inclusion of MOLP in diets which has previously been shown to produce a similar weight to conventional commercial feed in poultry (Gadzirayi et al., 2012). Similarly, the addition of sugar to the diet of *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) led to a significant increase in weight (Li et al., 2020), however, the variations in the quantity of sugar in the diets did not cause significant differences in weight of *R. differens*. Females attained higher weights compared to males fed on different diets similar to findings by Lehtovaara et al., (2017). Generally, male *R. differens* are smaller in size compared to their female counterparts (Matojo & Yarro, 2013). The wet adult weight of the female was comparable across the diets whereas the male *R. differens* fed on Diet 4 recorded significantly higher weights. The high crude fat and carbohydrate content in Diet 4 could have led to an increase in biomass in the male

grasshoppers fed on it. The fat content of insects correlates positively with the fat content of their diets. However, the accumulation of lipids in the body of females is utilized in oogenesis and egg maturation (Li et al. 2020).

Slight variations in diets influence reproductive performance of insects (Du et al., 2015). The mean preoviposition duration recorded in Diet 3 did not differ from the other diets. Preovipostion period recorded in Diets 2, 3 and 4 were within a similar range with 16 days previously reported (Brits & Throrton, 1981), however, *R. differens* fed on Diet 1 took almost twice as much time before laying eggs. This was probably due to the low nutrients contained in Diet 1. Prolonged pre-oviposition time is associated with poor nutrient quality of diets that delays oocyte development. Development of oocytes is under endocrine control that is triggered by correct nutrient levels in food (Ahmad & Nabi, 2012). This was demonstrated in *Oxya japonica* Thunberg (Orthoptera: Acrididae) where oocyte development was influenced by the nutritional requirements of the insect and the chemical composition of food (Ahmad & Nabi, 2012). On the other hand, an increase in sugar content of diet significantly reduced the pre-oviposition time in *H. axyridis* (Li et al. 2020). Pre-oviposition time of mites and lady birds decreased with different diet mixtures, which further corroborate these findings (Bonte et al., 2010; Muñoz-Cárdenas et al., 2014). Additionally, laying late is part of a survivorship strategy to increase longevity in female and chances of encountering more quality diet before oviposition (Hatt & Osawa, 2021). This was demonstrated in Diet 1 which recorded high female longevity in spite of delayed oviposition. The mean oviposition duration recorded in the study was much higher than the 32 days that was previously reported for *R. differens* (Brits & Throrton, 1981). The grasshoppers fed on diet 3 laid eggs over a significantly longer duration compared to other diets. Longer duration of oviposition is associated with high protein content in diets (Magara et al., 2019).

The highest fecundity of *R. differens* was comparable to the highest fecundity reported when the grasshopper was raised on an artificial diet mixture with six substrates (Malinga et al., 2018b); but 5.5 times higher than fecundity of *R. differens* reared on artificial diet mixed with their host plants (Leonard et al., 2022). Variations in fecundity in phytophagous insects are associated with variations in qualitative and quantitative amounts of nutrients in host plants (Roy & Barik, 2012b). It is possible that the differences arose from variations in the quality of diets used in the various studies. The

fecundity of *R. differens* fed on Diet 3 was higher but comparable to diets 1 and 2. The difference could be due to protein: sugar ratio of the diets. Diet 1, 2 and 3 were more protein biased compared to Diet 4. Egg production in phytophagous insects is better on balanced or slightly protein biased diets (Roeder & Behmer, 2014). High protein to carbohydrate ratio in diet results into high egg production due to substantial protein investment required for oogenesis in insects (Kim et al., 2020). The low egg production in grasshoppers fed on Diet 4 could also be due to high carbohydrate content that was almost two times higher compared to the other diets. The egg production rate in the grasshopper *Melanoplus sanguinipes* Fabricius (Orthoptera: Acrididae) displayed a negative response to increased carbohydrate (Joern & Behmer, 1998). This was however, contrary to findings on *H. axyridis* where egg production increased with an increase in sugar content in the diet (Li et al., 2020). Although the diversification of diet has been associated with high fecundity, grasshoppers fed on Diet 4, which was the most diversified in this study recorded the least number of eggs (Malinga et al., 2018). It's probable that the presence of MOLP in Diets 1, 2 and 3 contributed to better performance of these diets compared to Diet 4. Moringa leaves are rich in vitamins, minerals and essential amino acids which may have led to a higher fecundity in the diets with MOLP (Sodamade et al., 2017; Abbas et al., 2018). The inclusion of different nutrient classes in the diets of the Queensland fruitfly, *Bactrocera tryoni* Froggatt (Diptera: Tephritidae) resulted in substantial egg production (Fanson & Taylor, 2012a). There is however need for more work to determine the effects of supplementation of diets MOLP on the reproductive performance of edible grasshoppers.

Egg incubation duration was longer than reported in previous studies that assessed suitability of diverse egg hatching conditions (Ssepuuya et al., 2018; Egonyu et al., 2021; Leonard et al., 2021). Egg incubation period was shorter for *R. differens* raised on Diet 1 and 3 compared to control. Variations in nutrient composition of diets lead to differences in incubation period of insects (Li et al., 2020), where diets with limiting nutrients delay eclosion (Adler et al., 2013). This was contrary to our findings where egg incubation across diets was not consistent with their nutrient composition.

Percentage hatchability of eggs was higher in diets fortified with MOLP compared to control. Percentage of hatchability of eggs recorded in Diet 3 was comparable to Diet 2 but higher than Diets 1 and 4. Higher egg hatchability is correlated with high protein

content in cricket *S. icipe* (Magara et al., 2019). However, Diet 4, which had a higher protein content yielded a lower percentage of hatchability compared to Diet 1 that had the least protein content. Reduced hatchability in *Spodoptera exigua* was associated with irrational nutrition (Li et al., 2020). It's possible that addition of MOLP to diets accorded a reproductive advantage to *R. differens* based on other nutrients obtained from the plant which may have resulted in the large disparity in the number of eggs hatched between the MOLP fortified diets and the control. Previously, supplementation of poultry diet with moringa increased hatchability of eggs (Ashour et al., 2020) which may have been the case in this study. Many insects reared on artificial diets lose the ability to adapt and reproduce resulting into lower fertility (Li et al., 2020). This could explain the low fecundity and percentage hatchability that was observed in Diet 4. It is possible that this diet has a limiting effect on the reproductive performance of the grasshopper. However, there is need for further investigation before such a generalization can be made.

Longevity of adult *R. differens* was similar to longevity recorded by Malinga et al. (2018) and twice longer than the value recorded by Leonard et al. (2022). The highest adult longevity was recorded in Diet 3 which was comparable to Diet 4, whereas Diet 2 had the lowest longevity. The balance between protein and carbohydrate in diets is a key determinant of the relationship between diet and longevity. Excessive consumption of proteins relative to carbohydrates results in shorter lifespan in insects with high mortality rates reported in cockroaches, crickets, flies, ants and bees when confined to diets with higher protein contents relative to their requirements (Dussutour & Simpson, 2012). On the other hand, the reduction of protein to carbohydrate ratio is associated with increased lifespan in many taxa (Fanson & Taylor, 2012b). In spite of this evidence, it is difficult to attribute the differences observed in the study to variations in carbohydrate and protein contents of diets. The protein and carbohydrate content of Diet 3 and Diet 2 were comparable, but the average longevity recorded in Diet 3 was 1.7 times higher than Diet 2. The longevity recorded in Diet 1 that had a lower protein and carbohydrate content than all the diets was equally 1.3 times higher than Diet 2. These differences may have been influenced by micronutrients that were present in the diets. Micronutrients are beneficial for insects and influence their survival in diverse ways (Kuo & Fisher, 2022). However, there is a need for further investigation before a generalization is made. The longevity of male and female grasshoppers raised on the different diets was comparable. This was similar to findings of Magara et al. (2019) but contrary to studies on *Diacrisia*

casignetum Kollar (Lepidoptera: Arcriidae) where female longevity was longer than for male (Roy & Barik, 2012a). The findings were inconsistent with outcome of study conducted on *B. tryoni* which demonstrated that alteration of diet decreased female longevity (Fanson & Taylor, 2012a).

5.2. Effects of rearing cage designs on production of *R. differens*.

The findings of this study demonstrated that the different cage types could be used to successfully rear *R. differens*. The cage types had diverse effects on wet adult weight, development time, growth rate and survival of *R. differens*. Different cage types are associated with variations in microhabitat factors including temperature, humidity and light conditions that influence growth, development and survival of insects in different ways (Cohen, 2018). Wooden cage type was shown to have higher temperature and lower humidity compared to plastic rearing cages in an experiment that reared crickets; whereas netted cage types simulated the temperature and humidity conditions of the rearing environment (Härkönen et al., 2010; Ngonga et al., 2021). Although the temperature and humidity conditions of the rearing room was controlled, literature documents that this is not adequate to influence the environmental conditions within rearing cages (Jackson, 1986), thereby highlighting the need for controlling of internal environment of rearing cages. Insects require optimum temperature and humidity condition to optimize their growth, development and survival. Lower temperature and higher humidity lowers the rate of development in insects whereas higher temperature and low humidity are more conducive for insects (Miech et al., 2016; Lehtovaara et al., 2018). The variations observed in the study could therefore be attributed to the differences in environmental conditions within the specific cage types.

Wet adult weight of *R. differens* differed significantly by cage type with higher weight recorded in the wooden cage while the netted cage recorded the least weight. The highest weight recorded in the study was higher than findings by Malinga et al. (2020) who recorded an average weight of 0.38g when *R. differens* was reared in plastic containers using diets comprising of mixture of *R. differens* host plants. However, this weight was lower than 0.51-0.6 g reported by other authors who reared *R. differens* in assorted plastic containers using artificial diet mixtures (Sorjonen et al., 2020; Leonard et al., 2022; Malinga et al., 2022). The weights recorded in *R. differens* reared on artificial diet mixtures in assorted plastic containers were equally higher than *R. differens* reared in plastic cage in the study. It's probable that our rearing diet did not optimize weight gain

in *R. differens* compared to the diets reported by the other authors. There is also a possibility of a synergistic effect between diet and rearing environment (cage type) which could explain the higher weight gain in the wooden cage compared to the plastic and netted cages in this study.

A shorter development period occurred in *R. differens* reared in wooden and plastic cages compared to the netted cage even though the differences were not significant. Development period (52 days) was shorter than previously reported values in diverse lab where *R. differens* was reared in plastic containers using artificial diets (Malinga et al., 2018b, 2020; Leonard et al., 2022). This could be an indicator of the suitability of the novel diet and cage types on rearing of *R. differens*. These findings corroborates findings by Allahyari et al., (2022) who established that container type had no effects on the development time of immature stages of *Helicoverpa armigera* when rearing was done in Petri dishes or cubic containers.

A faster growth rate occurred in *R. differens* reared in the wooden cage compared to the other cages. This was commensurate to the higher weight observed in this cage type. It's probable that the micro habitat factors associated with the wooden cage were more advantageous for the growth of *R. differens*. Growth rate of insects is influenced by factors such as quality of feed, temperature and humidity among other things (Barragan-Fonseca et al., 2018; Alemneh & Getabalew, 2019). Since *R. differens* were reared on a similar diet, the variations could thus be attributed to varied conditions within the cage types. Contrary to our findings, rearing container type influenced growth rate of other ectothermic organisms such as fish. However, the differences were attributed to accessibility of feed in the diverse cage types (Barcellos et al., 2004). The higher weight gain, shorter development time and faster growth rate observed in the wooden cages implies that this cage type was more conducive for the development of *R. differens* nymphs. The Wooden cage probably retained more heat and less humidity which was more favourable for the growth and development of the grasshopper as demonstrated by Ngonga et al. (2021) who recorded better performance in crickets reared in wooden cage compared to plastic cage. On the contrary plastic containers are associated with higher humidity which may not be conducive for insects (Bueno et al., 2006).

Survival of *R. differens* reared in netted cage was almost twice higher than those reared in the wooden cage despite the higher weight, shorter development time and higher daily

growth rate observed in the latter. Mesh cages simulate natural conditions of rearing environments (Härkönen et al., 2010). It's probable that the temperature and humidity conditions in the netted cage was more similar to the controlled conditions in the rearing room which translated to higher survival of nymphs reared in this cage type. Optimal survival of *R. differens* occurs within a temperature range of 29-30℃ which was within the same range as the temperature in the rearing room (Lehtovaara et al., 2018). However, the longer development time recorded in the netted cage type contradicts findings by these authors. It is possible that the wooden cage allowed in less light which may have resulted in cannibalism among *R. differens* reared in this cage type. This species is associated with high rates of cannibalism which mostly occurs at night (in limited light). Thus, the lower intensity of light in this cage probably simulated night conditions which led to the low survival rate recorded (Egonyu et al., 2021; Fombong, 2022). The differences in survival of nymphs in the diverse cage types corroborated findings by other authors who found that rearing container type influenced the survival of honey bees, *Habrobracon hebetor and Hyblaea puera* when reared under laboratory conditions (Ghimire & Phillips, 2010; Huang et al., 2014; Jose et al., 2014). Contrary to these findings, container type did not affect the survival of immature stages of *Orius insidiosus* and *Helicoverpa armigera* (Bueno et al., 2006; Allahyari et al., 2022). The rate of survival in the different cage types ranged between 33-63% which was comparable to 38-65% survival rate reported from previous studies who reared *R. differens* in assorted plastic containers (Ssepuuya et al. 2018; Malinga et al. 2020; Leonard et al. 2022). However, the survival rate was lower than 84-100% survival recorded by Malinga et al. (2022) and Sorjonen et al. (2020). These differences could be attributed to the different types of diets that were utilized by the authors.

5.3. Efficacy of a novel trapping technology for mass harvesting of *R. differens*

The novel trap yielded significantly lower *R. differens* catches compared to the local trap. This difference could be attributed to the difference in the design of the two traps. Whereas the trapping surface of the local trap is a folded iron sheet, the trapping surface of the novel trap was made of a reflective PVC tarpaulin material. Upon landing on the iron sheet, the grasshoppers encounter difficulty in obtaining traction and therefore slide into the collection drum (Ssepuuya, 2019). Contrary to this, the collection surface of the novel trap provided more traction for the grasshoppers thereby enabling some of them to fly away instead of sliding into the collection bag. The trapping surface ceased to be water

proof after repeated use hence was easily soaked by rain and mist which provided more grip for the grasshoppers that were able to land, patch and fly away rather than fall into the collection bag. The traps remained outdoors throughout the harvesting season, it was observed that the reflective surface of the novel trap begun to wear out over time probably due to the effects of sunlight and rainfall.

In addition to this, the local traps (collection drums and iron sheets) are sprinkled with water and cassava flour which render the walls of the drum slippery thereby preventing the collected *R. differens* from crawling out (Okia et al., 2017; Sengendo et al., 2021a). The addition of these substances on the drums is implicated in contamination of *R. differens* samples with incidences of increased microbial load some of which are known disease causing pathogens (Labu et al., 2021; Ssepuuya et al., 2019b). The collected drums are mostly old and rusted, addition of cassava flour thus combines with rusted substance and dirt thereby reducing the safety of *R. differens*. Addition of waste oil on the drums to prevent the escape of *R. differens* harvest also occurs among commercial harvesters, however, waste oil is carcinogenic thereby enhancing safety concerns (Sengendo et al., 2021a). In spite of its low efficiency, the novel trap therefore presents a safer alternative for harvesting of *R. differens* and can be modified to enhance the quantity of *R. differens* catches which will in turn reduce the safety concerns associated with the local trapping technique.

Although a restraining netting material was used to prevent the escape of *R. differens* trapped in the collection bag of the novel trap, it's probable that some *R. differens* crawled out of the collection bags through gaps that could have been presents between the restraining netting material and the collection bag. Contrary to our finding, Sengendo et al found that the efficiency of an alternative trapping technique for harvesting of *R. differens* was comparable to the local trapping technology. This was attributed to the presence of a funnel that was fitted in the trap to enhance the retention of grasshopper catches (Sengendo et al., 2021a).

More female than male *R. differens* catches were obtained from both traps, however, more females than male were collected using the novel trap compared to the local trap respectively. Higher quantities of female compared to male *R. differens* occur during the swarming phase (Matojo & Yarro, 2010). Swarming in *R. differens* enhances their reproduction due to increased availability of resources during favourable conditions

(Matojo & Njau, 2010). The proportion of gravid and non-gravid *R. differens* did not, however, differ among the traps, an indication that swarming is probably essential in reproduction and dispersion of the species. Fecundity in *R. differens* is optimized during this period due to increased availability of resources (Matojo & Yarro, 2010).

More non-target species were trapped in the novel trap compared to the local trap. This was contrary to findings by other authors who did not recorded a difference in the quantity of non-target species collected alongside *R. differens* (Sengendo et al., 2021a). Their trap filtered out non target species that were smaller in size. Seemingly the novel trap did not have this advantage although a netting material was used on one side of the trap to facilitate this kind of movement. This implies that the position of the netting material may not have been appropriate to allow the escape of such species. Therefore, the position of the netting material should be placed on the bottom of the collection bag to increase its efficiency in filtering out non target species.

5.4. Nutritional composition of *R. differens* harvested from various geographical sites in Uganda

The findings of this study demonstrate that *R. differens* is rich in macromolecules such as proteins, fats and carbohydrates. The grasshopper is abundant in essential amino acids, unsaturated fatty acids, minerals, vitamins, and flavonoids that are critical for human health and nutrition. However, the nutrient profile of *R. differens* differed by geographical area of collection. The differences in nutritional profile of insects arise from factors such as developmental stage, origin and diet of insects (Kouřimská & Adámková, 2016; Govorushko, 2019; Salama, 2020). A swarm of *R. differens* are composed of adult insects which possess wings and therefore are able to fly; this implies that the observed variations in nutrient profile did not arise due to differences in the development stage of *R. differens* since swarms are comprised of adult insects (Matojo & Yarro, 2013). *R. differens* analysed in the study were purchased from commercial harvesters from five different districts in Uganda which are found in different agro ecological zones with diverse soil types and climatic conditions (Kabasiita et al., 2022; Kasangaki et al., 2022). *Ruspolia differens* swarms are thought to be recruited from local non swarming populations which are known to feed on locally available host plants (Opoke et al., 2019b). The chemical composition of plants is influenced by soil types in plant soil feedback interactions (Van der Putten et al., 2013; Joy et al., 2015). It's probable that the chemical composition of the host plants from the different geographical locations were varied due to diverse soil

types which may account for the diversity of nutritional profile of *R. differens* across the districts. The nutritional profile of *R. differens* is a reflection of host plants or diets on which they are reared (Lehtovaara et al., 2017; Rutaro et al., 2018b). Based on this evidence, it is probable that *R. differens* collected from the respective sites fed on diverse host plants that contained diverse nutrients which ultimately resulted in the variation observed in their nutrient profile.

The crude protein content of *R. differens* ranged between 28.2-44.8%. This is comparable and higher than protein content of commonly consumed plant and animal proteins such as soybeans, eggs, fish, chicken, beef and mutton which have a protein content ranging between 13-32% (Stadlmayr et al., 2012). The high crude protein content of *R. differens* makes it a suitable alternative that can be used to supplement existing protein sources in the efforts to curb food insecurity and malnutrition especially in Sub Saharan Africa (Kekeunou et al., 2020). Crude protein values of *R. differens* obtained from Kabale, Mbarara and Hoima corroborated findings of other authors (Kinyuru et al., 2010; Siulapwa et al., 2012; Bbosa et al., 2019). However, the crude protein content was lower than values observed by Fombong et al. (Fombong et al., 2017).

The crude fat content of *R. differens* was higher than values recorded for common meat sources (Williams et al., 2016; Orkusz, 2021). Crude fat content of *R. differens* from Masaka was significantly higher than those collected from the other sites. The high crude fat content of *R. differens* obtained from Masaka exceeded previously recorded values for *R. differens* (≤ 48%) and other edible grasshopper species (Kinyuru et al., 2010; Rumpold & Schlüter, 2013a; Ssepuuya et al., 2017). Crude fat content of *R. differens* from the other sites however, corroborated finding by some authors (Ssepuuya et al., 2017; Bbosa et al., 2019); and lower than values reported by others (Kinyuru et al., 2010; Siulapwa et al., 2012).

The high carbohydrate content of *R. differens* obtained from the different localities exceeded the previously reported values (1.4-3.7%) (Fombong et al., 2017; Ssepuuya et al., 2017; Bbosa et al., 2019). These values were, however, within the same range as 7.5-28.2% recorded in other edible grasshopper species including *Oxya fuscovittata* (Orthoptera: Acrididae), *Oxya hyla hyla* (Orthoptera: Acrididae) and *Boopedon flaviventris* (Orthoptera: Acrididae) (Anand et al., 2008; Blásquez et al., 2012; Ghosh et al., 2016). Carbohydrate content of *R. differens* obtained from Kampala was higher than

those collected from the other locations. The high fat and carbohydrate content of the grasshopper supplies energy that's vital for the human body (Hlongwane et al., 2020). Carbohydrates derived from insects are also rich in polysaccharides that enhance immunity in man (Kouřimská & Adámková, 2016). Limited data was obtained on carbohydrate content of common animal protein sources.

The crude fibre content of *R. differens* from the five districts was lower than quantities (6-13%) recorded by several researchers (Bbosa et al., 2019; Ssepuuya et al., 2017); but within the same range as the values (4-5%) observed by Kinyuru et al. (2010). Crude fibre content of *R. differens* from Mbarara was significantly higher than that of grasshoppers obtained from Masaka and Kampala but comparable to those collected from Hoima and Kabale.

Ruspolia differens contained more energy compared to beef, chicken and pork (Orkusz, 2021). Limited data exists on energy content of *R. differens*, however, the values obtained in *R. differens* from the different sites were higher than findings by other researchers (Bbosa et al., 2019). However, the energy content observed in *R. differens* from Hoima, Kabale, Mbarara and Kampala were lower than values recorded by some authors (Siulapwa et al., 2012). The highest energy content occurred in *R. differens* from Masaka which was probably due to the high crude fats and carbohydrate content of *R. differens* collected from the location.

The lowest ash content observed in *R. differens* collected from Masaka and Kampala was lower than previously recorded values for the species and other edible grasshopper species (Kinyuru et al., 2010; Rumpold & Schlüter, 2013a). The ash content of *R. differens* obtained from Hoima, Kabale and Mbarara corroborated findings by Kinyuru et al. (2010) but were contrary to findings by other authors who recorded higher (Fombong et al., 2017; Ssepuuya et al., 2017) and lower ash content in *R. differens* (Siulapwa et al., 2012). The ash content of *R. differens* exceeded values recorded in beef, chicken and pork (Williams et al., 2016; Orkusz, 2021).

The moisture content of *R. differens* collected from the different sites was lower than 50.4 -71.2% previously reported (Kinyuru et al., 2010; Ssepuuya et al., 2017). The authors analysed raw, wild collected grasshoppers that may have had a higher moisture content compared to the oven dried samples analysed in this study. However, the moisture

content of *R. differens* collected from Masaka and Kampala was higher than the previous value obtained for oven dried samples (Fombong et al., 2017).

Fatty acids play an integral role in diverse physiological activities in living organisms and supply energy for various functions (De Carvalho and Caramujo, 2018). *Ruspolia differens* recorded high concentration of saturated fatty acids such as palmitic acid, lauric acid, methyl 16 pentacosenoate, methyl heneicosanoate and methyl nanodecanoate that varied between the areas of collection. Palmitic acid has been observed as the most abundant unsaturated fatty acid in *R. differens*; however, this was only true for *R. differens* obtained from Masaka and Kampala (Rutaro et al., 2018a; Rutaro et al., 2018b; Malinga et al., 2020). The high quantity of lauric acid, methyl 16 pentacosenoate, methyl heneicosanoate and methyl nanodecanoate observed in *R. differens* obtained from some of the sites was contrary to the low proportions reported by other researchers (Fombong et al., 2017; Rutaro et al., 2018b; Ssepuuya et al., 2020). Although oleic acid has been reported as the most abundant monounsaturated fatty acid in *R. differens*, our findings showed that it was only abundant in *R. differens* collected from Mbarara (Cheseto et al., 2020; Rutaro et al., 2018b; Ssepuuya et al., 2019a). Other abundant monounsaturated fatty acids observed in *R. differens* collected from the other areas included elaidolinolenic, Methyl cis-13-eicosenoate and Methyl cis-10-heptadecenoate. These compounds have previously reported in *R. differens* oil and products made from their oils (Cheseto et al., 2020). Linoleic acid was the most abundant polyunsaturated fatty acid similar to findings by other authors (Ssepuuya et al., 2019a; Cheseto et al., 2020; Malinga et al., 2020). Polyunsaturated fatty acids are implicated in the prevention of cancer, cardiovascular diseases and diabetic neuropathy, however, these compounds cannot be manufactured by mammals and must therefore be supplied through diet (Yorek, 2018 Govorushko, 2019; da Silva Lucas et al., 2020). Therefore, the inclusion of *R. differens* can be essential in supplying these polyunsaturated fatty acids.

Ruspolia differens samples contained almost all essential amino acids most of which are not found in plant protein sources (Zielińska et al., 2015). This makes them a suitable alternative to plant proteins for fortification of food products such as flour and baked products in efforts to reduce malnutrition. It can also be utilised as a raw product in food processing to supply essential amino acids (Köhler et al., 2019; Ochieng et al., 2022). Other than leucine, the value of amino acids was lower than quantities obtained from pork, chicken and beef; and was lower than the daily requirements for adults (Orkusz,

2021; Rumpold & Schlüter, 2013a; World Health Organization & United Nations University, 2007). The inclusion of *R. differens* in diets can therefore supplement the essential amino acids supplied from the animal protein sources. The quantities of essential amino acids leucine, arginine, threonine, valine, histidine, isoleucine and phenylalanine did not vary significantly between the five districts. Quantities of leucine obtained were comparable to values reported in Kenya and Uganda but higher than values recorded in *R. differens* collected from Zambia (Siulapwa et al., 2012; Fombong et al., 2017; Ssepuuya, Smets, et al., 2019a). However, the quantities of arginine, threonine, valine, histidine, isoleucine and phenylalanine, methionine and lysine were lower than previously recorded values (Siulapwa et al., 2012; Fombong et al., 2017;Ssepuuya et al., 2019a). Similarly, the quantities of non-essential amino acids glutamic acid, aspartic acid, alanine, cysteine, arginine, glycine, proline, serine and tyrosine detected in *R. differens* collected from the different sites was lower than values reported by other researchers (Siulapwa et al., 2012; Fombong et al., 2017; Ssepuuya et al., 2019a). The low amino acid content recorded in the study could be attributed to the heat processing (oven drying) method that was applied prior to analysis of the samples. Processing is associated with alteration of nutritional profile of *R. differens* (Fombong et al., 2017; Nyangena et al., 2020).

Ruspolia differens showed a rich profile of macro and micro minerals that are vital for human health and development (Kinyuru et al., 2010; Mwangi et al., 2018; Silva et al., 2019). Minerals function as co-factor for diverse enzymes that are essential for different physiological processes in the human body (Gupta & Gupta, 2014). Minerals are required in diverse quantities and obtained through diet (Silva et al., 2019). Consumption of *R. differens* can therefore mitigate mineral deficiencies in man. Phosphorous content of *R. differens* collected from Hoima, Kabale, Masaka and Mbarara was lower than the least previously recorded values (121mg/100) (Kinyuru et al., 2010). The significantly higher value of phosphorus recorded in *R. differens* collected from Kampala was higher than values observed by Kinyuru et al. (2010) but lower than findings by Siulapwa et al. (2012) and Fombong et al. (2017). The quantity of phosphorus obtained in *R. differens* collected from Masaka was almost 2.5 times lower than the least recorded quantity in the other sites. Significantly higher potassium content was obtained in *R. differens* collected from Kabale which exceeded the highest value previously observed in the grasshopper (Fombong et al., 2017). The least potassium content occurred in *R. differens* collected

from Masaka which was lower than previously recorded value (Kinyuru et al., 2010). The potassium content of *R. differens* obtained from Kampala, Hoima and Mbarara was comparable to findings documented by other researchers (Fombong et al., 2017; Ssepuuya et al., 2019a). A higher content of calcium occurred in grasshoppers from Mbarara followed by Hoima, Kabale and Kampala. This was comparable to quantities observed by Ssepuuya et al. (2019a), higher than values obtained by Kinyuru et al. (2010) but lower than findings of other authors (Fombong et al., 2017; Ssepuuya et al., 2017). The highest zinc content was observed in *R. differens* obtained from Kampala followed by Hoima, Mbarara and Kabale which concurred with previous reports (Kinyuru et al., 2010; Fombong et al., 2017; Ssepuuya et al., 2019a). The significantly lower values of Zinc reported in grasshoppers from Masaka was lower than previously observed values for the species, however the quantity was higher compared to values recorded for *Zonocerus variegatus* (Orthoptera: Pyrgomorphidae), *Melanoplus foedus* (Orthoptera: Acrididae) and *Trimerotropis pallidipennis* (Orthoptera: Acrididae) (Ladeji et al., 2003; Finke, 2015; Oibiokpa et al., 2017). The iron content of *R. differens* analysed in the study was lower than quantities observed by Fombong et al. (2017). Grasshoppers obtained from Hoima contained the highest iron content followed by those collected from Kabale. These grasshoppers recorded a higher iron content compared to values recorded by Ssepuuya et al. (2019) and Kinyuru et al. (2010) while grasshoppers collected from Mbarara and Kampala contained iron within the same range reported by the authors (13-42mg/100g). Grasshoppers collected from Masaka contained significantly lower quantity of iron compared to the other sites albeit higher than values observed by Siulapwa et al. (2012). The low iron content of *R. differens* from Masaka exceeded the 0.2-0.7mg/100g obtained from *Cyrtacanthacris aeruginosa* (Orthoptera: Acrididae), *M. foedus* and *Z. variegatus* (Ladeji et al., 2003;Alamu et al., 2013; Oibiokpa et al., 2017). *R. differens* was more abundant in zinc and iron compared to other animal proteins, therefore, the consumption of *R. differens* can curb zinc and iron deficiencies that are prevalent among children and women of child bearing age (Mwangi et al., 2018; Orkusz, 2021).

Ruspolia differens collected from the various locations had a rich and varied profile of vitamins such as niacin, riboflavin, folic acid and pantothenic which occur in abundant quantities in edible insects compared to animal protein sources (Ordoñez-Araque & Egas-Montenegro, 2021; Orkusz, 2021). They also contained pyridoxine, retinol, tocopherol and Vit B 12. Vitamins are essential for metabolism in the human body, however, they

cannot be manufactured in the body. They are therefore continually supplied to human bodies through diet (Alamu et al., 2013; Kekeunou et al., 2020). Inclusion of *R. differens* in diet would therefore supplement the food sources of the micronutrients. The highest amount of niacin was obtained from *R. differens* collected from Kabale, however, it was not detected in grasshoppers obtained from Masaka and Kampala. The values observed in the study were higher than previous quantities reported for *R. differens* (Kinyuru et al., 2009) and other edible grasshopper species such as *Spheranium sp* (Orthoptera: Pyrgomorphidae), *T. pallidipennis* and *Brachystola magna* (Orthoptera: Romaleidae) (Blásquez et al., 2012; Finke, 2015; Zamudio-Flores et al., 2019). However, the niacin content of the grasshoppers was lower than the niacin content of *O. hyla hyla* (Ghosh et al., 2016). Riboflavin quantity of *R. differens* collected from Kabale, Masaka and Kampala were within the same range observed in *R. differens* and other edible grasshoppers (Kinyuru et al., 2009; Blásquez et al., 2012; Oibiokpa et al., 2017). However, *R. differens* collected from Hoima and Mbarara contained higher riboflavin than the previously recorded values.

Higher quantities of Pantothenic acid were observed in *R. differens* collected from Kampala, Hoima and Kabale compared to those obtained from Mbarara and Masaka. The high panthothenic acid content observed in this study corroborates reports that insects are a rich source of pantothenic acid, however, there was limited data on the quantity of pantothenic acid in edible insects (Rumpold & Schlüter, 2013a; Kumar et al., 2022). The quantities of pantothenic acid observed were higher than values obtained in pallid winged grasshoppers and *Acheta domestica* (Orthoptera: Gryllidae) (Rumpold & Schlüter, 2013b; Finke, 2015). The highest pyridoxine content occurred in grasshoppers obtained from Kabale followed by Hoima while those from Mbarara and Kampala recorded comparatively lower values. Higher quantities of pyridoxine were observed in *R. differens* analysed in the study compared to previous ones (Kinyuru et al., 2009). The pyridoxine quantity in the grasshoppers was equally higher than content observed in other edible grasshoppers (Hyun et al., 2012; Finke, 2015; Zamudio-Flores et al., 2019). Grasshoppers collected from Kabale, Hoima and Mbarara recorded higher content of folic acid compared to those obtained from Masaka and Kampala. Folic content of 79-137.6 mg/100g observed in the study was more than 100 folds higher than quantities recorded for *R. differens* and other grasshopper species (Kinyuru et al., 2009; Finke, 2015; Zamudio-Flores et al., 2019).

Vitamin B12 content of *R. differens* analysed exceeded quantities observed in *R. differens* by other authors (Kinyuru et al., 2009; Ssepuuya et al., 2019a). It was, however, within the same range as quantities recorded in *M. foedus* (Oibiokpa et al., 2017). Retinol content was higher in *R. differens* collected from Mbarara, Hoima and Masaka. These quantities were higher than values recorded for *R. differens*, *O. hyla hyla* and *B. magna* but lower than quantities observed in *M. foedus* (Kinyuru et al., 2009; Ghosh et al., 2016; Oibiokpa et al., 2017). Gamma tocopherol was observed in grasshoppers collected from Hoima, Kabale and Kampala but was not detected in grasshoppers obtained from Masaka and Mbarara.

Flavonoid content observed in the study was within the same range previously recorded value in processed *R. differens* (Ochieng et al., 2022). Flavonoids are rich sources of antioxidants which possess anti-microbial, anti-inflammatory, anti-allergenic and anti-cancer properties, critical for human health (Pal & Dubey, 2013; Cheseto et al., 2020). The flavonoid content of the grasshopper exceeds quantities of flavonoids obtained in several vegetables and fruits in some countries (Ssepuuya et al., 2020).

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

This study found that *Ruspolia differens* accepted all the diet substrates tested including *M. oleifera* and lake shrimps that have previously not been utilized in formulation of artificial diets for production of the grasshopper. Among the formulated diet, the most efficient diet for production of *R. differens* was Diet 3 which comprised wheat bran, maize bran, MOLP, *ochong'a* and soy bean meal. These substrates are cheap and readily available in the region. MOLP can easily be grown by farmers and obtained at no cost; with an added advantage of its other diverse uses. This diet significantly reduced the developmental time of *R. differens*, improved survival, increased fecundity and hatchability, and extended the longevity of *R. differens.* Thus, it provides a viable option that can be utilized by local farmers in the domestication of *R. differens*.

There were mixed effects on production of *R. differens* when reared in different cage designs. The wooden cage type was more conducive for *R. differens* production due to higher weight, shorter development time and faster growth rate observed, however it recorded a higher mortality compared to other cage types. The netted cage on the other hand yielded a higher survival compared to the other cage designs. Thus, netted cage conferred some advantages that the wooden cage lacked in enhancing survival of *R. differens*. Effective production would therefore require a cage design that has the advantages derived from the two cage designs.

The local trapping technology was more efficient that the novel trap in mass harvesting of *R. differens.* The novel trap collected and retained fewer grasshoppers catches while it collected more non target species compared to the local trapping technology.

Nutritional profile of *R. differens* collected from different geographical locations was highly robust and variable. The proximate, fatty acid, amino acids, minerals, vitamins and flavonoids was rich and diverse across geographical areas of collection.

6.2. Recommendations

Diet 3 provides a suitable and promising alternative feedstock, which would be affordable to everyone including the resource limited and vulnerable segment of the communities where *R. differens* is widely consumed. Further optimization of this diet would enable

year-round mass production of *R. differens* to overcome challenges and bridge the gap of unsustainable dependence on erratic seasonal swarms of these grasshoppers. Further studies are recommended to establish the impact of Diet 3 on the nutritional quality and sensory properties of *R. differens* for improved nutrition and health of consumers.

The wooden cage types should be modified to enhance the survival of *R. differens*. The temperature and humidity conditions in the cage should also be ascertained to determine their effects on production of *R. differens*. There is need for development and assessment of efficacy of a cheap, affordable rearing cage from locally available materials that can exploit the favourable features of the wooden and netted cage types in order to optimize the production of *R. differens*.

There is need for further modification and improvement of the novel trap in order to improve its performance in mass trapping of *R. differens*. Increasing the efficiency of the novel trap calls for modification of the trapping components to enhance the collection and retention of grasshopper catches. This will provide an efficient and safe alternative for mass harvesting of *R. differens*. Seeing that the local trapping technology is more efficient in mass harvesting of *R. differens*, the safety concerns associated with the local trapping technology should be addressed.

The robust nutritional profile of *R. differens* indicates that the consumption of *R. differens* can supplement the existing animal protein sources to supply macro and micro molecules that are critical in curbing the rising food security and malnutrition especially in sub Saharan Africa. The variability of the nutrient profile of *R. differens* from different regions highlights the need for pooling the collection and processing of *R. differens* from different regions in order to capitalize on its benefits across different regions and seasons.

6.3. Recommendation for further studies

1. The efficacy of Diet 3 should be tested under semi-field conditions to assess its effects on development, survival and reproductive performance of *R. differens* before a generalization can be made.
2. There is need for development and assessment of effects of a cage design that has the beneficial features of the wooden and netted cages for the production of *R. differens.*

3. A safe and efficient alternative trapping technology should be developed and tested to enhance safe harvesting of *R. differens.*
4. The causes of variations in the nutritional composition of *R. differens* across geographical areas of collection should be further explored. Such knowledge would provide great insight in the formulation of diets that can enhance the nutrient composition of *R. differens* under domestication for sustainable production.

REFERENCES

Abbas, K. R., Elsharbasy, F. S., & Fadlelmula, A. A. (2018). Nutritional Values of Moringa oleifera, Total Protein, Amino Acid, Vitamins, Minerals, Carbohydrates, Total Fat and Crude Fiber, under the Semi-Arid Conditions of Sudan. *Journal of Microbial & Biochemical Technology*, *10*(2), 56–58.

Achonga, B., Lagat, J., & Akuja, T. (2011). Evaluation of the diversity of crop and livestock enterprises among agro-biodiversity farmer field schools (ABD-FFS) and Non-ABD-FFS households in Bondo District, Kenya. *Applied Biosciences*, *38*, 2586–2591.

Adler, M. I., Cassidy, E. J., Fricke, C., & Bonduriansky, R. (2013). The lifespan-reproduction trade-off under dietary restriction is sex-specific and context-dependent. *Experimental Gerontology*, *48*(6), 539–548.

Agea, J. G., Biryomumaisho, D., Buyinza, M., & Nabanoga, G. N. (2008). Commercialization of Ruspolia nitidula (nsenene Grasshoppers) In Central Uganda. *African Journal of Food, Agriculture, Nutrition and Development*, *8*(3), 319–332.

Ahmad, T., & Nabi, S. (2012). On the food preferences and application of Dyar's law to different hopper instars of Choroedocus illustris Walker (Orthoptera: Acrididae). *Italian Journal of Zoology*, *79*(4), 598–606.

Alamu, T. O., Amao, O. A., Oke, A. O., & Lawal, O., (2013). Diversity and nutritional status of edible insects in Nigeria: A review. *International Journal of Biodiversity and Conservation*, *5*(4), 215–222.

Alemneh, T., & Getabalew, M. (2019). Factors Influencing the Growth and Development of Meat Animals. *International Journal of Animal Science*, *3*(3), 1048.

Allahyari, R., Aramideh, S., & Safaralizadeh, M. (2022). Improving mass rearing of *Helicoverpa armigera* (Lepidoptera: Noctuidae) by feeding neonates on chickpea plant. *11*(1), 61–69.

Anand, H., Ganguly, A., & Haldar, P. (2008). Potential Value of Acridids as High Protein Supplement for Poultry Feed. *International Journal of Poultry Science*, *7*(7), 722–725.

AOAC. (2012). Official Method of Analysis of AOAC International. (W. Latimer (ed.); 19th Editi). AOAC International.

Ashour, E. A., El-Kholy, M. S., Alagawany, M., El-Hack, M. A. E., Mohamed, L. A., Taha, A. E., El Sheikh, A. I., Laudadio, V., & Tufarelli, V. (2020). Effect of dietary supplementation with moringa oleifera leaves and/or seeds powder on production,

egg characteristics, hatchability and blood chemistry of laying Japanese quails. *Sustainability (Switzerland)*, *12*(6), 1–9.

Ayieko, M., Oriaro, V., & Nyambuga, I. (2010). Processed Products of Termites and Lake Flies: Improving Entomophagy for Food Security within the Lake Victoria Region. *African Journal of Food, Agriculture, Nutrition and Development*, *10*(2), 1–14.

Bailey, W. J., & Mccrae, A. W. R. (1978). The general biology and phenology of swarming in the East African tettigoniid Ruspolia differens (Serville) (Orthoptera). *Journal of Natural History*, *12*, 259–288.

Barcellos, L. J. G., Kreutz, L. C., Quevedo, R. M., Fioreze, I., Cericato, L., Soso, A. B., Fagundes, M., Conrad, J., Baldissera, R. K., Bruschi, A., & Ritter, F. (2004). Nursery rearing of jundiá, Rhamdia quelen (Quoy & Gaimard) in cages: Cage type, stocking density and stress response to confinement. *Aquaculture*, *232*(1–4), 383–394.

Barragan-Fonseca, K. B., Dicke, M., & van Loon, J. J. (2018). Influence of larval density and dietary nutrient concentration on performance, body protein, and fat contents of black soldier fly larvae (Hermetia illucens). *Entomologia Experimentalis et Applicata*, *166*(9), 761–770.

Bbosa, T., Tamale Ndagire, C., Muzira Mukisa, I., Fiaboe, K. K. M., & Nakimbugwe, D. (2019). Nutritional Characteristics of Selected Insects in Uganda for Use as Alternative Protein Sources in Food and Feed. *Journal of Insect Science (Online)*, *19* (6).

Bhatnagar-Panwar, M. Bhatnagar-Mathur, P. Bhaaskarla, V., Reddy, S., & Sharma, K. (2013). Rapid, accurate and routine HPLC method for large-scale screening of pro-vitamin A carotenoids in oilseeds. *Journal of Plant Biochemistry and Biotechnology*.

Blásquez, J. R.-E., Moreno, J. M. P., & Camacho, V. H. M. (2012). Could Grasshoppers Be a Nutritive Meal? *Food and Nutrition Sciences*, *03*(02), 164–175.

Bong, L. J., Neoh, K. B., Lee, C. Y., & Jaal, Z. (2014). Effect of diet quality on survival and reproduction of adult paederus fuscipes (Coleoptera: Staphylinidae). *Journal of Medical Entomology*, *51*(4), 752–759.

Bonte, J., Vangansbeke, D., Maes, S., Bonte, M., Conlong, D., & Clercq, P. De. (2012). Moisture source and diet affect development and reproduction of orius thripoborus and orius naivashae, two predatory anthocorids from Southern Africa. *Journal of*

Insect Science, *12*(1), 1–16.

Bonte, M., Samih, M. A., & de Clercq, P. (2010). Development and reproduction of Adalia bipunctata on factitious and artificial foods. *BioControl*, *55*(4), 485–491.

Brits, J. A., & Throrton, C. H. (1981). The biology of Ruspolia differens (Serville) (Orthoptera: Tettigonidae) in South Africa. *Phytophylactica*, *13*, 169–173.

Bueno, C. H., Mendes, S. M., & Carvalho, S. (2006). Evaluation of a Rearing-Method for the Predator Orius Insidiosus Related papers. *Bulletin of Insectology*, *59*(1), 1–6.

Campbell, C. R., & Plank, C. O. (1991). Sample Preparation. *Plant Analysis Reference Procedures for the Southern Region of the United States*, *Southern C* (Bulletin No. 368), 1–13.

Chadare, F. J., Madode, Y. E., Fanou-Fogny, N., Kindossi, J. M., Ayosso, J. O. G., Honfo, S. H., Kayodé, A. P. P., Linnemann, A. R., & Hounhouigan, D. J. (2018). Indigenous food ingredients for complementary food formulations to combat infant malnutrition in Benin: a review. *Journal of the Science of Food and Agriculture*, *98*(2), 439–455.

Cheseto, X., Baleba, S. B. S., Tanga, C. M., Kelemu, S., & Torto, B. (2020). Chemistry and sensory characterization of a bakery product prepared with oils from African edible insects. *Foods*, *9*(6).

Clissold, F. J., & Simpson, S. J. (2015). Temperature, food quality and life history traits of herbivorous insects. *Current Opinion in Insect Science*, *11*, 63–70.

Cohen, A. C. (2018). Ecology of Insect Rearing Systems: A Mini-Review of Insect Rearing Papers from 1906-2017. *Advances in Entomology*, *0* (02), 86–115.

da Silva Lucas, J. A., Menegon de Oliveira, L., da Rocha, M., & Prentice, C. (2020). Edible insects: An alternative of nutritional, functional and bioactive compounds. *Food Chemistry*, *311*, 126022.

Das, M., & Mandal, S. K. (2013). Assessment of Nutritional Quality and Anti-nutritinal Composition of two Edible Grasshopper (Orthoptera: Acrididae) - A Search for new food alternative. *International Journal of Medicine and Pharmaceutical Sciences*, *3*(5), 31–48.

De Carvalho, C. C. Caramujo, M. J. (2018). The various roles of fatty acids. *Molecules*, *23*(10), 2583.

de Groote, H., Nyanamba, T., & Wahome, R. (2010). Quality protein maize for the feed industry in Kenya. *Outlook on Agriculture*, *39* (4), 291–298.

Dewanto, V., Xianzhong, W., Kafui, K. A., & Rui, H. L. (2002). Thermal Processing

Enhances the Nutritional Value of Tomatoes by Increasing Total Antioxidant Activity. *J. Agric. Food Chem*, *50* (10), 3010–3014.

Di Mattia, C., Battista, N., Sacchetti, G., & Serafini, M. (2019). Antioxidant activities in vitro of water and liposoluble extracts obtained by different species of edible insects and invertebrates. *Frontiers in Nutrition*, *6*, 1–7.

Dobermann, D., Swift, J. A., & Field, L. M. (2017). Opportunities and hurdles of edible insects for food and feed. *Nutrition Bulletin*, *42*(4), 293–308.

Du, Y. L., Ai, P. P., Lang, L. X., Zhang, M. Z., & Sun, S. L. (2015). Development of an artificial diet for rearing conogethes punctiferalis (Lepidoptera: Crambidae). *Journal of Entomological Science*, *50*(2), 89–98.

Durst, P., Johnson, D. V., Leslie, R. N., & Shono, K. (2010). Forest insects as food: humans bite back. In P. B. Durst, D. V Johnson, R. N. Leslie, & K. Shono (Eds.), *Proceedings of a workshop on Asia-Pacific resources and their potential for development 19-21 february 2008, Chiang Mai, Thailand* (pp. 23–154). FAO.

Dussutour, A., & Simpson, S. J. (2012). Ant workers die young and colonies collapse when fed a high-protein diet. *Proceedings of the Royal Society B: Biological Sciences*, *279* (1737), 2402–2408.

Egonyu, J. P., Miti, M. M., Tanga, C. M., Leonard, A., & Subramanian, S. (2021). Cannibalism, oviposition and egg development in the edible long-horned grasshopper, Ruspolia differens (Orthoptera: Tettigoniidae) under laboratory conditions. *Journal of Insects as Food and Feed*, (1), 89–97.

El-hack, M. E. A., Alagawany, M., Elrys, A. S., Desoky, E. M., Tolba, H. M. N., Elnahal, A. S. M., Elnesr, S. S., & Swelum, A. A. (2018). Effect of Forage Moringa oleifera L . (moringa) on Animal Health and Nutrition and Its Beneficial Applications in Soil , Plants and Water Purification. *Agriculture*, 1–22.

Elhassan, M., Wendin, K., Olsson, V., & Langton, M. (2019). Quality aspects of insects as food-Nutritional, sensory, and related concepts. *Foods*, *8*(3), 1–14.

Fanson, B. G., & Taylor, P. W. (2012a). Additive and interactive effects of nutrient classes on longevity, reproduction, and diet consumption in the Queensland fruit fly (Bactrocera tryoni). *Journal of Insect Physiology*, *58*(3), 327–334.

Fanson, B. G., & Taylor, P. W. (2012b). Protein:carbohydrate ratios explain life span patterns found in Queensland fruit fly on diets varying in yeast:sugar ratios. *AGE*, *34*(6), 1361–1368.

Fanzo, J. (2012). The Nutrition Challenge in Sub-Saharan Africa. *UNDP - Regional*

Bureau for Africa - Working PAper, January, 1–68.

FAO. (2003). Food energy-methods of analysis and conversion factors. *Food and Agriculture Organization of the United Nations Technical Workshop Report*, *77*, 89.

Ferrero, A., Torreblanca, A., & Garcerá, M. D. (2017). Assessment of the effects of orally administered ferrous sulfate on Oncopeltus fasciatus (Heteroptera: Lygaeidae). *Environmental Science and Pollution Research*, *24*(9), 8551–8561.

Fielding, D. J., & Defoliart, L. S. (2007). Growth, development, and nutritional physiology of grasshoppers from subarctic and temperate regions. *Physiological and Biochemical Zoology*, *8*(6), 607–618.

Finke, M. D. (2015). Complete nutrient content of three species of wild caught insects, pallid-winged grasshopper, rhinoceros beetles and white-lined sphinx moth. *Journal of Insects as Food and Feed*, *1*(4), 281–292.

Fombong, F. T. (2022). (Anti)Nutritional, Techno-functional and Antimicrobial Properties of the edible bush cricket, Ruspolia differens. KU Leuven.

Fombong, F. T., Van Der Borght, M., & Broeck, J. Vanden. (2017). Influence of freeze-drying and oven-drying post blanching on the nutrient composition of the edible insect Ruspolia differens. *Insects*, *8*(3).

Gadzirayi, C. T., Masamha, B., Mupangwa, J. F., & Washaya, S. (2012). Performance of broiler chickens fed on mature Moringa oleifera. *International Journal of Poultry Science*, *11*(1), 5–10.

Gahukar, R. T. (2016). Edible Insects Farming: Efficiency and Impact on Family Livelihood, Food Security, and Environment Compared With Livestock and Crops. In *Insects as Sustainable Food Ingredients*. Elsevier Inc.

Ganesan, K., Sukalingam, K., & Xu, B. (2017). Impact of consumption of repeatedly heated cooking oils on the incidence of various cancers- A critical review. *Critical Reviews in Food Science and Nutrition*, *59*(3), 488–505.

Ghimire, M. N., & Phillips, T. W. (2010). Mass rearing of Habrobracon hebetor Say (Hymenoptera : Braconidae) on larvae of the Indian meal moth , Plodia interpunctella (Lepidoptera : Pyralidae): effects of host density , parasitoid density , and rearing containers. *Journal of Stored Products Research*, *46* (4), 214–220.

Ghosh, S., Haldar, P., & Kumar Mandal, D. (2016). Evaluation of nutrient quality of a short horned grasshopper, Oxya hyla hyla Serville (Orthoptera: Acrididae) in search of new protein source. *Journal of Entomology and Zoology Studies*, *4*(1), 193–197.

Ghosh, S., Haldar, P., & Mandal, D. K. (2014). Suitable food plants for mass rearing of

the short-horn grasshopper Oxya hyla hyla (Orthoptera: Acrididae). *European Journal of Entomology*, *111*(3), 448–452.

Global Market Insights. (2016). Edible Insects Market Size Set to Exceed USD 520mn by 2023, With Over 40% Growth from 2016 to 2023. PRNewswire, 05th July 2016.

Govorushko, S. (2019). Global status of insects as food and feed source: A review. *Trends in Food Science and Technology*, *91* (July 2018), 436–445.

Gupta, U. C., & Gupta, S. C. (2014). Sources and deficiency diseases of mineral nutrients in human health and nutrition: a review. *Pedosphere.*, *24*(1), 13–38.

Hanboonsong, Y., Jamjanya, T., & Durst, P. B. (2013). *S*ix-legged livestock: edible insect farming, collection and market in Thailand. RAP publication, 3(8).

Härkönen, L., Härkönen, S., Kaitala, A., Kaunisto, S., Kortet, R., Laaksonen, S., & Ylönen, H. (2010). Predicting range expansion of an ectoparasite–the effect of spring and summer temperatures on deer ked Lipoptena cervi (Diptera: Hippoboscidae) performance along a latitudinal gradient. *Ecography*, *33*(5), 906–912.

Harvey, J. A., Cloutier, J., Visser, B., Ellers, J., Wäckers, F. L., & Gols, R. (2012). The effect of different dietary sugars and honey on longevity and fecundity in two hyperparasitoid wasps. *Journal of Insect Physiology*, *58*(6), 816–823.

Hatt, S., & Osawa, N. (2021). High variability in pre-oviposition time independent of diet available at eclosion: A key reproductive trait in the ladybird beetle Harmonia axyridis (coleoptera: Coccinellidae) in its native range. *Insects*, *12*(5).

Hernández, L. M. A., Todd, E. V., Miller, G. A., & Frederickson, M. E. (2012). Salt intake in amazonian ants: Too much of a good thing? *Insectes Sociaux*, *59*(3), 425–432.

Hlongwane, Z. T., Slotow, R., & Munyai, T. C. (2020). Nutritional composition of edible insects consumed in africa: A systematic review. *Nutrients*, *12*(9), 1–28.

Horwitz, W. (Ed.). (2000). Official Methods of Analysis of AOAC International (17th editi). AOAC International.

Hosotani, K., & Kitagawa, M. (2003). Improved simultaneous determination method of beta-carotene and retinol with saponification in human serum and rat liver. *J Chromatogr B Analyt Technol Biomed Life Sci*, *791*(1–2), 305–313.

Huang, S. K., Csaki, T., Doublet, V., Dussaubat, C., Evans, J. D., Gajda, A. M., Gregorc, A., Hamilton, M. C., Kamler, M., Schiesser, N., Sohr, A. R., Tanner, G., Tozkat, C., Williams, G. R., Wu, L., Zheng, H., & Chen, Y. P. (2014). Evaluation of Cage

Designs and Feeding Regimes for Honey Bee (Hymenoptera : Apidae) Laboratory Experiments. *Journal of Economic Entomology*, *107*(1), 54–62.

Huang, X., Ullah, H., & Wang, Y. (2020). Growth performance and transcriptomic response of Calliptamus abbreviatus Ikonn (Orthoptera: Acrididae) to suitable and unsuitable host plant species. *Arthropod-Plant Interactions*, *14*(5), 605–612.

Huang, X., Whitman, D. W., Ma, J., McNeill, M. R., & Zhang, Z. (2017). Diet alters performance and transcription patterns in Oedaleus asiaticus (Orthoptera: Acrididae) grasshoppers. *PLoS ONE*, *12*(10), 1–20.

Hyun, S. H., Kwon, K. H., Park, K. H., Jeong, H. C., Kwon, O., Tindwa, H., & Han, Y. S. (2012). Evaluation of nutritional status of an edible grasshopper, oxya chinensis formosana. *Entomological Research*, *42*(5), 284–290.

Jackson, J. J. (1986). Rearing and Handling of Diabrotica virgifera and Diabrotica undecimpunctata howardi. In J. L. Krysan & T. A. Miller (Eds.), Methods for the Study of Pest Diabrotica (1st ed., pp. 25–47). Springer New York.

Joern, A., & Behmer, S. T. (1997). Importance of dietary nitrogen and carbohydrates to survival, growth, and reproduction in adults of the grasshopper Ageneotettix deorum (Orthoptera: Acrididae). *Oecologia*, *112*(2), 201–208.

Joern, A., & Behmer, S. T. (1998). Impact of diet quality on demographic attributes in adult grasshoppers and the nitrogen limitation hypothesis. *Ecological Entomology*, *23*(2), 174–184.

Jongema, Y. (2017). World list of edible insects. *Wageningen University*, 1–100. https://www.wur.nl/en/Research-Results/Chair-groups/Plant-Sciences/Laboratory-of-Entomology/Edible-insects/Worldwide-species-list.htm

Jose, B. K., Sudheendrakumar, V. V, & Sajeev, T. V. (2014). Evaluation of various captive spaces for the rearing of the teak defoliator (Hyblaea puera).

Joy, E. J. ., Martin, R. B., Scott, D. Y., Colin, R. B., Allan, D. . C., Louise, A., Barlow, T. S., & Watts, M. J. (2015). Soil type influences crop mineral composition in Malawi. *Science of the Total EnvironmentEnvironment*, *505*, 587–595.

Kababu, M., Mweresa, C. K., Subramanian, S., Egonyu, J. P., & Tanga, C. M. (2023). Variability in nutrient composition of the edible long-- horned grasshopper (Ruspolia differens) in Uganda and its potential in alleviating food insecurity. *Food Science and Nutrition*, *March*, 1–17. https://doi.org/10.1002/fsn3.3346

Kabasiita, J. K., Opolot, E., & Malinga, G. (2022). Quality and Fertility Assessments of Municipal Solid Waste Compost Produced from Cleaner Development Mechanism

Compost Projects: A Case Study from Uganda. *Agriculture*, *12*(5), 582.

Kasangaki, P., Sarah Otim, A., P'Odyek Abila, P., Angiro, C., Chemurot, M., & Kajobe, R. (2022). The presence of varroa in Uganda and knowledge about it by the beekeeping industry. *Journal of Apicultural Research*, *54*(4), 373–377.

Kasozi, K. I., Namazi, C., Basemera, E., Atuheire, C., Odwee, A., Majalija, S., & Kateregga, J. N. (2019). Inorganic pollutants in edible grasshoppers (Ruspolia nitidula) of Uganda and their major public health implications. *African Health Sciences*, *19*(3), 2679–2691.

Kekeunou, S., Simeu-Noutchom, A., Mbadjoun-Nziké, M., Achu-Loh, M. B., Akono-Ntonga, P., Wandji, A. C., & Tamesse, J. L. (2020). Nutritional Composition of African Edible Acridians. In A. A. Mariod (Ed.), African Edible Insects As Alternative Source of Food, Oil, Protein and Bioactive Components (pp. 169–193). Springer Nature.

Kekeunou, S., Weise, S., & Messi, J. (2010). Effect of 13 single and eight mixed host plant diets on survival, post-embryonic development and morphology of variegated grasshopper in laboratory. *Entomological Research*, *40*(1), 8–17.

Kelemu, S., Niassy, S., Torto, B., Fiaboe, K., Affognon, H., Tonnang, H., Maniania, N. K., & Ekesi, S. (2015). African edible insects for food and feed: Inventory, diversity, commonalities and contribution to food security. *Journal of Insects as Food and Feed*, *1*(2), 103–119.

Kim, K., Jang, T., Min, K. J., & Lee, K. P. (2020). Effects of dietary protein:carbohydrate balance on life-history traits in six laboratory strains of Drosophila melanogaster. *Entomologia Experimentalis et Applicata*, *168*(6–7), 482–491.

Kinyuru. J, N., Kenji, G. ., Muhoho, S. N., & Ayieko, M. (2010). Nutritional potential of longhorn grasshopper (Ruspolia differens) consumed in the Lake Victoria Region of East Africa. *Journal of Agriculture, Science and Technology*, *12*(1), 32–46.

Kinyuru, J. N., Broeck, J. V, Ayieko, M., Fombong, F., & Ng'ang'a, J. (2018). *Ruspolia grasshopper production systems in East Africa and their nutritional properties* (*Technical Brief # 1: Issue December*).

Kinyuru, J. N., Kenji, G. M., Njoroge, S. M., & Ayieko, M. (2009). Effect of processing methods on the in vitro protein digestibility and vitamin content of edible winged termite (Macrotermes subhylanus) and grasshopper (Ruspolia differens). *Food and Bioprocess Technology*, *3*(5), 778–782.

Köhler, R., Kariuki, L., Lambert, C., & Biesalski, H. K. (2019). Protein, amino acid and

mineral composition of some edible insects from Thailand. *Journal of Asia-Pacific Entomology*, *22*(1), 372–378.

Kouřimská, L., & Adámková, A. (2016). Nutritional and sensory quality of edible insects. *NFS Journal*, *4*, 22–26.

Kubiriza, G. K., Akol, A. M., Arnason, J., Sigurgeirsson, Snorrason, S., Tómasson, T., & Thorarensen, H. (2018). Practical feeds for juvenile Nile tilapia (Oreochromis niloticus) prepared by replacing Rastrineobola argentea fishmeal with freshwater shrimp (Caridina nilotica) and mung bean (Vigna radiata) meals. *Aquaculture Nutrition*, *24*(1), 94–101.

Kumar, P., Mehta, N., Abubakar, A. A., Kumar, A., Kaka, U., Sharma, N., Sazili, A. Q., Pateiro, M., Kumar, M., & Lorenzo, J. M. (2022). Potential Alternatives of Animal Proteins for Sustainability in the Food Sector Potential Alternatives of Animal Proteins for Sustainability in the. *Food Reviews International*, 1–26.

Kuo, C., & Fisher, B. L. (2022). A Literature Review of the Use of Weeds and Agricultural and Food Industry By-Products to Feed Farmed Crickets (Insecta ; Orthoptera; Gryllidae). *Frontiers in Sustainable Food Systems*, *5*(January), 1–14.

Labu, S., Subramanian, S., Khamis, F. M., Akite, P., Kasangaki, P., Chemurot, M., Tanga, C. M., Ombura, F. L. O., & Egonyu, J. P. (2021). Microbial contaminants in wild harvested and traded edible long-horned grasshopper, Ruspolia differens (Orthoptera: Tettigoniidae) in Uganda. *Journal of Insects as Food and Feed*, *7*(7), 1131–1141.

Ladeji, O., Mariam, S., & Hugh, M. (2003). Proximate chemical analysis of Zonocerus variegatus (Giant grasshopper). *Nigerian Journal of Biotechnology*, *14*(1), 42-45–45.

Lee, K. P. (2015). Dietary protein: Carbohydrate balance is a critical modulator of lifespan and reproduction in Drosophila melanogaster: A test using a chemically defined diet. *Journal of Insect Physiology*, *75*(February), 12–19.

Lehtovaara, V. J. ., Valtonen, A., Sorjonen, J., Hiltunen, M., Rutaro, K., Malinga, G. M., Nyeko, P., & Roininen, H. (2017). The fatty acid contents of the edible grasshopper Ruspolia differens can be manipulated using artificial diets. *Journal of Insects as Food and Feed*, *3*(4), 253–262.

Lehtovaara, V. J., Roininen, H., & Valtonen, A. (2018). Optimal Temperature for Rearing the Edible Ruspolia differens (Orthoptera: Tettigoniidae). *Journal of Economic Entomology*, *111*(6), 2652–2659.

Lehtovaara, V. J., Tahvanainen, J., Sorjonen, J., Valtonen, A., & Roininen, H. (2019). Space and Shelter Requirement of Nymphs in the Mass-Rearing of the Edible Ruspolia differens (Orthoptera: Tettigoniidae). *Journal of Economic Entomology*, *112*(4), 1–7.

Leonard, A., Egonyu, J. P., Tanga, C. M., Kyamanywa, S., Ekesi, S., Khamis, M. ., & Subramanian, S. (2022). Host Plant-Based Artificial Diets Enhance Development, Survival and Fecundity of the Edible Long-Horned Grasshopper Ruspolia differens (Orthoptera: Tettigoniidae). *Journal of Insect Science*, *22* (2), 1–7.

Leonard, A., Egonyu, J. P., Tanga, C. M., Kyamanywa, S., Tonnang, H. Z. E., Azrag, A. G. A., Khamis, F. M., Ekesi, S., & Subramanian, S. (2021). Predicting the current and future distribution of the edible long-horned grasshopper Ruspolia differens (Serville) using temperature-dependent phenology models. *Journal of Thermal Biology*, *95*, 102786.

Leonard, A., Khamis, F. M., Egonyu, J. P., Kyamanywa, S., Ekesi, S., Tanga, C. M., Copeland, R. S., & Subramanian, S. (2020). Identification of Edible Short- and Long-Horned Grasshoppers and Their Host Plants in East Africa. *Journal of Economic Entomology*, *113*(5), 2150–2162.

Li, D. Y., Ai, P. P., Du, Y. L., Sun, S. L., & Zhang, M. Z. (2015). Effects of different host plants on the development and reproduction of Yellow Peach Moth, Conogethes punctiferalis (Guenée, 1854) (Lepidoptera: Crambidae). *Austral Entomology*, *54*(2), 149–153.

Li, Y., Wang, S., Liu, Y., Lu, Y., Zhou, M., Wang, S., & Wang, S. (2020). The Effect of Different Dietary Sugars on the Development and Fecundity of Harmonia axyridis. *Frontiers in Physiology*, *11*, 1–12.

Lindroth, R. L., Barman, M. A., & Weisbrod, A. V. (1991). Nutrient deficiencies and the gypsy moth, Lymantria dispar: Effects on larval performance and detoxication enzyme activities. *Journal of Insect Physiology, 37*(1), 45–52.

Magara, H. J. O., Tanga, C. M., Ayieko, M. A., Hugel, S., Mohamed, S. A., Khamis, F. M., Salifu, D., Niassy, S., Sevgan, S., Fiaboe, K. K. M., Roos, N., & Ekesi, S. (2019). Performance of Newly Described Native Edible Cricket Scapsipedus icipe (Orthoptera: Gryllidae) on Various Diets of Relevance for Farming. *Journal of Economic Entomology*, *112*(2), 653–664.

Malinga, G., Acur, A., Ocen, P., Holm, S., Rutaro, K., Ochaya, S., Kinyuru, J. N., Eilenberg, J., Roos, N., Valtonen, A., Nyeko, P., & Roininen, H. (2022). Growth

and Reproductive Performance of Edible Grasshopper (Ruspolia differens) on Different Artificial Diets. *Journal of Economic Entomology*, 1–7.

Malinga, G., Lehtovaara, V. J., Valtonen, A., Nyeko, P., & Roininen, H. (2019). Developing Mass Egg-Laying Medium for the Edible Ruspolia differens (Orthoptera: Tettigonidae). *Economic Entomology*, 1–4.

Malinga, G., Valtonen, A., Hiltunen, M., Lehtovaara, V. J., Nyeko, P., & Roininen, H. (2020). Performance of the African edible bush-cricket, Ruspolia differens, on single and mixed diets containing inflorescences of their host plant species. *Entomologia Experimentalis et Applicata*, *168*(6–7), 448–459.

Malinga, G., Valtonen, A., Lehtovaara, V., Rutaro, K., Opoke, R., Nyeko, P., & Roininen, H. (2018a). Diet acceptance and preference of the edible grasshopper ruspolia differens (Orthoptera: Tettigoniidae). *Applied Entomology and Zoology*, *53*(2), 229–236.

Malinga, G., Valtonen, A., Lehtovaara, V., Rutaro, K., Opoke, R., Nyeko, P., & Roininen, H. (2018b). Mixed artificial diets enhance the developmental and reproductive performance of the edible grasshopper, ruspolia differens (Orthoptera: Tettigoniidae). *Applied Entomology and Zoology*, *53*(2), 237–242.

Massa, B. (2015). Taxonomy and distribution of some katydids (Orthoptera Tettigoniidae) from tropical Africa. *ZooKeys*, *524*(2015), 17–44.

Matojo, N. D. (2017). A Review Work on How to Differentiate the Longhorn Grasshoppers Ruspolia A Review Work on How to Differentiate the Longhorn Grasshoppers Ruspolia differens and Ruspolia nitidula (Orthoptera : Tettigoniidae). *Journal of Applied Life Sciences International*, *15*(2), 1–4.

Matojo, N. D., & Hosea, K. M. (2013). Phylogenetic Relationship of the Longhorn Grasshopper Ruspolia differens Serville (Orthoptera: Tettigoniidae) from Northwest Tanzania Based on 18S Ribosomal Nuclear Sequences. *Journal of Insects*, *2013*, 1–5.

Matojo, N. D., & Njau, M. (2010). Plasticity and biosystematics of swarming of the conehead Ruspolia differens Serville (Orthoptera : Conocephalidae). *International Journal of Integrative Biology*, *9*(2), 97–103.

Matojo, N. D., & Yarro, J. G. (2010). Variability in polymorphism and sex ratio of the conehead Ruspolia differens Serville (Orthoptera: Conocephalidae) in north-west Tanzania. *International Journal of Integrative Biology*, *9*(3), 131–136.

Matojo, N. D., & Yarro, J. G. (2013). Anatomic Morphometrics of the “Senene”

Tettigoniid Ruspolia differens Serville (Orthoptera : Conocephalidae) from North-West Tanzania. *International Shcolarly Research Notices*, *2013*.

Mccrae, A. W. R. (1982). Characteristics of Swarming in the African Edible Bush-Cricket Ruspolia Differens (Serville) (Orthoptera, Tettigonidea). *Journal of the East Africa Natural History Society and National Museum*, *December*.

Melgar-Lalanne, G., Hernández-Álvarez, A. J., & Salinas-Castro, A. (2019). Edible Insects Processing: Traditional and Innovative Technologies. *Comprehensive Reviews in Food Science and Food Safety*, *18*, 1166–1191.

Melo-Ruiz, V., Sandoval-Trujillo, H., Quirino-Barreda, T., Sánchez-Herrera, K., Díaz-García, R., & Calvo-Carrillo, C. (2015). Chemical composition and amino acids content of five species of edible Grasshoppers from Mexico. *Emirates Journal of Food and Agriculture*, *27*(8), 654–658.

Mertz, E. T., Hassen, M. M., Cairns-Whittern, C., Kirleis, A. W., Tu, L., & Axtell, J. D. (1984). Pepsin digestibility of proteins in sorghum and other major cereals. *Proceedings of the National Academy of Sciences of the United States of America*, *81*, 1–2.

Meutchieye, F., Tsafo, K. E. C., & Niassy, S. (2016). Inventory of edible insects and their harvesting methods in the Cameroon centre region. *Journal of Insects as Food and Feed*, *2*(3), 145–152.

Meyer-Rochow, V. B., Gahukar, R. T., Ghosh, S., & Jung, C. (2021). Chemical Composition, Nutrient Quality and Acceptability of Edible Insects Are Affected by Species, Developmental Stage, Gender, Diet, and Processing Method. *Foods*, *10*(1036).

Miech, P., Berggren, Lindberg, J. E., Chhay, T., Khieu, B., & Jansson, A. (2016). Growth and survival of reared Cambodian field crickets (Teleogryllus testaceus) fed weeds, agricultural and food industry by-products. *Journal of Insects as Food and Feed*, *2*(4), 285–292.

Mmari, M. W., Kinyuru, J. N., Laswai, H. S., & Okoth, J. K. (2017). Traditions, beliefs and indigenous technologies in connection with the edible longhorn grasshopper Ruspolia differens (Serville 1838) in Tanzania. *Journal of Ethnobiology and Ethnomedicine*, *13*(60), 1–11.

Moyo, B., Masika, P. J., Hugo, A., & Muchenje, V. (2016). Nutritional characterization of Moringa (Moringa oleifera Lam.) leaves. *African Journal of Biotechnology*, *10*(60), 12925–12933.

Mugo-Bundi, J., Oyoo-Okoth, E., Ngugi, C. C., Manguya-Lusega, D., Rasowo, J., Chepkirui-Boit, V., Opiyo, M., & Njiru, J. (2015). Utilization of Caridina nilotica (Roux) meal as a protein ingredient in feeds for Nile tilapia (Oreochromis niloticus). *Aquaculture Research*, *46*(2), 346–357.

Mukherjee, R., Chakraborty, R., & Dutta, A. (2016). Role of fermentation in improving nutritional quality of soybean meal - A review. *Asian-Australasian Journal of Animal Sciences*, *29*(11), 1523–1529.

Muñoz-Cárdenas, K., Fuentes, L. S., Cantor, R. F., Rodríguez, C. D., Janssen, A., & Sabelis, M. W. (2014). Generalist red velvet mite predator (Balaustium sp.) performs better on a mixed diet. *Experimental and Applied Acarology*, *62*(1), 19–32.

Musundire, R., Osuga, I. M., Cheseto, X., Irungu, J., & Torto, B. (2016). Aflatoxin contamination detected in nutrient and anti-oxidant rich edible stink bug stored in recycled grain containers. *PLoS ONE*, *11*(1), 1–16.

Mutayoba, S. K., Dierenfeld, E., Mercedes, V. A., Frances, Y., & Knight, C. D. (2011). Determination of chemical composition and ant-nutritive components for tanzanian locally available poultry feed ingredients. *International Journal of Poultry Science*, *10*(5), 350–357.

Mwangi, M. N., Oonincx, D. G. A. B., Stouten, T., Veenenbos, M., Melse-Boonstra, A., Dicke, M., & Van Loon, J. J. A. (2018). Insects as sources of iron and zinc in human nutrition. *Nutrition Research Reviews*, *31*(2), 248–255.

Mwesigwa, R., Mutetikka, D., & Kugonza, D. R. (2012). Performance of growing pigs fed diets based on by-products of maize and wheat processing. *Tropical Animal Health and Production*, *45*(1), 441–446.

Nash, W. J., & Chapman, T. (2014). Effect of dietary components on larval life history characteristics in the medfly (Ceratitis capitata: Diptera, Tephritidae). *PLoS ONE*, *9*(1).

Ngonga, C. A., Gor, C. O., Okuto, E. A., & Ayieko, M. A. (2021). Growth Performance of Acheta Domesticus and Gryllus Bimaculatus Production Reared under Improvised Cage System for Increased Returns and Food Security. *Journal of Insects as Food and Feed*, *7*(3), 301–310.

Nyakeri, E. M. (2018). *Optimization of Production of Black Soldier Fly Larvae (Hermetia illucens, L) For Fish Feed Formulation*. Jaramogi Oginga Odinga University of Science and Technology.

Nyangena, D. N., Mutungi, C., Imathiu, S., Kinyuru, J., Affognon, H., Ekesi, S., Nakimbugwe, D., & Fiaboe, K. K. M. (2020). Effects of Traditional Processing Techniques on the Nutritional and Microbiological Quality of Four East Africa. *Foods*, *9*, 574.

Ochieng, B. O., Anyango, J. O., Nduko, J. M., Cheseto, X., Mudalungu, C. M., Khamis, F. M., Ghemoh, C. J., Egonyu, P. J., Subramanian, S., Nakimbugwe, D., Ssepuuya, G., & Tanga, C. M. (2022). Dynamics in nutrients, sterols and total flavonoid content during processing of the edible Long-Horned grasshopper (Ruspolia differens Serville) for food. *Food Chemistry*, *383*, 132397.

Oibiokpa, F. I., Akanya, H. O., Jigam, A. A., & Saidu, A. (2017). Nutrient and Antinutrient Compositions of Some Edible Insect Species in Northern Nigeria. *Fountain Journal of Natural and Applied Sciences*, *6*(1), 9–24.

Okia, C. A., Odongo, W., Nzabamwita, P., Ndimubandi, J., Nalika, N., & Nyeko, P. (2017). Local knowledge and practices on use and management of edible insects in Lake Victoria basin, East Africa. *Journal of Insects as Food and Feed*, *3*(2), 83–93.

Oonincx, D. G. A. B., van Itterbeeck, J., Heetkamp, M. J. W., van den Brand, H., van Loon, J. J. A., & van Huis, A. (2010). An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. *PLoS ONE*, *5*(12), 1–7.

Opoke, R., Malinga, G. M., Rutaro, K., Nyeko, P., Roininen, H., & Valtonen, A. (2019). Seasonal pattern in population dynamics and host plant use of non - swarming Ruspolia differens Serville (Orthoptera : Tettigoniidae). *Journal of Applied Entomology*, *143*(4), 371–379.

Opoke, R., Nyeko, P., Malinga, G. M., Rutaro, K., Roininen, H., & Valtonen, A. (2019). Host plants of the non-swarming edible bush cricket Ruspolia differens. *Ecology and Evolution*, *9*(7), 3899–3908.

Ordoñez-Araque, R., & Egas-Montenegro, E. (2021). Edible insects: A food alternative for the sustainable development of the planet. *International Journal of Gastronomy and Food Science*, *23*.

Orinda, M. A., Mosi, R. O., Ayieko, M. A., & Amimo, F. A. (2017). Growth performance of Common house cricket (Acheta domesticus) and field cricket (Gryllus bimaculatus) fed on agro-byproducts. *Journal of Entomology and Zoology Studies*, *5*(6), 1664–1668.

Orkusz, A. (2021). Edible insects versus meat—nutritional comparison: Knowledge of

their composition is the key to good health. *Nutrients*, *13*(4).

Pal, D., & Dubey, P. (2013). Flavonoids: A powerful and abundant source of antioxidants. *International Journal of Pharmacy and Pharmaceutical Sciences*, *5*(3), 95–98.

Pastor, B., Cickova, H., Kozanek, M., Martinez-Sanchez, A., Takac, P., & Rojo, S. (2011). Effect of the size of the pupae, adult diet, oviposition substrate and adult population density on egg production in Musca domestica (Diptera: Muscidae). *European Journal of Entomology*, *108*(4), 587–596.

Paul, A., Frederich, M., Uyttenbroeck, R., Hatt, S., Malik, P., Lebecque, S., Hamaidia, M., Miazek, K., Goffin, D., Willems, L., Deleu, M., Fauconnier, M. L., Richel, A., De Pauw, E., Blecker, C., Monty, A., Francis, F., Haubruge, É., … Danthine, S. (2016). Grasshoppers as a food Source: A review. *Biotechnologie, Agronomie, Société et Environnement*, *20* (AgricultureIsLife), 337–352.

Peterson, T. N., Welti, E. A. R., & Kaspari, M. (2021). Dietary sodium levels affect grasshopper growth and performance. *Ecosphere*, *12*(3).

R Core Team. (2020). *R: A language and environment for statistical computing* (4.1.2). R Foundation for Statistical Computing. https://www.r-project.org/.

Rho, M. S., & Lee, K. P. (2014). Geometric analysis of nutrient balancing in the mealworm beetle, Tenebrio molitor L. (Coleoptera: Tenebrionidae). *Journal of Insect Physiology*, *71*, 37–45.

Roeder, K. A., & Behmer, S. T. (2014). Lifetime consequences of food protein-carbohydrate content for an insect herbivore. *Functional Ecology*, *28*(5), 1135–1143.

Roy, N., & Barik, A. (2012a). Influence of four host-plants on feeding, growth and reproduction of Diacrisia casignetum (Lepidoptera: Arctiidae). *Entomological Science*, *16*(1), 112–118.

Roy, N., & Barik, A. (2012b). The impact of variation in foliar constituents of sunflower on development and reproduction of Diacrisia casignetum Kollar (Lepidoptera: Arctiidae). *Psyche (London)*, *2012*.

Rumpold, B. A., & Schlüter, O. K. (2013a). Nutritional composition and safety aspects of edible insects. *Molecular Nutrition and Food Research*, *57*(5), 802–823.

Rumpold, B. A., & Schlüter, O. K. (2013b). Potential and challenges of insects as an innovative source for food and feed production. *Innovative Food Science and Emerging Technologies*, *17*, 1–11.

Rutaro, K., Malinga, G. M., Lehtovaara, V. J., Opoke, R., Nyeko, P., Roininen, H., & Valtonen, A. (2018). Fatty acid content and composition in edible Ruspolia differens feeding on mixtures of natural food plants. *BMC Research Notes*, *11*(1), 1–6.

Rutaro, K., Malinga, G. M., Lehtovaara, V. J., Valtonen, A., Kwetegyeka, J., Nyeko, P., & Roininen, H. (2018). The fatty acid composition of edible grasshopper Ruspolia differens (Serville) (Orthoptera: Tettigoniidae) feeding on diversifying diets of host plants. *Entomological Research*, *48*(6), 490–498.

Rutaro, K., Malinga, G. M., Opoke, R., Lehtovaara, V. J., Omujal, F., Nyeko, P., Roininen, H., & Valtonen, A. (2018). Artificial diets determine fatty acid composition in edible Ruspolia differens (Orthoptera: Tettigoniidae). *Journal of Asia-Pacific Entomology*, *21*(4).

Salama, S. (2020). Nutrient Composition and Bioactive Components of the Migratory Locust (Locusta migratoria). In A. Mariod (Ed.), *African Edible Insects As Alternative Source of Food, Oil, Protein and Bioactive Components* (pp. 231–239). Springer Nature.

Schabel, H. G. (2010). Forest insects as food: A global review. In P. B. Durst, D. V.Johnson, R. N. Leslie, & K. Shono (Eds.), *Forest insects as food: Humans bite back* (pp. 37–64). FAO.

Selaledi, L., Hassan, Z., Manyelo, T. G., & Mabelebele, M. (2021). Insects' Production, Consumption, Policy, and Sustainability: What Have We Learned from the Indigenous Knowledge Systems? *Insects*, *12*(432), 1–18.

Sengendo, F., Subramanian, S., Chemurot, M., Tanga, C. M., & Egonyu, J. P. (2021). Efficient Harvesting of Safe Edible Grasshoppers: Evaluation of Modified Drums and Light-Emitting Diode Bulbs for Harvesting Ruspolia differens (Orthoptera: Tettigoniidae) in Uganda. *Journal of Economic Entomology*, *114*(2), 676–683.

Sengendo, F., Subramanian, S., Kidoido, M., Chemurot, M., Tanga, C., & Egonyu, J. P. (2021). Cost–benefit analysis of improved light trap for harvesting the edible grasshopper, Ruspolia differens (Orthoptera: Tettigoniidae): Evidence from Uganda. *International Journal of Tropical Insect Science*, *41*(3), 1913–1921. https://doi.org/10.1007/s42690-021-00505-8

Shobana, K., Murugan, K., & Naresh Kumar, A. (2010). Influence of host plants on feeding, growth and reproduction of Papilio polytes (The common mormon). *Journal of Insect Physiology*, *56*(9), 1065–1070.

Shrivastava, S. K., Prakash, A., Rao, J., Saxena, S., & Arif, M. (2019). Grasshopper: an

Untapped Halal Food Source for Future India. *J. Appl. Zool. Res*, *30*(2), 93–132.

Silva, C. S., Moutinho, C., Ferreira da Vinha, A., & Matos, C. (2019). Trace minerals in human health: Iron, zinc, copper, manganese and fluorine. *International Journal of Science and Research Methodology*, *13*(3), 57–80.

Silva, R. B., Zanuncio, J. C., Serrão, J. E., Lima, E. R., Figueiredo, M. L. C., & Cruz, I. (2009). Suitability of different artificial diets for development and survival of stages of the predaceous ladybird beetle Eriopis connexa. *Phytoparasitica*, *37*(2), 115–123.

Singleton, V. L., & Rossi, J. J. . (1965). Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16*, 144–158.

Sirimungkararat, S., Saksirirat, W., Nopparat, T., & Natongkham, A. (2010). Edible products from eri and mulberry silkworms in Thailand. In P. B. Durst, D. V. Johnson, R. N. Leslie, & & K. Shono (Eds.), *Forest insects as food: Humans bite back* (pp. 189–200). FAO.

Siulapwa, N., Mwambungu, A., Lungu, E., & Sichilima, W. (2012). Nutritional Value of Four Common Edible Insects in Zambia. *International Journal of Science and Research 3*(6), 2319–7064.

Sodamade, A., Owonikoko, A. D., & Owoyemi, D. (2017). Nutrient contents and mineral composition of Moringa oleifera Seed. *International Journal of Chemical Studies*, *5*(2), 205–207.

Sorjonen, J. M., Lehtovaara, V. J., Immonen, J., Karhapää, M., Valtonen, A., & Roininen, H. (2020). Growth performance and feed conversion of Ruspolia differens on plant-based by-product diets. *Entomologia Experimentalis et Applicata*, *168*(6–7), 460–471.

Sorjonen, J. M., Valtonen, A., Hirvisalo, E., Karhapää, M., Lehtovaara, V. J., Lindgren, J., Marnila, P., Mooney, P., Mäki, M., Siljander-Rasi, H., Tapio, M., Tuiskula-Haavisto, M., & Roininen, H. (2019). The plant-based by-product diets for the mass-rearing of Acheta domesticus and Gryllus bimaculatus. *PLoS ONE*, *14*(6).

Ssepuuya, G. (2019). Shelf Life , sensorial , and nutritional quality of the long-horned grasshopper Ruspolia differens Serville.

Ssepuuya, G., Kagulire, J., Katongole, J., Kabbo, D., Claes, J., & Nakimbugwe, D. (2021). Suitable extraction conditions for determination of total anti-oxidant capacity and phenolic compounds in Ruspolia differens Serville. *Journal of Insects as Food and Feed*, *7*(2), 1–10.

Ssepuuya, G., Mukisa, I. M., & Nakimbugwe, D. (2017). Nutritional composition, quality, and shelf stability of processed Ruspolia nitidula (edible grasshoppers). *Food Science and Nutrition*, *5*(1), 103–112.

Ssepuuya, G., Nakimbugwe, D., De Winne, A., Smets, R., Claes, J., & Van Der Borght, M. (2020). Effect of heat processing on the nutrient composition, colour, and volatile odour compounds of the long-horned grasshopper Ruspolia differens serville. *Food Research International*, *129*.

Ssepuuya, G., Smets, R., Nakimbugwe, D., Van Der Borght, M., & Claes, J. (2019). Nutrient composition of the long-horned grasshopper Ruspolia differens Serville: Effect of swarming season and sourcing geographical area. *Food Chemistry*, *301*.

Ssepuuya, G., Tanga, C. M., Yekko, I., Sengendo, F., Ndagire, C. T. T., Fiaboe, K. K. M., Karungi, J., & Nakimbugwe, D. (2018). Suitability of egg hatching conditions and commonly available food plants for rearing the long-horned grasshopper Ruspolia differens Serville (Orthoptera: Tettigoniidae). *Journal of Insects as Food and Feed*, *4*(4), 253–261.

Ssepuuya, G., Wynants, E., Verreth, C., Crauwels, S., Lievens, B., Claes, J., Nakimbugwe, D., & Van Campenhout, L. (2019). Microbial characterisation of the edible grasshopper Ruspolia differens in raw condition after wild-harvesting in Uganda. *Food Microbiology*, *77*, 106–117.

Stadlmayr, B., Charrondiere, U. R., Enujiugha, V. N., Bayili, R. G., Fagbohoun, E. G., Samb, B., Baddy, P., Barikmo, I., Ouattara, F., Oshaug, A., Akinyele, I., Annor, G. A., Bomfeh, K., Ene-Obong, H., Smith, I. F., Thiam, I., & Burlingame, B. (2012). West African food composition table.

Suk, Y. O., & Washburn, K. W. (1995). Effects of Environment on Growth, Efficiency of Feed Utilization, Carcass Fatness, and Their Association. *Poultry Science*, *74*, 285–296.

Thermo Fisher Scientific. (2010). Determination of water – and fat – soluble vitamins by HPLC, Knowledge Creation Diffusion Utilization.

Thomas, B. (2013). Sustainable harvesting and trading of mopane worms (Imbrasia belina) in Northern Namibia: an experience from the Uukwaluudhi area. *International Journal of Environmental Studies*, *70*, 494–502.

Valtonen, A., Malinga, G. M., Junes, P., Opoke, R., Lehtovaara, V. J., Nyeko, P., & Roininen, H. (2018). The edible katydid Ruspolia differens is a selective feeder on the inflorescences and leaves of grass species. *Entomologia Experimentalis et*

Applicata, *166*(7), 592–602.

Van der Putten, W. H., Bardgett, R. D., Bever, J. D., Bezemer, T. M., Casper, B. B., Fukami, T., Kardol, P., Klironomos, J. N., Kulmatiski, A., Schweitzer, J. A., Suding, K. ., & Wardle, D. A. (2013). Plant–soil feedbacks: the past, the present and future challenges. *Journal of Ecology*, *101*(2), 265–276.

van Huis, A. (2013). Potential of Insects as Food and Feed in Assuring Food Security. In *Annual Review of Entomology* (Vol. 58, Issue 1, pp. 563–583).

van Huis, A. (2015). Edible insects contributing to food security? *Agriculture and Food Security*, *4*(1), 1–9.

van Huis, A. (2022). Cultural significance of locusts, grasshoppers, and crickets in sub-Saharan Africa. *Journal of Ethnobiology and Ethnomedicine*, *18*(1), 1–19.

van Huis, A., & Oonincx, D. G. A. B. (2017). The environmental sustainability of insects as food and feed. A review. *Agronomy for Sustainable Development*, *37*(5).

van Huis, A., van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G., & Vantomme, P. (2013). *Edible insects: Future prospects for food and feed security*.

World Health Organization & United Nations University (2007). P*rotein and amino acid requirements in human nutrition* (Vol.935).World Health Orgarnization.

Williams, J. P., Williams, J. R., Kirabo, A., Chester, D., & Peterson, M. (2016). Nutrient Content and Health Benefits of Insects. In *Insects as Sustainable Food Ingredients*. Elsevier Inc.

Yen, A. L. (2009). Edible insects: Traditional knowledge or western phobia? *Entomological Research*, *39*(5), 289–298.

Yorek, M. A. (2018). The potential role of fatty acids in treating diabetic neuropathy. *Current Diabetes Reports*, *18*(10), 1–10.

Zamudio-Flores, P. B., Tirado-Gallegos, J. M., Espino-Díaz, M., Ochoa-Reyes, E., Hernández-Centeno, F., Hernández-Gonzaléz, M., López-De la Peña, H. Y., Salgado-Delgado, R., García-Cano, V. G., & Sánchez-Ortíz, O. (2019). Food supplements from a Grasshopper: A developmental stage-wise evaluation of amino acid profile, protein and vitamins in Brachystola magna (Girard). *Emirates Journal of Food and Agriculture*, *31*(7), 561–568.

Zhang, Z., Stout, M. J., Shang, H., & Pousson, C. (2004). A Method for Rearing the Rice Water Weevil , Lissorhoptrus oryzophilus (Coleoptera: Curculionidae), in the Laboratory. *The Coleopterists Bulletin*, *58*(4), 644–651.

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid

contents in mulberry and their scavenging effects on Superoxide radicals. *Food Chem*, *64*, 555–559.

Zhu, D. H., Zhao, Q., & Tanaka, S. (2013). Influence of male presence on reproductive parameters of Locusta migratoria (orthoptera: Acrididae) females. *Annals of the Entomological Society of America*, *106*(1), 66–71.

Zielińska, E., Baraniak, B., Karaś, M., Rybczyńska, K., & Jakubczyk, A. (2015). Selected species of edible insects as a source of nutrient composition. *Food Research International*, *77*, 460–466.

APPENDICES

Appendix 1: University ERC approval

JARAMOGI OGINGA ODINGA
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DIVISION OF RESEARCH, INNOVATION AND OUTREACH
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BONDO

OUR REF: JOOUST/DVC-RIO/ERC/E2

7th November, 2019

Margaret Orengo Kababu
SAFS
JOOUST

Dear Ms. Kababu,

RE: APPROVAL TO CONDUCT RESEARCH TITLED *"OPTIMIZING MASS REARING CONDITIONS OF THE EDBILBE GRASSHOPPER RUSPOLIA DIFFERENS"*

This is to inform you that JOOUST ERC has reviewed and approved your above research proposal. Your application approval number is **7/14/ERC/11/19-20.** The approval period is from 6th November, 2019 – 5th November, 2020.

This approval is subject to compliance with the following requirements:

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations and violations) are submitted for review and approval by JOOUST IERC.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to NACOSTI IERC within 72 hours of notification.
iv. Any changes. anticipated or otherwise that may increase the risks of affected safety or welfare of study participants and others or affect the integrity of the research must be reported to NACOSTI IERC within 72 hours.
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to JOOUST IERC.

Prior to commencing your study, you will be expected to obtain a research permit from National Commission for Science, Technology and Innovation (NACOSTI) https:oris.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely,

Prof. Francis Aiga'wa
Chairman, JOOUST ERC

Cc: Deputy Vice-Chancellor, RIO Director, BPS Dean, SAFS Director, INSEFOODS

Appendix 2: NACOSTI research permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **382819**

Date of Issue: **16/February/2021**

RESEARCH LICENSE

This is to Certify that Ms.. Margaret Kababu of Jaramogi Oginga Odinga University of Science and Technology, has been licensed to conduct research in Homabay, Siaya on the topic: Optimizing Mass rearing conditions of the edible grasshopper Ruspolia differens for the period ending : 16/February/2022.

License No: **NACOSTI/P/21/5103**

382819

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

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Appendix 3: Publication 1

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ORIGINAL ARTICLE

Food Science & Nutrition WILEY

Variability in nutrient composition of the edible long-horned grasshopper (*Ruspolia differens*) in Uganda and its potential in alleviating food insecurity

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Abstract

Ruspolia differens Serville (Orthoptera: Tettigonidae) is a highly nutritious and luxurious insect delicacy that is consumed as a food source in many African countries. However, the nutrient profile of *R. differens* in different geographical regions have received limited research interest. Here, we provide comprehensive evidence of geographical impact on the nutrient profile of *R. differens* and its potential to meet the recommended dietary intake of the population. Our results demonstrated that proximate composition, fatty acids, amino acids, minerals, vitamins, and flavonoid contents of *R. differens* collected from five districts in Uganda varied considerably. The crude protein (28–45%), crude fat (41–54%), and energy (582–644 Kj/100 g) contents of *R. differens* exceed that reported from animal origins. The highest crude protein, crude fat, and carbohydrate contents of *R. differens* were recorded in Kabale, Masaka, and Kampala, respectively. A total of 37 fatty acids were identified with linoleic acid (omega-6 fatty acid) being the most abundant polyunsaturated fatty acid in *R. differens* from Kabale, Masaka, and Mbarara. All essential amino acids were recorded in *R. differens*, particularly histidine with values exceeding the daily requirement for adults. Mineral and vitamin content differed significantly across the five districts. The highest quantity of flavonoids was recorded in *R. differens* from Hoima (484 mg/100 g). Our findings revealed that *R. differens* could be considered as functional food ingredients capable of supplying essential macro- and micronutrients that are critical in curbing the rising food insecurity and malnutrition in the regions.

KEYWORDS
edible grasshoppers, food security, functional food ingredient, geographical location, *Ruspolia differens*, vulnerable communities

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Innovative feedstocks for optimal mass production of the edible long-horned grasshopper, *Ruspolia differens*

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The edible long-horned grasshopper *Ruspolia differens* Serville (Orthoptera: Tettigoniidae) is a highly nutritious food source consumed in over 20 African countries. Its occurrence is highly seasonal, and wild harvesting is carried out using locally designed and inefficient light traps, thus limiting sustainable utilization as an important food source. To ensure year-round production and availability of *R. differens*, we evaluated the effects of low-cost and affordable diets based on agricultural by-products on their growth performance, survival, fecundity, and longevity. A total of four diets with varying ratios of agricultural by-products were evaluated: Diet 1 [33.3% maize bran (MB) + 33.3% wheat bran (WB) + 33.3% *Moringa oleifera* leaf powder (MOLP)], Diet 2 [25% MB + 25% WB + 25% MOLP + 25% shrimp powder (SP)], Diet 3 [20% MB + 20% WB + 20% MOLP + 20% SP + 20% soya bean meal], and Diet 4 ("control"—routinely used diet). The grasshoppers were subjected to the diets from the 1st nymphal instar (24-h-old stages) through adult stages until death. Diet 3 had the highest crude protein content (28%) and digestibility (74.7%). *R. differens* fed Diet 3 had the shortest development time (57 days) [$p < 0.001$], highest survival (87%) [$p < 0.001$], and maximum longevity (89 days) [$p = 0.015$] and fecundity (247 eggs/female) [$p = 0.549$] across the various diets. Female survival rate (59%) on Diet 3 was significantly higher compared to the males (41%). The adult female weight gain was significantly higher compared to males fed on different diets. Percentage hatchability of eggs was not significantly different when females were fed Diet 3 and Diet 2. There was a significantly positive correlation between longevity and fecundity of *R. differens* reared on Diet 2 and 3. These diets could be further optimized and fine-tuned for improved cost-effective mass production of *R. differens* continent-wide to reduce dependence on erratic and poor seasonal harvest during swarms.