PERFORMANCE OF AMARANTH ACCESSIONS AGAINST MOISTURE STRESS AND KEY INSECT PESTS AND THEIR INDIGENOUS PARASITOIDS IN ARUSHA, TANZANIA

STEPHEN TARMOGIN OMBURO OTHIM (MSc. Crop Prot. Ento)

A99/38397/2016

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN AGRICULTURAL ENTOMOLOGY OF KENYATTA UNIVERSITY.

NOVEMBER 2019

DECLARATION

I Stephen Tarmogin Omburo Othim declare that this thesis is my original work and has not been presented for the award of a degree in any other university or any other award.

Signature ……Date…….

Declaration by supervisors

We confirm that the work reported in this thesis was carried out by the candidate under our supervision and has been submitted with our approval as supervisors.

Signature…….....Date ……

Dr. Ruth Kahuthia-Gathu

Department of Agricultural Science and Technology

Kenyatta University (KU)

Signature…….....Date ……

Dr. Komi Kouma Mokpokpo Fiaboe

Plant Health Theme

International Centre of Insect Physiology and Ecology (*icipe*)

Signature .....Date ……

Dr. Srinivasan Ramasamy

Entomology department

World Vegetable Centre (WorldVeg)

DEDICATION

Dedicated to my loving and dearest wife *Diana Nyanting'a* who has been my greatest source of inspiration and whose tolerance and sacrifice has seen me complete this thesis.

ACKNOWLEDGEMENTS

Greatest appreciation to the Almighty God who Has been faithful and brought me to this great achievement.

I extend my deepest appreciation to my loving wife Diana Nyanting'a for her endless motivation and moral support during my research.

I sincerely thank my supervisors Dr. Ruth Kahuthia-Gathu of Kenyatta University (KU), Dr. Komi Fiaboe of International Centre of Insect Physiology and Ecology (*icipe*)/International Institute of Tropical Agriculture (IITA) and Dr. Srinivasan Ramasamy of World Vegetable Center (WorldVeg) for their guidance, advice and criticism that really helped me in my research and write-up. It is through their effort that this thesis acquired shape and was completed.

I thank the African Indigenous Vegetables – Integrated Pest Management (AIV-IPM) project teams both at *icipe* and Worldveg Arusha for their support and the Capacity building team at *icipe* for continuous support, training and guidance.

I thank *icipe*, WorldVeg and KU for granting me an opportunity to conduct my research at their campuses and for the entire infrastructure that was at my disposal during the research.

Special thanks to The Federal Ministry for Economic Cooperation and Development (BMZ) for sponsoring my research through the AIV-IPM project.

Special thanks to my loving mum Florence Othim, my siblings and my friends for their moral support throughout my entire period of study.

LIST OF ABBREVIATIONS AND ACRONYMS

AIV:	African indigenous vegetable
ANOVA:	Analysis of variance
ASL:	Above sea level
Bt:	*Bacillus thuringiensis*
CRD:	Completely randomized design
DAFF:	Department of Agriculture Fisheries and Forestry
DM:	Dry mass
EPF:	Entomopathogenic fungi
ESA:	Eastern and Southern Africa
GLM:	General linear model
HCDA:	Horticultural Crops Development Authority
HPR:	Host plant resistance
HSD:	Honestly significant difference
icipe:	International Centre of Insect Physiology and Ecology
IPM:	Integrated Pest Management
KU	Kenyatta University
LA:	Leaf area
LAR:	Leaf area ratio
LL:	Leaf length
LMR:	Leaf mass ratio
LSD:	Least significant difference

LW:	Leaf width
NAFIS:	National Farmers Information Services
NPV:	Nucleopolyhedrovirus
NRC:	National Research Council
PAA:	Phenylacetaldehyde
RCBD:	Randomized complete block design
RGR:	Relative growth rate
RH:	Relative humidity
RMR:	Root mass ratio
RR:	Relative risk/Risk ratio
RSR:	Root to shoot ratio
SLA:	Specific leaf area
WHC:	Water holding capacity
WorldVeg:	World Vegetable Centre

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABSTRACT

Amaranths are African indigenous vegetables (AIVs) that have recently gained importance as a food source in Africa owing to the high nutritional value of their leaves and grains. Production of this crop is, however, limited by arthropod pests especially the lepidopteran leaf-webbers, leaf-worms and stem weevils. The use of insecticides for their management is uneconomical and also present health and environmental concerns. Host plant resistance (HPR) to insects is an effective, economical and environmentally friendly alternative that is poorly understood and unexploited among AIVs. The aim of this study was to assess *Amaranthus* accessions for resistance to leaf-webbers and stem weevils, their tolerance to water stress conditions and the performance of indigenous parasitoids on selected accessions. Field and laboratory experiments were conducted at the World Vegetable Centre (WorldVeg) in Arusha Tanzania with 36 accessions and lines of amaranth. Accessions VI036227, RVI00027, VI054569, VI033487, VI044432, VI048076, VI049639, VI049530 and VI049698 had high levels of pest resistance with significantly lower infestations (≤ 11.11±2.14%) and damage (≤ 68.06±3.90%) by leaf-webbers and leaf-worms. The accession VI036227 was found to be highly resistant against *Spoladea recurvalis*, exhibiting exemplary antibiosis by causing 100% larval mortality despite not being deterrent for oviposition. The accessions VI048076, VI056563 and VI047555-B demonstrated moderate resistance against the pest for specific parameters including low oviposition, moderate early stage larval mortality and reduced adult longevity. The relative growth rate (RGR) of accessions VI033479, VI049698 and VI056563 were not significantly affected by the three soil water levels (40%, 60% and 90% water holding capacity (WHC)). *Apanteles hemara* performed well on all the other moderately resistant accessions except VI056563 that recorded lower parasitism rates compared to the susceptible accession. The longevity of the parasitoid was significantly extended on the resistant accessions compared to the susceptible one. The functional response curve exhibited by *A. hemara* corresponded to type II functional response with an asymptote at the density of 30 larvae. *Apanteles hemara* parasitism was significantly higher in 1-2-day-old compared to 3-4-day-old larvae (P=0.04). Thus, accessions VI036227 and VI049698 were identified to be highly resistant to leaf-webbers in addition to 24 moderately resistant ones while VI047517-B, VI036227 and VI056563 had low levels of resistance against stem weevils. Accessions VI033479, VI049698 and VI056563 were also tolerant to moisture stress. The identified pest resistant and water stress-tolerant amaranth accessions from this study are recommended for multiplication and release to farmers to alleviate the effects of pests and drought. These can also be used in breeding programs to improve locally cultivated varieties. The identified parasitoids can also be reared and released into farmer fields to synergize host plant resistance.

CHAPTER ONE. GENERAL INTRODUCTION

1.1. Background information

Amaranthus sp. (Caryophyllales: Amaranthaceae) is known as an orphaned, neglected or underutilized crop among other African indigenous vegetables (AIVs). However, amaranth has been exploited as a vegetable, grain, animal feed, and as an ornamental in most parts of the world (NAFIS, 2011). The leaves have a high energy value and are richly endowed with protein, calcium, potassium, iron, ascorbic acid, lysine, vitamins A, B and C, and have also shown potential benefits as medicinal plants (Costea *et al*., 2004; Ouma, 2004). The grains are equally highly nutritious and are largely used in feeding children and the elderly to boost their immunity by supplying the much-needed micro-nutrients, and as a major source of relief for the lactose-intolerant individuals (Gikonyo *et al*., 2011; Amicarelli and Camaggio, 2012). Amaranth is also rich in Squalene, a special component of amaranth oil which is used as an important ingredient of cosmetics preparation in pharmaceutical industries, as a lubricant in servicing computers, and production of edible oil for domestic use (NAFIS, 2011; Amicarelli and Camaggio, 2012).

Amaranthus is a large genus that includes three recognized sub-genera and nearly 75 species with between 4,000 to 6,000 varieties. These varieties are distributed all over the world and can grow over a very broad range of climatic conditions (Infonet-biovision, 2018).

In East Africa, amaranth has for a long time been considered as a weed and therefore neglected like several other AIVs by most people who have found a replacement in exotic varieties of vegetables (Ekesa *et al*., 2009). However, a rise in its consumption and demand has been reported over the past decade due to increasing awareness of its nutritional and medicinal richness and as a source of income for both small scale and large-scale farmers (Ouma, 2004; Kagali *et al*., 2013). In Kenya, for example, both leaf and grain amaranth were cultivated in a total area of 1,806 Hectares with a net production of 13,134 Metric tons valued at USD 3,444,057 in 2016 (HCD, 2015-2016).

The production of amaranth in several regions is however affected by numerous arthropod pests and diseases that limit its productivity (Aderolu *et al*., 2013). Pests reported to infest amaranth across the world include beet webworm *Spoladea recurvalis* F., southern beet webworm *Herpetogramma bipunctalis* F., Cotton leaf roller *Sylepta derogata* F., and *Psara basalis* Walker (Lepidoptera: Crambidae); southern armyworm *Spodoptera eridania* Stoll, beet armyworm *S. exigua* Hübner, fall armyworm *S. frugiperda* J.E. Smith, cotton leafworm *S. litura* F., cotton bollworm *Helicoverpa armigera* Hübner, and cutworm *Agrotis* sp., (Lepidoptera: Noctuidae); *Aspavia armigera* F. and southern green stink bug *Nezara viridula* L., (Hemiptera: Pentatomidae), Leaf miners *Liriomyza* sp. (Diptera: Agromyzidae), *Cletus* sp. (Hemiptera: Coreoidae), *Hypolixus nubilosus* Boheman, *H. truncatulus* F. (Coleoptera: Curculionidae), *Epilachna elaterii* Rossi (Coleoptera: Coccinellidae), *Hyphantria cunea* Drury (Lepidoptera: Arctiidae) and

aphids (Homoptera: Aphididae) (Clarke-Harris *et al.,* 2004; Sharma and Ramamurthy, 2009; James *et al.,* 2010; Aderolu *et al.,* 2013).

Spoladea (*Hymenia*) *recurvalis* F. (Lepidoptera: Crambidae) and other Lepidopteran leaf webbers including *H. bipunctalis, P. basalis* have been reported to be the most damaging pests of amaranth world-wide (Clarke-Harris *et al*., 2004; Sharma and Ramamurthy, 2009; Aderolu *et al*., 2013). They usually occur in various combinations within any given field but in most cases, one pest species usually predominates (Clarke-Harris *et al*., 2004; Aderolu *et al*., 2013). These pests cause severe damage to the crop which sometimes leads to complete yield loss (James *et al*., 2010).

Management of the pests that attack amaranths has been majorly through the use of synthetic insecticides which lead to environmental degradation, pollution and pest resistance to pesticides. In addition, indiscriminate use of pesticides has led to resurgence of secondary pests, decimation of natural enemies and high residues on the produce which pose serious challenges to human and animal health (Chahal *et al*., 1997; Arivudainambi *et al*., 2010; Srinivasan, 2012). Clarke-Harris *et al*. (2004) observed failure of insecticides in the field in managing the lepidopteran pests of amaranth and attributed this failure to pesticide resistance especially to pyrethroids. Since most farmers use insecticides extensively and indiscriminately, there is a high likelihood that most of these lepidopteran pests will continue to build resistance against a broader range of insecticides (Srinivasan, 2012). There is, thus, a great need to establish new control strategies

which are cost effective, environmentally safe and which do not pose health risks to humans. Various integrated pests management (IPM) strategies have been suggested in different regions but their adoption is, however, low due to their limited effectiveness compared to chemical insecticides (Srinivasan, 2012).

Host plant resistance (HPR) is still inadequately explored in vegetable production systems while it offers a potentially low-cost, practical and long-term solution for maintaining lower populations of pests and thereby reducing crop losses (Bellotti and Arias, 2001). In addition to being cost effective, HPR is also compatible with virtually every other control strategy including the use of natural enemies such as parasitoids (Eigenbrode and Trumble, 1994). Due to the neglect of most AIVs including amaranths, very limited studies have been conducted to establish HPR to pests in these crops. This study thus sought to identify amaranth accessions that are resistant to leaf-webbers and stem weevils, assess their effects on indigenous natural enemies and their tolerance to soil water stress with an aim of improving yields to ensure food and nutritional security and reduced production costs.

1.2. Statement of the problem

The nutritive value of amaranths and their potential to promote health and alleviate poverty in Eastern Africa and other parts of the continent has seen a rise in their demand and popularity. Within the East African region, amaranth is mainly grown by small holder farmers who can barely afford synthetic chemicals to manage amaranth leaf-webbers and stem weevils which are the major

devastating pests in the region. Apart from being expensive, synthetic insecticides can cause health and environmental risks that could be avoided through implementation of suitable and sustainable IPM alternatives. A combination of host plant resistance and the use of natural enemies could be one of the most sustainable solutions to the management of amaranth pests. However, the subject of HPR has barely been explored on African indigenous vegetables (AIVs) and still remains an unexploited area. With more than 4,000 varieties of *Amaranthus* grown worldwide, it would be impossible to miss a few varieties possessing resistant or pest non-preference traits. However, no studies have been conducted to screen for pest resistance in amaranths, specifically the amaranth leaf-webbers and stem weevils. How these resistant accessions would affect the performance of the indigenous natural enemies is also still unknown. The imminent challenge of climate change which has brought about unpredictable weather patterns and persistent drought conditions, especially in Africa, necessitate that the plants be tolerant to drought stress. Only a few studies have explored drought tolerant amaranth varieties, but no study has combined drought tolerance and pest resistance traits in amaranth accessions/lines/varieties.

In addition to screening for pest resistance, it is important to understand the mechanisms employed by the resistant amaranth accessions, their horticultural traits, their effects on the biology and reproduction of the pests and possible effects on the performance of natural enemies. These will aid in the development of good

quality lines of amaranth that not only confer resistance to pests but also provide good yields to farmers.

1.3. Significance of the study

Amaranth leaf-webbers and stem weevils are a great menace to small-holder farmers in Africa and the world at large. Identification of resistant accessions of amaranth and their dissemination will form a basis for poverty alleviation among the poor farmers by economic empowerment through reduction of production costs especially those related to pest control, thereby improving yields. Use of resistant accessions will lead to a reduction in the use of pesticides which normally lead to environmental pollution, pest resurgence and decimation of natural enemies. Identified indigenous natural enemies will be used in conjunction with the resistant accessions to lower the pest populations without causing harm to the environment. With the imminent challenge of climate change which has led to extended periods of drought in most parts of Africa, drought tolerant accessions will be recommended to ensure food and nutritional security in such areas. Plant breeding programs will also acquire information regarding the specific resistance traits and mechanisms exhibited by the resistant accessions and their desirable horticultural traits which they can further capitalize on during the development of other resistant lines and varieties. The information will also help to inform policies related to seed production and pest management in vegetable production.

1.4. Research questions

i. Which lepidopteran defoliators and stem weevils attack amaranth in Arusha Tanzania and are there indigenous parasitoids associated with them?

ii. Which accessions of amaranth are resistant to lepidopteran defoliators and stem weevils?

iii. In what ways do resistant amaranth accessions affect the biology of leaf-webbers?

iv. How do pest resistant amaranth accessions also tolerate water stress?

v. How is the performance of the leaf-webbers' indigenous parasitoids affected by pest resistant amaranth accessions?

vi. Does a leaf-webber's age and density affect the performance of indigenous parasitoids?

1.5. Research objectives

1.5.1. General objective

To assess *Amaranthus* accessions for resistance to leaf-webbers and stem weevils, tolerance to water stress conditions and evaluate the performance of indigenous parasitoids on selected accessions to establish a sustainable IPM strategy for amaranth pests in Arusha, Tanzania.

1.5.2. Specific objectives

i. To assess the occurrence and diversity of amaranth lepidopteran defoliators and stem weevils and their associated parasitoids in Arusha, Tanzania.

ii. To evaluate amaranth accessions for resistance against leaf-webbers and stem weevils under field conditions.

iii. To assess the possible mechanisms underlying resistance in amaranth accessions through their effects on the biology of leaf-webbers infesting amaranths in Arusha, Tanzania.

iv. To evaluate the selected resistant amaranth accessions for water stress tolerance in Arusha, Tanzania.

v. To assess the performance of identified indigenous parasitoids of leaf-webbers on selected resistant amaranth accessions in Arusha, Tanzania.

vi. To evaluate the effects of age and density of a selected pest host on the performance a selected indigenous parasitoid in Arusha Tanzania.

1.6. Null hypotheses

i. There are neither lepidopteran defoliators nor stem weevils attacking amaranth in Arusha, Tanzania.

ii. Amaranth accessions are not resistant against leaf-webbers and weevils under field conditions.

iii. Selected amaranth accessions do not have any effects on the biology of leaf-webbers infesting amaranths.

iv. The selected amaranth accessions are not tolerant to water stress and do not possess desirable horticultural traits.

v. Resistant amaranth accessions against leaf-webbers have no effect on performance of indigenous parasitoids.

vi. There are no effects of host age and density on the performance of selected indigenous parasitoid.

CHAPTER TWO. LITERATURE REVIEW

2.1 Description and uses of amaranth

Amaranthus is a large genus that includes three recognized sub-genera and nearly 75 species with between 4,000 to 6,000 varieties. Of major economic importance is the subgenus Amaranthus proper, which comprises the three species mainly grown for grain production: *Amaranthus hypochondriacus* L., *A. cruentus* L., and *A. caudatus* L (NRC, 1984). Other species of amaranths are grown for use as leafy vegetables, as feed, as potherbs, or as ornamentals (NRC, 1984; Trucco and Tranel, 2011). Amaranth is a very adaptable crop with resistance to drought, tolerance to a broad range of temperatures, and resistance to insect pests and diseases (Infonet-biovision, 2018). It thrives well at different elevations and on soils with variable levels of nutrients thus having worldwide distribution (Amicarelli and Camaggio, 2012; Infonet-biovision, 2018).

Amaranth has for many centuries been abandoned, neglected and underutilized as a potential source of food by various communities, researchers and policy makers (NRC, 1984; Adebooye and Opabode, 2004; Amicarelli and Camaggio, 2012). According to Yang and Keding (2012) and Lotter *et al*. (2014), a shift toward exotic vegetables in Africa has been mainly driven by the perception that modernized urban dwellers should not eat indigenous vegetables meant for the poor. There has however been an increasing interest and popularity of amaranths and other AIVs among various groups including researchers, conservationists, consumers and governments (Amicarelli and Camaggio, 2012). This is as a result

of changes in perception as an outcome of the realization of their richness and importance to human nutrition, medicine and nature (Adebooye and Opabode, 2004).

Versatile amaranth is exploited not only for its grains but also its leaves. The leaves have a high energy value and are rich in protein, calcium, potassium, iron, ascorbic acid**,** lysine and vitamins A, B and C (Ouma, 2004; Amicarelli and Camaggio, 2012; Lotter *et al*., 2014; Mbwambo *et al*., 2015). Notably, lack of vitamin A has led to nutritional deficiency in the tropics and blindness in many children (NRC, 1984; World Bank, 2011). In Tanzania for example, 24% of preschool-aged children and 15% of pregnant women are deficient in vitamin A while the rates of anaemia among preschool-aged children and pregnant women are 72% and 58%, respectively (World Bank, 2011). Compared to other potherbs, amaranth ranks among the best in calcium and iron and is an ideal source of vitamin A which could help to reduce the burden of malnutrition (Lotter *et al*., 2014; NRC, 1984). Amaranth leaves possess a spinach-like flavour and can be consumed fresh as vegetable in salads or mixed with other vegetables, boiled (potherbs) or fried in oil and consumed with meat or fish; they can be purred to provide base for sauces and baby food or dried to be used as spice (NRC, 1984; Amicarelli and Camaggio, 2012).

Amaranth grain protein is one of unusual quality because it contains high amounts of the amino acid lysine; nearly twice the lysine content of wheat protein, three times that of maize, and as much as is found in milk (NRC, 1984; Mlakar *et*

al., 2010;). The grains of amaranth can be used in numerous recipes including popped amaranth snack, porridge, chapati (flat bread), bread, cakes, biscuits, scones, pizzas and pancakes and also milled to be used in gruel (NRC, 1984; Mbwambo *et al*., 2015). Amaranth grains possess unique chemical composition and are different from other cereals in that they contain high amounts of proteins, amino acids and fats. The absence of gluten in amaranth proteins makes them most preferred in the diet for people suffering from celiac disease (gluten intolerance) (NRC, 1984; Mlakar *et al*., 2010; Amicarelli and Camaggio, 2012).

Amaranth grain, especially from *Amaranthus cruentus*, can be used to produce oil which has various health benefits such as improvement of circulatory system, increasing body energy, reducing pain, improved skin, lessening wrinkles, control of chronic diseases such as arthritis, allergies, diabetes, and asthma (Kirby *et al*., 2010). Furthermore, the oil can be used for healing of burns, infections and skin lesions, reduction of various symptoms of cancer, increasing white blood cells, and enhancing excretion of mercury and clearing of eczema (Kirby *et al*., 2010). In addition, Squalene, a component of amaranth oil which is a terpenoid and a precursor of cholesterol biosynthesis has led to increased attention in amaranth by pharmaceutical industries because of its health and cosmetic benefits (Amicarelli and Camaggio, 2012). Naturalists and conservationists also have an interest in the crop as an alternative to obtaining it from sharks which are a major source of Squalene (Amicarelli and Camaggio, 2012). Due to such growing

interests from various sectors, amaranth farmers can have an advantage of increased demand and therefore get better economic returns from amaranth.

Mature whole plants of *A. retroflexus* have been recommended as animal feed, providing 20 to 30% protein and over 40% soluble carbohydrates in above-ground tissue (Costea *et al*., 2004). Unprocessed amaranth grain can also be used in feeding poultry (NRC, 1984). Amaranth grains also possess useful medicinal properties, are used in phytoremediation, and also used as a source of genes for breeding programs to improve cultivated species (Costea *et al*., 2004). Due to its qualities of being inexpensive, drought tolerant, early maturing, easy to harvest and highly nutritive nature (NAFIS, 2011), amaranth could be fronted as a suitable supplement to maize farming in East Africa following the staple's unreliable supply that has aggravated food insecurity.

2.2 Varieties of amaranth

Amaranth has the highest number of cultivated species and varieties compared to all the other indigenous tropical leafy vegetables such as nightshade and spider plants among others (AVRDC, 2004). Some of the most common commercial amaranths are selections of *Amaranthus tricolor* which emanate in various leaf colours including white (light green), dark green, red, purple and variegated (AVRDC, 2004). More than 20 species of amaranth are consumed as vegetable or grain and people have different preferences for the different amaranth species. Across the world, varieties of *Amaranthus tricolor* L., *A. blitum* L., *A. spinosus* L., and *A. viridis* L. are consumed (Ebert *et al*., 2011). *Amaranthus*

cruentus, *A. dubius* and *A. blitum* are the most common vegetable varieties in Africa, Asia, China and India (NRC, 1984; Costea *et al*., 2003). The main varieties grown for grain are *A. cruentus*, *A. hypochondriacus* and *A. caudatus* (NRC, 1984; Shroyer *et al*., 1990). Most of the other varieties are majorly weeds and are not grown for economic purposes. The key weedy types are *A. viridis*, *A. spinosis*, *A. retroflexus*, and *A. hybridus*, with *A. retroflexus* ("pigweed") being one of the world's worst weeds (NRC, 1984).

2.3 Growing conditions of amaranth

Amaranth species can grow from sea level to 3,200 metres above sea level (ASL); only *A. caudatus* is known to grow well at altitudes above 2,500 m asl (NRC, 1984). They require temperature range of 16 to 35°C, with minimum temperatures of 15 to 17°C for seed germination (NRC, 1984). Amaranth can be grown during both wet and dry seasons, though irrigation is normally required during the dry season. It can, nonetheless, withstand drought after the plant has been established (NRC, 1984; DAFF, 2010). It is also adapted to low and medium humidity (Infonet-biovision, 2018). Amaranth thrives best in loam or silty-loam soils with good water-retention ability, but it can also grow on a broad range of soil types and soil moisture intensities. They can stand a soil pH from 4.5 to 8 and obliges proper land preparation and a well-prepared bed for good growth (AVRDC, 2004). This broad adaptability of amaranth is because it belongs to the C_4 group of dicotyledons whose pathways allow very high photosynthetic efficiency in a broad range of temperatures and moisture availability (NRC, 1984;

Amicarelli and Camaggio, 2012; Moskova, 2013). Amaranths can thus, fit into an all year-round production system that always ensures availability of nutritionally rich food for nutritionally deficient societies worldwide (Weinberger and Msuya, 2004).

Amaranth is planted either by direct seeding or transplanting depending on availability of seeds, labour and growing season (NRC, 1984; Infonet-biovision, 2018). Seedbeds should be of good tilth, well drained, and fairly level to prevent rain from washing away the tiny seeds or seedlings. Seeds must be planted no more than 1cm deep, and the seedbeds should have fine soil without large clods (NRC, 1984). Once the crop is established, the broad leaves usually form a canopy that inhibits development of weeds. However, weeding can also be done mechanically.

2.4 Major pests of amaranth

Lepidopteran defoliators and amaranth stem weevils have been reported in several studies to cause damage to the crop around the world (Clarke-Harris *et al*., 2004; James *et al*., 2010; Aderolu *et al*., 2013). Two distinct groups of lepidopteran defoliators have been frequently reported to cause losses in amaranths in several countries around the world. The first group is the leaf-webbers or webworms whose larvae fold, web or glue amaranth leaves using their silken webs as they feed within the leaves (Batra and Bhattacherjee, 1960; James *et al*., 2010). The second group is composed of leaf-worms which usually occur as occasional pests of amaranth. Their larvae also feed on amaranth leaves, causing windowing

on leaves, but unlike webworms, they do not glue or fold amaranth leaves (Mureithi *et al*., 2017). Major leaf-worms attacking amaranths belong to the family Noctuidae and include *Spodoptera littoralis* (Boisduval), S. *exigua* and *H. armigera* among others (Clarke-Harris and Fleischer, 2003; Clarke-Harris *et al*., 2004).

2.4.1 Amaranth leaf-webbers and their host crops

Amaranth leaf-webbers also known as webworms mostly belong to the family Crambidae and they characteristically fold or glue leaves together using their silken webs as they feed on the crop. Some examples of leaf-webbers include *S. recurvalis, U. ferrugalis, P. basalis, H. bipunctalis* and *Achyra rantalis* Guenee among others (Clarke-Harris *et al*., 2004; Arivudainambi *et al*., 2010; James *et al*., 2010; Grovida, 2015). They are widely distributed across the world and are found in the tropics and sub-tropical regions, including Africa, Asia, and Australia (Shirai, 2006; Bailey, 2007; De Prins and De Prins, 2014). They are also found in America and the Neotropics and have also been reported in the temperate regions including Belgium and Denmark (Bailey, 2007; Aderolu *et al*., 2013). In Africa, they have been reported in Cameroon, DR Congo, Equatorial Guinea, Ethiopia, Gambia, Kenya, La Reunion, Madagascar, Morocco, Mozambique, Namibia, Niger, Nigeria, Rwanda, Ghana, Senegal, Sierra Leone, Somalia, South Africa, Sudan, Togo, Tanzania, Zambia, Mauritius, Seychelles, Lesotho, Comoros and Zimbabwe (De Prins and De Prins, 2014).

Apart from the *Amaranthus* spp., the leaf-webbers have been reported on other crops such as the adzuki beans (*Vigna angularis* Willd), mung beans/ green grams (*V. radiata* L.), soy beans (*Glycine max* L.), sugar beet (*Beta vulgaris* L.), silver beet (*B. Vulgaris* var. *cicla* L.), spinach (*Spinacia oleracea* L.), purslane (*Portulaca* sp.), black pigweed (*Trianthema portulacastrum* L.), goosefoot (*Chenopodium* sp.), watermelon (*Citrullus lanatus* Thunb. var. *lanatus* Matsum and Nakai), aubergine/ eggplant (*Solanum melongena* L.), peanut (*Arachis hypogaea* L.), cotton (*Gossypium* sp.), and maize (*Zea mays* L.) (Bailey, 2007; James *et al*., 2010; De Prins and De Prins, 2014). Kahuthia-Gathu (2013), observed yield losses of up to 100% on spinach *Spinacia oleracea* L., family Amaranthaceae from *Spoladea recurvalis* infestations. The pest also infests wild hosts such as devils horse whip A*chyranthes aspera* L. (Amaranthaceae) (Kahuthia-Gathu, 2013). *Spoladea recurvalis* has been observed to feed voraciously on leaves of desert horsepurslane *Trianthema portulacastrum* L. leading to complete destruction of the weed and is therefore thought of as a potential biocontrol agent of the weed (Martin *et al*., 2004; Baltazar, 2009; Kedar and Kumaranag, 2013).

2.4.2 Amaranth leaf-webbers: Description, biology and damage

The amaranth leaf-webbers lay their eggs singly or in batches in grooves of leaf veins. The eggs differ in colour from white, cream to yellowish depending on the species (Seham *et al*., 2006; Grovida, 2015). The female adults of *S. recuvalis*

and *U. ferrugalis* can lay between 200 to 400 eggs during their lifespan (Ki-Yeol *et al*., 2002; Seham *et al*., 2006), and they usually have overlapping generations within a year. The eggs of *S. recurvalis* hatch after 5 - 7 days at 18.6 ± 2°C and 70 ± 5% Relative Humidity (RH) (Seham *et al*., 2006) (Figure 2.1), *U. ferrugalis* hatch in 5 ± 0.35 days at 25°C (Ki-Yeol *et al*., 2002) and *H. bipunctalis* in 5.59 days (Diez-Rodríguez *et al*., 2013). The first and second instar larvae feed on the epidermis of the leaves skeletonising the tissue and thereafter consume the entire leaf with the third instar being the most destructive in *S. recurvalis* (Aderolu *et al*., 2013). The larvae undergo five instars before they reach a pre-pupation stage and finally pupation which occurs in the soil (Grovida, 2015; Seham *et al*., 2006). The larval period in *S. recurvalis*, *U. ferrugalis* and *H. bipunctalis* lasts 24-30, 10-25 and 26-37 days, respectively, depending on temperature while the pupal period ranges between 15-18, 5-16 and 13-37 days, respectively (Ki-Yeol *et al*., 2002; Seham *et al*., 2006; Diez-Rodríguez *et al*., 2013) (Figure 2.1).

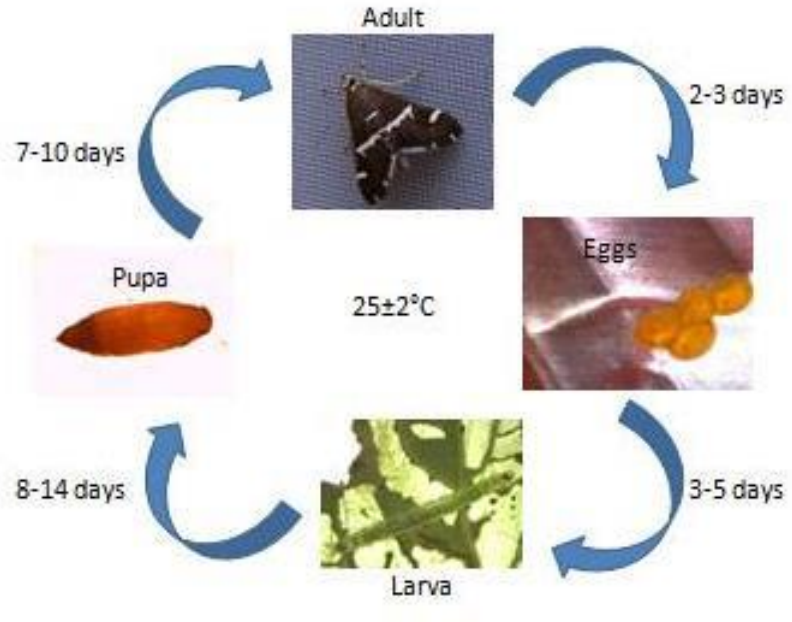

Figure 2.1: Life cycle of *Spoladea recurvalis* at 25 ± 2°C and 60 ± 10% RH

Spoladea recurvalis is also known as the Hawaiian beet webworm. It is largely restricted to plants in the family Chenopodiacea (Grovida, 2015). The life stages have been described by Clarke-Harris *et al*. (1998) and Grovida (2015) as follows: The adult is a dark brown moth with two white translucent bands on the forewings and one on the hind wings; these bands form a continuous arch pattern when the wings are spread. The forewing also bears one elongate and two small white spots distally and the wingspan is about 17-23 mm. The margin of the front is alternating dark and light and there are also narrow light bands on the abdomen. The eggs are elliptical, scale-like, shiny translucent yellow sacs, deposited singly or in rows of several eggs. The egg measures 0.6mm long, 0.5mm wide and

0.25mm in height and are normally laid on the lower surface of leaves adjacent to leaf veins (Clarke-Harris *et al*., 1998; Grovida, 2015).

The larvae are a translucent green with the gut visible through the integument as a pulsating dark green band. There are two longitudinal white wavy lines sandwiching the green band formed by the gut (Clarke-Harris *et al*., 1998). The head capsule is light coloured though a few dark spots are found on the head and thoracic plate (Grovida, 2015). Young larvae of *S. recurvalis* feed beneath the leaves and occasionally spin light webs in which they rest. The body bears numerous stout hairs over the length of its body but lacks the dark spots found with such hairs on many webworms (Grovida, 2015). In the pre-pupal stage, the larva changes colour from green to yellow to brown to bright pink. The larva webs the leaf around itself using silken threads and pupates there. Pupae are 8-10mm long and straw coloured (Clarke-Harris *et al*., 1998).

Leaf-webbers usually wrap young leaves in a loose web and feed within the protection of the web (Grovida, 2015). Young larvae of leaf-webbers feed only on the epidermis of the leaves skeletonising the tissues. After the second instar, they consume the entire leaf and eventually defoliate the plant (Grovida, 2015) (Plate 2.1). For example, the larvae of *S. recurvalis* skeletonises the foliage leaving only the main leaf veins intact and rolls amaranth leaves into distinctive leaf shelters, form webbing on leaves and leave frass on the leaves (James *et al*., 2010; Grovida, 2015). The webbing and rolling of the leaves deprive the crops of essential physiological processes and often leads to the death of the plant.

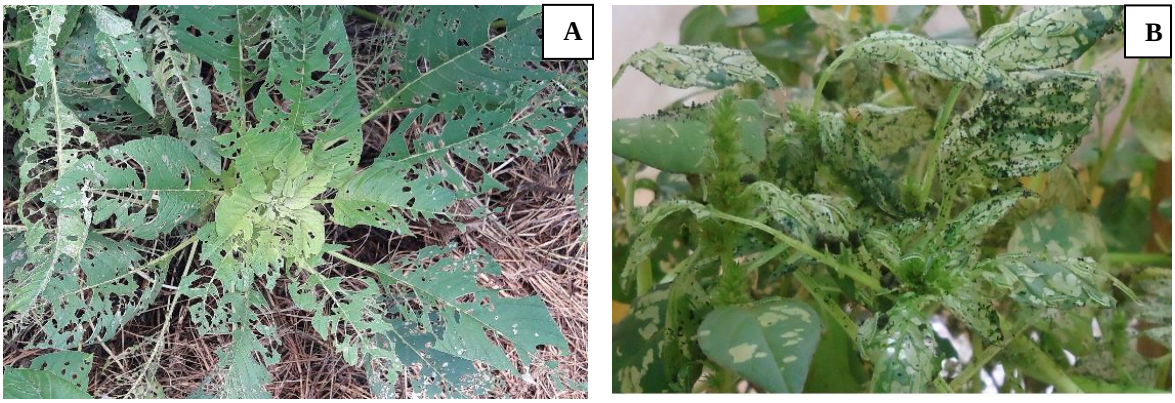

Plate 2.1: Damage caused by *Spoladea recurvalis* on amaranths A: windowing and skeletonization of leaves by older larvae B: damage caused by young larvae feeding on the leaf epidermis and the black frass they deposit

Udea ferrugalis larvae glue leaves together while feeding between them during which they leave dark frass on the leaves and also cause windowing of the leaves they have fed on. As they near pupation, they roll the leaves to form a protective covering for their pupae. *Psara basalis* larvae scrape epidermal and palisade tissues of leaves, web the leaves with silken threads resulting to drying of the webbed leaves (Grovida, 2015). *Eretmocera impactella* webs leaves with white silken threads and remains hidden in the folds while feeding on the inside.

2.4.3 Amaranth stem weevils: Description, biology and damage

Weevils belonging to the order Coleoptera and family Curculionidae are among the most prevalent pests of amaranth in the world with both their adults (as leaf feeders) and larvae (as root and/or stem borers) causing considerable damage to the crop (Louw *et al*., 1995). Several species including *Hypolixus haerens*

Boheman, *H. truncatulus* (Plate 2.2), *Gastroclisus* sp., and *Neocleonus sannio* Herbst., have been observed to cause damage in amaranth (Louw *et al*., 1995; Tara *et al*., 2009; García *et al*., 2011). Adults of *H. haerens* chew semi-circles out of the leaf edges, create windows in the leaf lamina, and also feed on the growing tips of the plant. They also leave visible faecal material as small brown blotches all over the plants (Louw *et al*., 1995).

Plate 2.2: Amaranth stem weevil *Hypolixus truncatulus* (Mag. X4)

Adults of *H. truncatulus* eat up tender margins of leaves, making irregular scratches on tender stem branches and sometimes eat up all the inner contents of stem leaving behind only the epidermis and hypodermal tissues (Tara *et al*., 2009). The larvae cause damage by tunnelling through the stems in a zig-zag fashion, thereby reducing the vitality and vigour of the plants, impairing their standing capacity and sometimes causing desiccation when the stems rapture (Plate 2.3)

(Louw *et al*., 1995; Tara *et al*., 2009). Pupation usually occurs within the larval tunnels and often leads to galling of the stems after which the adults emerge by biting holes through the galls, causing further weakening of the plant (Louw *et al*., 1995; Tara *et al*., 2009).

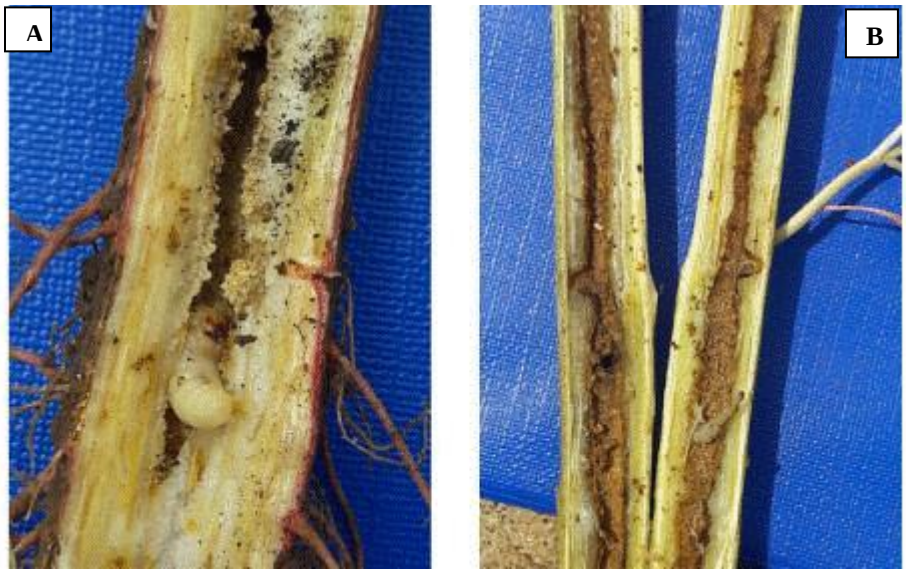

Plate 2.3: Damage caused by amaranth stem weevils *Hypolixus* sp. on amaranths A: larva of amaranth stem weevil within a tunnel at the root zone of the stem B: tunnels and frass left by larvae of stem weevils on amaranth

2.5 Management of amaranth pests

Pests of Amaranth are difficult to control because of their intrinsic biology and ecology as some stay and feed within the stems (Louw *et al*., 1995; Tara *et al*., 2009; Othim *et al*., 2018). Control by only chemical or biological means is difficult to achieve and a combination of these and other tactics are often needed in an Integrated pest management (IPM). The latter is an approach that can not only reduce pesticide application but also ensure adequate control is attained (Wheeler, 2002; Pappas *et al*., 2017). To achieve adequate levels of control, growers also need to use microbial insecticides, cultural control, variety selection, parasitoids, and adjusting planting schedules (James *et al*., 2007; Zehnder *et al*., 2007). The conservation and enhancement of populations of natural enemies are cornerstones of successful IPM programmes as they reduce populations of primary pests, limit pest damage and keep secondary pests below the economic threshold.

2.5.1 Cultural and physical/mechanical control

Cultural practices such as crop rotation, farm sanitation, application of manure and adjusting of planting schedules go a long way in reducing populations of webworms (James *et al*., 2007). The webworms can also be controlled using different types of enhanced traps. Light traps have been used to trap these nocturnal moths which are later killed (Viqar and Ali, 2012). Various traps baited with chemical/floral lures have also been used. They include the UniTraps, AgriSense and PontyPridd traps which consist of a white bucket covered by a

yellow cone and a green lid, with the lures placed within the traps in polypropylene vials, and holes in the lids to provide release of volatile chemicals at different rates (Landolt *et al*., 2011a; Landolt *et al*., 2011b). In addition, Vaportape® (2,2-Dichlorovinyl dimethyl phosphate) killing agent is incorporated within the trap (Landolt *et al*., 2011a).

2.5.2 Use of synthetic pesticides/ Chemical control

Growers of amaranth mostly rely on synthetic insecticides to control pests (Clarke-Harris *et al*., 2004; Losenge, 2005; Arivudainambi *et al*., 2010). Organochlorides, organophosphates, pyrethroids and carbamates including Lambda-cyhalothrin, Dimethoate, Endosulfan, Abamectin, Chlorpyriphos, Spinosad and Carbaryl are widely used across the world and in Kenya in the management of agricultural pests including pests of amaranth such as *S. recurvalis* and other leaf webbers (Losenge, 2005; Aderolu *et al*., 2013; Kagali *et al*., 2013).

Application of these insecticides is usually done indiscriminately resulting to environmental pollution and other undesirable effects (Clarke-Harris *et al*., 2004). Indiscriminate use of these pesticides has thus brought about pest resistance to pesticides and environmental pollution. Moreover, health concerns due to residue levels in vegetables and economic concerns have often been raised concerning these pesticides, thus the need for the development of effective, safe and sustainable IPM approaches (Chahal *et al*., 1997; Losenge, 2005; Arivudainambi *et al*., 2010; Srinivasan, 2012). The use of synthetic insecticides

also leads to the elimination of natural enemies. Natural enemies foraging for pests within farms sprayed with pesticides often risk coming into contact with the pesticide which may lead to their death or cause an indirect effect by impairing their performance (Srinivasan, 2012).

2.5.3 Botanical pesticides

The use of neem extracts from *Azadirachta indica* A. Juss has been reported to reduce the pest populations and also increase the yields of amaranth in Nigeria (Aderolu *et al*., 2013). Neem contains the active ingredient Azadiractin which acts as an antifeedant and a pest repellent (Aderolu *et al*., 2013). Apart from mortality, the effects of botanical pesticides on pest insects include feeding reduction, developmental alteration, reproductive abnormalities, and behavioural changes (Monstreal-Ceballos *et al*., 2018).

Herbal extracts from Karra *Cleistanthus collinus* Roxb., Asian spider flower *Cleome viscosa* L., cat's whiskers *Gynandropsis pentaphylla* DC., and Creat *Andrographis paniculata* Nees., have also been used in the management of *S. recurvalis* (Arivudainambi *et al*., 2010). China berry *Melia azedarach* L., has also been reported to enhance the attraction of the parasitoid *Cotesia (plutellae) vestalis* Kurdjumov (Hymenoptera: Braconidae) when sprayed to control diamondback moth *Plutella xylostella* L., (Lepidoptera: Plutellidae) in cabbage (Srinivasan, 2012).

2.5.4 Microbial control agents

Entomopathogenic fungi (EPF) play a vital role in managing insect pests in the humid tropics (Srinivasan, 2012). Several reports have confirmed the effectiveness of EPF against various pests on vegetables. For instance, ovicidal and pupicidal effects have been reported in some lepidopteran pests (Srinivasan, 2012). *Paecilomyces farinosus* (Holmsk) have also been shown to infect and kill larvae of leaf webbers like *Psara basalis* Walker and *S. recurvalis* on amaranth (Kuruvilla and Jacob, 1980; James *et al*., 2007).

Microbial pesticides based on the soil-borne bacterium *Bacillus thuringiensis* (Berliner) (*Bt*) are among the most widely used groups of biopesticides (Srinivasan, 2012). *Bacillus thuringiensis* formulations have been found to be effective against several lepidopteran pests when used solely or in combination with other biological control agents (Srinivasan, 2012). According to Srinivasan (2012), *Bt* preparations are a promising alternative to conventional insecticides because of their high toxicity to certain pests and their compatibility with IPM strategies due to their narrow host specificity, high amenability to genetic engineering and being harmless to non-target organisms (Tabashnik, 1994). Delplanque and Gruner (1975), reported that *Bt* preparations are also used in the management of leaf webbers and have been effective against *S. recurvalis* and *H. bipunctalis*. In West Africa, microbial pesticides based on *B. bassiana* isolates from Benin are being developed to control larvae of leaf caterpillars, *Psara basalis* and *S. recurvalis* on amaranth (James *et al*., 2010).

2.5.5 Use of plant volatiles

Certain secondary metabolites in plants act as deterrents for generalist feeders or attractants for specialist feeders (Srinivasan, 2012). Phenylacetaldehyde (PAA), a flower volatile and attractant for many nectar-seeking moths is the most effective biochemical lure for moths (Landolt *et al*., 2011b). In addition, PAA lure was observed to attract both sexes of moths, unlike the sex pheromones which normally attract a particular sex (Landolt *et al*., 2011b). By itself, PAA attracts many noctuid species and thus appears to be the main attractant volatile in some flowers. Various field trials with PAA and other floral lures such as linalool, *cis*-jasmone, methyl-2-methoxybenzoate, isobutanol, β-myrcene (BM) and methyl salicylate (MS), have shown effectiveness in attracting *S. recurvalis*, *U. ferrugalis*, *A. rantalis*, *Udea profundalis* Packard, *Ostrinia nubilalis* Hübner and *Pyrausta orphisalis* Walker, among other Crambidae (Maini and Burgio, 1990; Landolt *et al*., 2014; Landolt *et al*., 2011b). Other than Crambidae, these floral lures also attract Noctuidae such as *Chrysodeixis eriosoma* Doubleday, *Autographa biloba* Doubleday, *Mythimna unipuncta* Haworth, *Mamestra brassicae* L., *Agrotis exclamationis* L., *Amphipyra pyramidea* L., (Tóth *et al*., 2010, Landolt *et al*., 2011a; Landolt *et al*., 2011b). However, Othim *et al*. (2018) found PAA to be ineffective in the management of *S. recurvalis* in East Africa.

2.5.6 Host plant resistance

Host plant resistance (HPR) in vegetable production has been fully exploited as a strategy in the management of pests in most vegetable production systems (Eigenbrode and Trumble, 1994). Pest resistance in crops has been widely studied in recent decades and resistance traits in some of the vegetables including tomatoes *Solanum lycopersicum* L. (Solanales: Solanaceae), carrots *Daucus carota* L. (Apiales: Apiaceae), lettuce *Lactuca sativa* L. (Asterales: Asteraceae), okra *Abelmoschus* spp. (Malvales: Malvaceae) and onion *Allium cepa* L. (Asparagales: Amaryllidaceae) are well documented (Eigenbrode and Trumble, 1994; Srinivasan and Uthamasamy, 2005 Abang *et al*., 2014; Abang *et al*., 2016;Njau *et al*., 2017; Rakha *et al*., 2017a; Rakha *et al*., 2017b; Rakha *et al*., 2017c). However, HPR in most AIVs has not been given much attention. That notwithstanding, some reports and observations have been made regarding possible resistance by certain accessions/lines of amaranth against the leaf-webbers. For example, NRC (1984) reported that *A. hypochondriacus* exhibited greater resistance to pest damage when compared to *Amaranthus cruentus*. Othim *et al*. (2018) also reported lower levels of infestation and damage on Abuku Var.2 amaranth line compared to Abuku Var.8. There is, however, very scanty information on the role played by resistant amaranth varieties/ lines/ accessions in the management of lepidopteran leaf webbers and weevils.

2.5.7 Use of natural enemies

Natural enemies composed of both predators and parasitoids play a very important role in keeping pest population under check. A number of parasitoids have been reported to be associated with webworms. These include the egg parasitoids *Trichogramma* species (Hymenoptera: Trichogrammatidae); larval parasitoids *Apanteles* sp. *Cardiochiles* sp., and *Phanerotoma* sp., *Cotesia marginiventris* Cresson (Hymenoptera: Braconidae), *Campoletis* sp., *Venturia infesta* Cresson (Hymenoptera: Ichneumonidae), and *Prosopodopsis* sp. (Diptera: Tachinidae) have been reared on *S. recurvalis* (James *et al*., 2010; Kedar and Kumaranag, 2013; Grovida, 2015). Bhattacherjee and Ramdas (1964) reported parasitism of 11.46% on *S. recurvalis* by *Apanteles delhiensis* Mues and Subba-Rao (Hymenoptera: Braconidae). Narayanan *et al*. (1957) also reported parasitism of up to 62% by *Apanteles* sp. on *S. recurvalis*. In Kenya, certain natural enemies such as *Dentichasmias busseolae* Heinr (Hymenoptera: Ichneumonidae) and *Iphiulax varipalpis* (Hymenoptera: Braconidae) have been associated with pests of amaranths but information relating the natural enemies to specific pests is still lacking (Kagali *et al*., 2013). Furthermore, no studies have been conducted to assess the effect of pest resistant amaranth varieties on the performance of these natural enemies.

2.5.7.1 Biology of *Apanteles* species

Apanteles sp. is a solitary endoparasitoid of lepidopteran larvae. The eggs are laid in the larva of a lepidopteran host by the female inserting its ovipositor through the caterpillar's integument. The eggs usually float on the body cavity of the host before they hatch into larvae (Cardona and Oatman, 1975). Once the eggs hatch, the parasitoid larvae feed on the haemolymph of the host, secondary metabolites and lastly on the vital organs of the host.

The larval period of *Apanteles hemara* Nixon, *A. subandinus* Blanchard and *A. myeloenta* Wilkinson takes 6-10, 8-10 days and 7-10 days, respectively, while the pupal period takes 3-6 days, 4-6 days and 11-19 days, respectively (Figure 2.2). Before pupation, the larva cuts its way along the lateral line of the host, exits from the host body and spins a white silken cocoon just next to the killed host (Cardona and Oatman, 1975). The adult emerges from the pupal cocoon and mates after feeding. Development time depends on temperature and relative humidity (RH) (Cardona and Oatman, 1975; Farahani *et al*., 2012). The females do not have a pre-oviposition period and can even lay eggs without mating though such eggs will only develop into males (Cardona and Oatman, 1975). *Apanteles* sp. has oviposition preference for the second instar larvae though other instars can also be parasitized (Cardona and Oatman, 1975; Farahani *et al*., 2012; Tunca *et al*., 2014a). The adult longevity depends on the diet and environmental conditions.

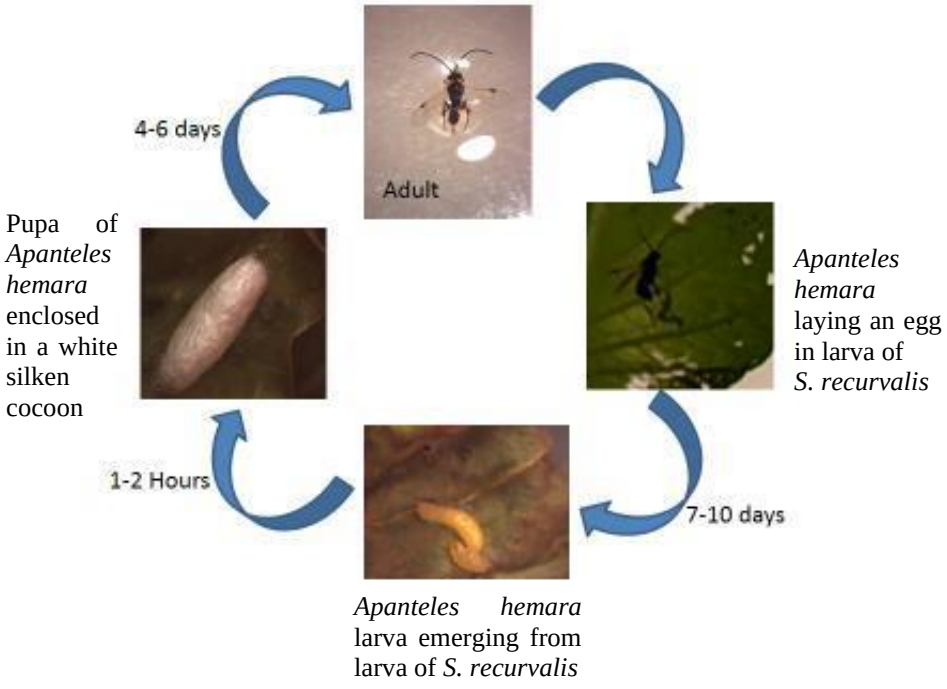

Figure 2.2: Life cycle of *Apanteles hemara* at 25 ± 2ºC and 60 ± 10 % RH

2.5.8 Integrated pest management

Integrated pest management (IPM) is a strategy that involves the combination of various crop protection methods to avoid pest infestations from reaching economically damaging levels (James *et al*., 2010). Botanical pesticides act as a synergistic component in several IPM strategies (Srinivasan, 2012). Evidence of this synergistic action has been reported between neem and microbial pesticides such as nucleopolyhedroviruses (NPVs) against common army worm (Nathan and Kalaivani, 2006), and between neem and entomopathogenic fungi

(*Beauveria bassiana* (Balsamo) Vuillemin) against common army worm (Mohan *et al*., 2007).

On the other hand, the alkaloids and other allelochemicals found in resistant plants can be toxic to parasitoids and predators or cause other negative effects such as induction of sterility in parasitoids (van Emden, 1991). Consequently, resulting in antagonistic effects between the two pest management strategies i.e. HPR and natural enemies. Nevertheless, complementary interaction between host plant resistance and parasitoids have been shown to result in higher pest mortality (van Emden, 1991). Botanical insecticides have also been shown, on one hand, to have detrimental effects on parasitoids while in other instances they have synergistic effects with the parasitoids (Tunca *et al*., 2014a; Monstreal-Ceballos *et al*., 2018). The varied results on compatibility of different pest management strategies are still open for further investigation.

CHAPTER THREE. MATERIALS AND METHODS

3.1 Study sites and laboratory conditions

The World Vegetable Center (WorldVeg) farm located at the Eastern and Southern Africa (ESA) hub at Arusha, Tanzania, 36.86°E, -3.374°S and 1,309 m above sea level (asl) was used for the assessment of the diversity of lepidopteran defoliators, stem weevils and their associated parasitoids. The open field screening of the amaranth accessions and lines for resistance against leaf-webbers and amaranth stem weevils was also conducted in the same location. This area experiences average temperatures of 19.5°C and an average rainfall of 1,098 mm per annum. The area receives bimodal rainfall with the long rainy season between March and May and the short rainy season between September and December. The site has a clay loamy soil with pH ranging between 6.0 and 6.7.

All the laboratory experiments were conducted at the WorldVeg ESA's entomology laboratory in Arusha. The laboratory conditions were maintained at 25 ± 2 °C, 50-70% RH and photoperiod of 12:12 h (light:darkness).

3.2 Plant material for colony maintenance

Selected accessions of amaranth for laboratory experiments and colony maintenance were grown in the screen houses located at the WorldVeg facility in Arusha, Tanzania. Seeds of the amaranth accessions were sown in plastic trays containing a substrate of soil and manure in the ratio 4:1. Two to three weeks after germination, the seedlings were transplanted into plastic pots of 10 cm diameter

(1000 cm^3) and maintained with regular watering for use in colony maintenance or in the experiments. *Amaranthus dubius* Mart. ex Thell. (Ex-zan variety), obtained from WorldVeg's ESA genebank in Arusha, Tanzania, was used for pests' colony maintenance.

3.3 Rearing of Spoladea recurvalis

A colony of *S. recurvalis* was established and maintained in the entomology laboratory at WorldVeg, Arusha on *A. dubius* for five generations prior to their experimental use. The adults and larvae of *S. recurvalis* were originally collected from amaranth fields within WorldVeg (-3.38° S, 36.8° E) in November and December 2015. Adult moths were placed in transparent perspex cages (40 × 40 × 45 cm) with a sliding door and a netting material at the back and on the sides for ventilation. The moths were fed on 10% honey solution soaked in cotton wool and provided with potted amaranth plants for oviposition.

The plants were replaced every 24 h and placed in separate holding cages (50 × 50 × 60 cm) made from transparent perspex material with netting at the back and on the sides for the eggs to hatch. Newly hatched larvae were left to feed on the live plants for three to four days and then transferred into plastic containers (15 × 7 × 5 cm) lined with paper towel and fine netting material on the lid for ventilation. Fresh amaranth leaves were supplied to the larvae daily for food until pupation. The pupae were incubated under similar laboratory conditions in the plastic containers until adult emergence.

3.4 *Apanteles hemara* colony

A colony of the koinobiont larval endoparasitoid, *A. hemara* was established in the laboratory at WorldVeg, Arusha from pupae obtained from *S. recurvalis* larvae collected as described in 3.1.3. Colonies were replenished (infused) with new field collections obtained every quarter to avoid effects of inbreeding such as selective mortality and other genetic defects. Adults were placed in a ventilated perspex cage (40 × 40 × 45 cm) with a sleeve on one side and fed with honey on strips of paper. The rearing was maintained at 25 ± 2°C, 50-70% RH, and 12:12 L:D photoperiod. Potted plants containing 3-day old larvae of *S. recurvalis* were then introduced into the cage for the parasitoids to oviposit. The exposed larvae were removed on a daily basis and placed in ventilated plastic boxes (15 × 7 × 5 cm) lined with paper towel. Fresh amaranth leaves were added into the plastic boxes as and when required until pupation. The parasitoid pupae were collected and transferred to clean Petri dishes (9-cm diameter), kept inside a perspex cage under similar conditions for adult emergence. These parasitoids were mass reared on *S. recurvalis* feeding on *A. dubius* (Ex-Zan) for several generations before their use for the experimental treatments.

3.5 Assessment of the occurrence and diversity of amaranth lepidopteran defoliators and stem weevils and their associated parasitoids and evaluation of amaranth accessions for resistance against leaf-webbers and stem weevils in open field conditions in Arusha, Tanzania

3.5.1 Cropping seasons

The first season of field screening was carried out during the long rainy season between March and June 2016, characterized by 22.5 ± 0.28°C, 544 mm total rainfall and 79.7 ± 0.79% relative humidity. The second screening was conducted during the short rainy season (which started late) between December 2016 and March 2017 characterized by 23.45 ± 0.19°C, 233 mm total rainfall and 78.34 ± 0.99% relative humidity.

3.5.2 Plant material

Eighteen (18) and thirty-six (36) amaranth accessions and lines (hereafter both referred to as accessions) were sown in the long and short rainy seasons, respectively, in trays in the screen house and transplanted into plots, when 3 weeks old. During both seasons, a susceptible check for lepidopteran defoliators, selected from a preliminary screening in Taiwan, was included among the accessions. This was assumed to be the susceptible check for the stem weevils, since resistance screening against amaranth stem weevils has never been conducted. The plots were manually constructed, ploughed using a hand hoe, after which the 3-week-old seedlings were transplanted with an inter-row spacing of 50 cm and intra-row

spacing of 20 cm to obtain 12 plants per row. Fertilizers were applied during the second week after transplanting nitrogen, phosphorous and potassium (NPK 20-10-10) at 200Kg/ha and 120KgN/ha Urea. Weeding was done manually once a month and watering done regularly for the duration of each growing season. No insecticides or fungicides were applied to the crops.

3.5.3 Experimental design and data collection

The trial was laid out in a randomized complete block design (RCBD) with three replications for each accession. During the long rainy season, the field was laid out into three blocks consisting of 35.5 × 3.5 m^2, each with a spacing of 2 m between the blocks. Each block contained 18 plots, each measuring 3.0 × 1.2 m^2 (2 rows per plot and 12 plants per row) with a spacing of 0.5 m between the plots. Eighteen amaranth accessions were randomly assigned to each plot. During the short rainy season, the field was laid out into three blocks of 64.2×3.5 m^2 each with a spacing of 2m between the blocks. Each block was then subdivided into 36 plots each measuring 3.0 × 1.2 m^2 with a spacing of 0.5m between plots where 36 amaranth accessions (Table 3.1) were assigned.

Non-destructive sampling was done weekly, starting from two weeks after transplanting (WAT). Eight plants were sampled randomly within each plot, visually scored for damage by leaf-webbers using a modified (0–5 instead of 0-7) assessment scale described by Gilbert and Grégoire (2003), where 0= 0%; 1= 1-20%; 2= 21-40%; 3= 41-60%; 4= 61-80% and 5= 81-100% of damage. Developmental stages of lepidopteran pests of amaranth including eggs, larvae,

pupae and pupae of associated parasitoids encountered were collected and incubated in the laboratory in ventilated plastic containers. The larvae were supplied with fresh amaranth leaves until adult pest/parasitoid emergence.

Destructive sampling was done once at the end of the season when the crop had reached maturity for stem weevil damage assessment. This involved cutting the stems of 8 randomly selected plants per accession at the base, approximately 1 cm below the ground level, and transferring to the laboratory for dissection to check for the developmental stages of the stem weevils. Both the main stem and the branches were also dissected to assess levels of stem weevil infestations and the associated damage. The number of weevils and their associated parasitoids within each stem and number of mined tunnels created by the weevils was recorded for each plant. The number of tunnels was recorded as a measure of severity of damage. The adults of Lepidoptera and stem weevils were identified using the available taxonomic keys described by Dugdale (1988) and Dombroskie (2011), while the parasitoids were identified at the Natural History Museum, UK. The voucher specimens are held at the WorldVeg ESA's entomology laboratory at Arusha, Tanzania.

Table 3.1: Amaranth accessions, and lines developed by single plant selection from germplasm collections studied in two seasons and their morphological characteristics in Arusha, Tanzania, 2016 and 2017

Gene bank code	Species	Type	Leaf color	Leaf shape	Country of origin	Number of branches per plant (mean)	Plant height (mean) cm	Leaf width (mean) cm	Leaf length (mean) cm	Petiole length (mean) cm	Days to flowering (weeks)[$]
VI033482*	*Amaranthus tricolor*	Accession	Green	Reniform	Malaysia	9.0	100.9	10.6	19.3	5.0	6
RVI00002	*A. cruentus*	Line	Green	Ovate	Zambia	12.5	122.6	6.6	16.5	10.2	4
RVI00005	*A. dubius*	Line	Reddish	Ovate	Tanzania	12.2	140.3	6.0	12.5	7.6	5
RVI00027	*Amaranthus* sp.	Line	Green	Ovate	Malawi	7.3	96.3	6.2	11.1	7.8	2
RVI00053	*A. dubius*	Line	Green	Ovate	Uganda	11.0	167.0	8.7	15.6	8.0	7
VI033477	*Amaranthus* sp.	Accession	Reddish	Ovate	Malaysia	9.2	99.7	7.9	12.3	5.2	6
VI033479	*Amaranthus* sp.	Accession	Green	Ovate	Malaysia	11.3	100.8	4.9	7.6	4.2	2
VI033487	*A. cruentus*	Accession	Green	Reniform	Malaysia	13.5	128.7	5.6	7.7	6.6	4
VI036225	*A. graecizans*	Accession	Green	Ovate	Hungary	15.4	77.2	1.6	3.2	2.3	3
VI036227	*A. blitoides*	Accession	Green	Oblanceolate	Hungary	15.8	67.4	1.2	3.2	1.6	4
VI044367	*A. cruentus*	Accession	Green	Lanceolate	Tanzania	9.1	123.5	5.8	13.7	10.2	5
VI044369	*A. hypochondriacus*	Accession	Green	Lanceolate	Ghana	13.7	129.0	6.1	17.0	9.2	4
VI044388	*A. graecizans*	Accession	Green	Oblanceolate	India	14.6	89.8	2.3	4.3	2.5	3
VI044432	*A. viridis*	Accession	Green	Cordate	Indonesia	11.0	102.9	4.5	6.9	4.0	2
VI044437-A	*A. cruentus*	Accession	Green	Lanceolate	Malaysia	11.5	89.5	5.5	13.0	7.2	2
VI044473	*A. palmeri*	Accession	Green	Obovate	Senegal	9.0	80.1	2.2	4.4	2.4	3
VI046233-A	*Amaranthus* sp.	Accession	Reddish	Lanceolate	Vietnam	8.0	142.1	6.8	17.0	10.6	5
VI047517-B	*A. tricolor*	Accession	Green	Ovate	Bangladesh	12.9	119.8	8.1	15.9	7.7	6

Table continues on the next page…

Continued from previous page

VI047555-B	*A. tricolor*	Accession	Green	Lanceolate	Vietnam	10.9	135.6	4.5	13.4	4.8	5
VI048076	*A. tricolor*	Accession	Green	Cordate	Bangladesh	13.1	130.1	8.0	13.5	7.1	6
VI048864-A	*A. viridis*	Accession	Green	Cordate	Thailand	10.2	95.1	4.1	5.8	3.6	2
VI048919	*Amaranthus* sp.	Accession	Green	Ovate	Thailand	11.6	126.1	3.7	7.0	4.3	3
VI049242	*Amaranthus* sp.	Accession	Green	Ovate	Thailand	11.6	87.8	4.4	5.8	3.7	2
VI049502	*Amaranthus* sp.	Accession	Green	Cordate	Thailand	10.0	103.1	4.7	6.8	4.1	2
VI049504	*Amaranthus* sp.	Accession	Green	Lanceolate	Thailand	12.1	134.2	3.0	6.6	3.2	3
VI049530	*Amaranthus* sp.	Accession	Green	Ovate	Thailand	10.4	89.2	4.3	6.4	3.9	2
VI049639	*A. viridis*	Accession	Green	Ovate	Thailand	11.3	91.7	4.2	6.1	3.5	2
VI049698	*A. viridis*	Accession	Green	Ovate	Thailand	12.4	100.5	3.9	5.5	3.4	2
VI050609-A	*A. tricolor*	Accession	Variegated	Cordate	Vietnam	9.8	129.1	9.2	11.7	6.8	5
VI050609-B	*A. tricolor*	Accession	Variegated	Ovate	Vietnam	8.5	140.9	9.6	15.0	6.1	5
VI054569	*A. gracilis*	Accession	Green	Ovate	Philippines	11.0	95.1	4.5	7.2	3.9	2
VI054798	*Amaranthus* sp.	Accession	Green	Ovate	Lao PDR	12.4	89.3	4.1	6.2	3.3	2
VI055127	*A. viridis*	Accession	Green	Ovate	Malaysia	11.4	108.1	5.6	10.3	6.6	3
VI055128	*A. viridis*	Accession	Green	Cordate	Malaysia	10.7	123.4	5.0	7.0	3.9	2
VI055135	*A. viridis*	Accession	Green	Cordate	Malaysia	10.8	92.0	5.0	7.2	4.1	2
VI056563	*Amaranthus* sp.	Accession	Reddish	Ovate	Bangladesh	9.7	136.9	9.0	17.1	8.3	6
Mean						11.2	110.7	5.5	10.0	5.5	
LSD (5%)						4.38	26.74	1.4	3.69	2.33	

*Susceptible check. $^{\$}$Days to flowering recorded from the date of transplanting

3.6 Assessing the possible mechanisms underlying resistance in amaranth accessions through their effects on the biology of leaf-webbers infesting amaranths

3.6.1 Amaranth accessions

Open field experiments were conducted to screen 31 amaranth accessions obtained from the WorldVeg gene bank in Taiwan and four improved lines from WorldVeg ESA for resistance against leaf-webbers as in the previous objective (section 3.5). In this objective, further screening for expression of resistance was conducted in a screen house was conducted on all 35 test accessions and one susceptible accession in choice assays. Morphological characteristics of the amaranth accessions tested are presented in Table 3.1. From both the open field assay previously conducted in section 3.5 and the screen house assays conducted in this objective, eight amaranth accessions exhibiting pest resistance and the susceptible accession were selected on the basis of damage incidence and severity, pest incidence (occurrence) and abundance (actual number of pests) and oviposition preference for in-depth assessment of their effects on selected biological traits of the pest. The susceptible accession was considered as such because it had the most severe damage and the highest pest incidence and abundance. The selected accessions were grown and maintained as described in section 3.2.

3.6.2 Choice bioassay for oviposition by *Spoladea recurvalis*

Choice assays were conducted in two sets since all the 36 accessions could not fit in a single cage. The experiment was conducted within a screen-house in a split plot design replicated six times. In the first set (set-up A), 17 test accessions and the susceptible accession were exposed to 25 mated female adults of *S. recurvalis* in a glass cage measuring 150 × 100 × 120 cm. In the second set (set-up B), established three weeks later, the remaining 18 test accessions and the susceptible accession were exposed to 25 mated female adults of *S. recurvalis* in the same glass cages used for the first set. One potted plant of each accession (seven weeks old) was randomly placed in each cage and left for 48 h for the moths to lay eggs. The plants were watered after 24 h and the moths provided with 10% honey solution on cotton plugs to feed. The plants were then removed from the cages and the leaves of each plant thoroughly inspected for the presence of eggs, which were counted with the aid of a dissecting microscope and recorded.

3.6.3 No-Choice bioassay for oviposition by *Spoladea recurvalis*

The eight most resistant accessions identified from the field experiments conducted in section 3.5 and the choice experiments in section 3.6.2 were assessed individually in a no-choice experiment in comparison with the susceptible accession (Table 3.2). One potted amaranth plant of each selected accession at six to seven weeks of age was exposed to two mated female moths of *S. recurvalis* from the stock culture for 48 h in the transparent perspex cages. During the exposure period, *S. recurvalis* adults were fed on 10% honey solution soaked in

cotton wool and the plants were watered adequately. After 48 h of exposure, the plants were removed from the cages and the leaves inspected for the presence of eggs under a dissecting microscope. The number of eggs on each amaranth accession was recorded. This experiment was replicated six times.

3.6.4 Effect of accession on weight gain of amaranth leaf-webber

One or two leaves (based on leaf size) from each of the eight selected amaranth accessions and the susceptible accession were exposed to one larva of *S. recurvalis* for 48 h in a Petri dish (8 cm diameter) lined with moistened filter paper. The leaves were obtained from amaranth accessions grown and maintained in the screen-house at six to seven weeks. The larvae were obtained from the laboratory stock culture and exposed at 3–5 days old to each accession. Prior to their exposure to the leaves, the larvae were deprived of food for 12 h. The weight of each larva was measured before and after 48 h of exposure to the leaves using a digital scale (Mettler AE200 analytical balance, Columbus, OH, USA). This experiment was replicated 12 times with each amaranth accession. The weight gain and percentage weight gain by *S. recurvalis* larvae on each accession was calculated.

Table 3.2: Resistance status, description and morphological characteristics of selected resistant amaranth accessions and lines

Amaranth accession code	Species	Type	Leaf colour	Leaf shape	Country of origin	Branches per plant (mean)	Plant height (mean) cm	Leaf width (mean) cm	Leaf length (mean) cm	Petiole length (mean) cm	Resistance status
VI033482	*A. tricolor*	Accession	Green	Reniform	Malaysia	9.0	100.9	10.6	19.3	5.0	S
RVI00053	*A. dubius*	Line	Green	Ovate	Uganda	11.0	167.0	8.7	15.6	8.0	MR
VI033479	*Amaranthus* sp.	Accession	Green	Ovate	Malaysia	11.3	100.8	4.9	7.6	4.2	MR
VI036227	*A. blitoides*	Accession	Green	Oblanceolate	Hungary	15.8	67.4	1.2	3.2	1.6	HR[$]
VI044437-A	*A. cruentus*	Accession	Green	Lanceolate	Malaysia	11.5	89.5	5.5	13.0	7.2	MR
VI047555-B	*A. tricolor*	Accession	Green	Lanceolate	Vietnam	10.9	135.6	4.5	13.4	4.8	MR
VI048076	*A. tricolor*	Accession	Green	Cordate	Bangladesh	13.1	130.1	8.0	13.5	7.1	MR
VI049698	*A. viridis*	Accession	Green	Ovate	Thailand	12.4	100.5	3.9	5.5	3.4	MR
VI056563	*Amaranthus* sp.	Accession	Reddish	Ovate	Bangladesh	9.7	136.9	9.0	17.1	8.3	MR
Mean						11.6	114.3	6.3	12.0	5.5	
LSD (5%)						6.1	35.2	1.5	4.8	1.6	

MR= Moderately resistant; HR= Highly resistant; S= Susceptible. [$]The highly resistant accession could not support development of the host pest hence was not included in the assessment of the parasitoid's performance.

3.6.5 Effects of selected amaranth accessions on the development of amaranth leaf-webber and adult longevity

The nine accessions tested in no-choice experiment were evaluated for their effect on larval development. Five neonate larvae of *S. recurvalis* were placed in a plastic Petri dish (8 cm diameter) lined with filter paper to absorb excess moisture. These were supplied daily with fresh leaves of the selected accessions until all larvae pupated or died. The pupae were then incubated under the same conditions until adult emergence. The emerged adults were placed in perspex cages and fed on 10% honey solution until they died. The assay was replicated 10 times for each selected amaranth accession. The data on larval, pupal and total developmental time, larval and pupal mortality and adult longevity were recorded for each accession. Early stage larval mortality was recorded as mortality within the first 36 h (when the larvae are not causing considerable damage) of exposure.

3.7 Evaluation of selected resistant amaranth accessions for water stress tolerance

3.7.1 Plant material and growth conditions

The experiments were carried out in a screen house at the World Vegetable Centre ESA in Arusha, Tanzania, between July and September 2017. Seven amaranth accessions/lines previously identified to be moderately resistant against the leaf-webber pests and one susceptible accession were selected for this study (Table 3.2). Seeds of the amaranth accessions were sown at five seeds per pot in 240 pots (25 cm diameter and 32 cm height) using surface soil obtained from the slopes of Mt. Meru near a forested region at Tengeru, Arusha. The soil collection was mixed thoroughly with sand and cow dung manure in the ratio 1:0.5:1. Each pot was then filled with similar amounts of the soil mixture and allowed to dry to a constant weight (7.4Kg). Ten randomly selected pots were then thoroughly watered and allowed to drain freely until the weight was constant. The difference between this weight and the soil dry weight was used to calculate the soil water holding capacity (WHC) and the amount of water needed to achieve 40%, 60% and 90% WHC. Before sowing, all the pots were well watered to 90% WHC to ensure seed germination. Shortly after the seeds germinated and developed two to three true leaves, the seedlings were thinned to one plant per pot and the watering treatment initiated four weeks after germination.

3.7.2 Experimental design and treatment management to determine the effect of water stress on amaranth

The experimental layout was a completely randomized design with three water supply regimes, eight amaranth accessions and 10 replications. The layout consisted of the control/well-watered group (90% WHC), moderately stressed (60% WHC) and severely stressed (40% WHC) group. The plants in the control/well-watered group were watered every 2 days to maintain 90% WHC until termination of the experiment. Both the moderately stressed and severely stressed plants were watered after 4, 8, 14, 21 and 28 days up to 60% and 40% WHC, respectively. The growth parameters such as plant height and leaf size were measured every two days while biomass parameters were measured at day 0, 14, 21 and 34 after initiation of stress treatments.

3.7.3 Growth and leaf parameters

The plant height was measured from the base of the stem at the soil level to the terminal bud of the main stem using a ruler. The number of branches and leaves, as well as the measurements for the leaf length (LL) and leaf width (LW), taken using a ruler, was recorded after every two days after initiation of stress until the experiment was terminated. The number of days taken by the plants to initiate flowering was also recorded for each accession.

3.7.4 Biomass parameters

Two seedlings of each accession were harvested after 8, 14, 21 and 34 days after initiation of water stress from all the treatment groups. The seedlings were washed to remove any debris; separated into roots, stems and leaves and their fresh weight determined. They were then dried to constant weight in an oven at 85°C for 36 - 48 hours. The dry mass (DM) of the roots, stems and leaves were determined for each plant. The total plant biomass was the sum of root, stem and leaf masses. Relative growth rate (RGR) was determined using the formula below:

$$RGR = \frac{lnDM_2 - lnDM_1}{t_2 - t_1}$$

where DM_1 and DM_2 represent the plant DM at time t_1 and t_2, respectively.

Total leaf area (LA) was estimated using leaf length and width measurements according to Kintomo and Ojo (2000) as:

$$LA = 1.1132\ (LL \times LW) + 0.0613$$

Root to shoot ratio (RSR) was calculated as the root dry mass divided by shoot dry mass. Leaf area ratio (LAR) was calculated as the total leaf area divided by the total plant dry mass of each plant. Specific leaf area (SLA) was obtained as the total leaf area divided by the dry mass of the leaves. The root mass ratio (RMR) was calculated as the root dry mass divided by the total plant dry mass. Leaf weight ratio (LWR) was obtained as the leaf dry mass divided by the total plant dry mass.

3.8 Assessment of the performance of indigenous parasitoids of amaranth leaf-webbers on selected resistant accessions in Tanzania

3.8.1 Amaranth accessions

Eight amaranth accessions exhibiting pest resistance and one susceptible accession were selected (Table 3.2) from the open field screening conducted in section 3.5 using 36 accessions obtained from the WorldVeg's genebanks in Shanhua, Taiwan and Arusha, Tanzania (Table 3.1). However, the most resistant accession VI036227 could not be tested as it led to 100% mortality of *S. recurvalis* larvae at an early stage. Therefore, seven moderately resistant accessions RVI00053, VI033479, VI044437-A, VI047555-B, VI048076, VI049698 and VI056563 were tested against a susceptible accession VI033482. For the purpose of pest colony maintenance and parasitoid colony maintenance, *A. dubius* (Ex-zan) obtained from the WorldVeg gene bank in Arusha was used. The selected accessions were raised in the screen house at WorldVeg in Arusha. The seeds were sown in plastic trays containing a substrate of soil and manure in the ratio 4:1. Two to three weeks after germination, the seedlings were transplanted into plastic pots of 10 cm diameter (1,000 cm^3) and maintained with daily watering for use in the experiments.

3.8.2 *Spoladea recurvalis* colony

A colony of *S. recurvalis* was established and maintained in the entomology laboratory at WorldVeg ESA, Arusha from field collections of larvae in amaranth fields as described in section 3.3. These were reared on *A. dubius* (Ex-zan) for at least five generations prior to their experimental use.

3.8.3 *Apanteles hemara* colony

A colony of *A. hemara* was established and maintained in the laboratory at WorldVeg, Arusha from pupal samples emerging from *S. recurvalis* larvae collected from amaranth fields as described in section 3.4. The parasitoids were maintained on larvae of *S. recurvalis* feeding on *A. dubius* (Ex-zan).

3.8.4 Assessment of the performance of *Apanteles hemara* on *Spoladea recurvalis* feeding on different amaranth accessions

Potted amaranth plants of the seven selected resistant and one susceptible accession were exposed to adult *S. recurvalis* for 24 h to oviposit. The plants were then kept in separate perspex cages (40 × 40 × 45 cm; six plants per cage) and with adequate ventilation until the eggs hatched. The hatched larvae were allowed to feed on the plants until their second instar before they were used in the experiment. A leaf/branch of each accession infested with 25 second instar larvae of *S. recurvalis* was cut and placed in a ventilated cylindrical container 10 cm diameter and 5 cm height. A two-day-old mated naïve (no prior contact with the pest) female parasitoid of *A. hemara* was then introduced into the container with *S.*

recurvalis larvae and allowed to parasitize the larvae for 24 h. The parasitoid was fed on honey smeared on a strip of paper during the 24 h period. After the 24 h of exposure to the parasitoid, the larvae of *S. recurvalis* were removed from the container and incubated in plastic boxes where they were supplied with fresh amaranth leaves and monitored daily until parasitoid or host pupation. The pupae were collected, counted and then placed in a clean plastic Petri dish under similar conditions and monitored daily until adult eclosion. The adults were then transferred into plastic vials (20 ml) covered with a netting material at the top where they were supplied with undiluted honey and monitored individually until they died. This procedure was followed with each accession and replicated six times. A control was also set up along each accession in which no parasitoid was introduced into the vial containing larvae.

The fitness parameters recorded to assess performance of the parasitoid on different accessions included: the number of parasitoid pupae and adult parasitoids, development time of the parasitoid, adult longevity, F_1 sex ratio, length of adult hind tibia and forewing (as indices of body size) of 20 randomly chosen parasitoids of each sex, as well as larval and pupal mortality.

3.9 Evaluation of the effects of host age and density on the performance of *Apanteles hemara*

3.9.1 Host plants

One improved breeder line of amaranth (*Amaranthus dubius*, line RVI00053) exhibiting moderate pest resistance and possessing desirable horticultural traits such as broad leaves and rapid growth, was selected from both open field and laboratory screening conducted in sections 3.5 and 3.6. For the purpose of pest colony maintenance and parasitoid colony maintenance, *A. dubius* (Ex-zan) obtained from the WorldVeg gene bank in Arusha was used as described in sections 3.3 and 3.4. The selected accession was raised in the screen house as described in section 3.2.

3.9.2 Amaranth leaf-webber colony

A colony of the amaranth leaf-webber was established and maintained in the entomology laboratory at WorldVeg, Arusha as described in section 3.3. Prior to the experiments, the parental generation of *S. recurvalis* were fed and reared on line RVI00053 under the same laboratory conditions.

3.9.3 *Apanteles hemara* colony

A colony of the koinobiont larval endoparasitoid, *A. hemara,* was established and maintained in the laboratory at WorldVeg, Arusha as described in section 3.4.

3.9.4 Assessing the effect of larval density of *Spoladea recurvalis* on parasitism and other life history parameters of *Apanteles hemara*

Four potted amaranth plants of line RVI00053 were exposed to 10 adult females of *S. recurvalis* in a ventilated perspex cage (40 × 40 × 45 cm) for 24 h to oviposit. The plants were then removed and kept in separate perspex cages of similar dimensions with adequate ventilation until the eggs hatched. The newly hatched larvae were allowed to feed on the plants until the second instar. A leaf infested with 10, 20, 30 or 40 second instar larvae of *S. recurvalis* was cut from the plant and placed in a ventilated cylindrical container (10 cm diameter and 5 cm height). A two-day-old mated naïve (no prior contact with the pest) female parasitoid of *A. hemara* was then introduced into the container with *S. recurvalis* larvae and allowed to oviposit for 24 h. The parasitoid was fed on honey smeared on a strip of paper during the 24 h period. After the 24 h of exposure, the larvae of *S. recurvalis* were removed from the container and incubated in ventilated plastic boxes (15 × 7 × 5 cm) where they were supplied with fresh amaranth leaves and monitored daily until parasitoid or host pupation. The cocoons were collected, counted and placed in a clean plastic Petri dish under similar conditions until adult eclosion. The adults were then transferred into plastic vials (20 ml) covered with a netting material at the top where they were supplied with undiluted honey and individually monitored until they died. This procedure was followed with each larval density and replicated five times. A control was also set up along each larval density in which no parasitoid was introduced. The laboratory conditions were

maintained at 25 ± 2°C, 50-70% RH, and 12:12 L:D photoperiod during the experiment.

3.9.5 Assessing the influence of larval age on parasitism and other life history parameters of *Apanteles hemara* reared on *Spoladea recurvalis*

Four potted amaranth plants of line RVI00053 were exposed to 10 adult females of *S. recurvalis* in a ventilated perspex cage (40 × 40 × 45 cm) for 24 h to oviposit. The plants were then removed and kept in separate perspex cages of similar dimensions with adequate ventilation until the eggs hatched. Upon hatching, 25 larvae at 1-2-days, 3-4-days, 5-6-days and 7-9-days old were transferred gently while on the amaranth leaf into a ventilated cylindrical container of 10 cm diameter and 5 cm height. A two-day-old mated naïve (no prior contact with the pest) female parasitoid of *A. hemara* was then introduced into the container with larvae for parasitization for 24h. The parasitoid was fed on honey smeared on a strip of paper during the 24 h period. After the 24 h of exposure to the parasitoid, the larvae of *S. recurvalis* were removed from the container and incubated in ventilated plastic boxes (15 × 7 × 5 cm) where they were supplied with fresh amaranth leaves and monitored daily until parasitoid or host pupation. The cocoons were collected using soft forceps, counted and placed in clean plastic Petri dishes under similar conditions and monitored daily until adult eclosion.

Upon emergence, the adults were then transferred into plastic vials (20 ml) covered with a netting material at the top where they were supplied with honey

and individually monitored until they died. The experiment was replicated five times with each larval age group and a control was set up along each larval age group in which no parasitoid was introduced.

3.9.6 Data parameters for both host density and host age assays

The parameters recorded to assess performance of the parasitoid at different larval densities and larval age group included: number of parasitoid pupae emerging from incubated larvae, the number of adult parasitoids that emerged, parasitoid's pupal mortality, the parasitoid development time, adult longevity, F_1 sex ratio, length of adult hind tibia and forewing (as indices of body size) of at least 15 randomly chosen parasitoids of each sex, as well as host larval and pupal mortalities. The length of forewing and hind tibia were measured using a stereo microscope LEICA EZ4D (Leica Microsystems Inc., Illinois, USA) at ×30 magnification.

3.10 Data analysis

One-way analysis of variance (ANOVA) in GENSTAT version 19.1 was used to compare the morphological characteristics of amaranth accessions including number of branches per plant, plant height, leaf length and width and petiole length. All other statistical analyses were conducted using R version 3.5.1 statistical software (R Development Core Team, Vienna, Austria). The weight gain (mg) and percentage weight gain and by larvae of *S. recurvalis*, larval and pupal mortalities, egg viability, fecundity and F_1 female proportions of *S. recurvalis* were analysed using one-way ANOVA. The effect of larval host (amaranth accession), larval host density and larval host age on laboratory parasitism rates, developmental time (larval, pupal and total), non-reproductive larval and pupal mortalities, female F_1 proportions, adult longevity and length of forewings and hind tibia of *A. hemara* were also analysed using one-way ANOVA.

Two-way repeated measures ANOVA was used to analyse morphological and biomass partitioning data from water stress treatment with water supply regimes and amaranth accessions as factors.

Abundance of lepidopteran defoliators, amaranth stem weevils and number of stem tunnels was analysed using the Generalised Linear Model (GLM) with the quasipoisson family and the log link function. Similarly, GLM with $\log_{10}$-link and Poisson distribution error was used to compare the number of eggs oviposited by female *S. recurvalis* moths from both choice and no-choice assays, number of days taken for larval, pupal and total development, and adult longevity of *S. recurvalis*

on various accessions. The number of days taken for larval and pupal development, duration of the whole development cycle and adult longevity of *A. hemara* at different host age and density were also analysed by GLM with log link and poisson distribution error. The effect of a factor for a GLM is reflected in the deviance (likelihood ratio test statistic) that has an appropriate chi-square distribution; hence the chi-square values are presented as test statistics.

The "Relative Risk/Risk Ratio" (RR), which is a ratio of the probability of having the pest lay an egg on the test amaranth accession relative to the susceptible accession, was calculated as an exponent of the coefficients obtained from the Poisson regressions. The data on pest incidence and damage caused by lepidopteran defoliators and stem weevils was analysed using GLM with the binomial family and the logit link. Pest (infestation) and damage incidence was calculated as the proportion or percentage of plants infested with the pest according to Ibeawuchi *et al*. (2007).

Severity of damage by lepidopteran defoliators was analysed using ordered logistic regression in GLM. Pearson's product-moment correlation test was used to determine the correlation between stem weevil abundance and tunnelling damage during the short rainy season. Percent parasitism on each accession in the open field was calculated as the number of parasitoids recovered divided by the total number of lepidopteran pests sampled. Species diversity of lepidopteran defoliators and their associated parasitoids on each accession during the two

seasons was determined using Shannon diversity index and Evenness (Magurran, 2004).

The instantaneous rate of increase (r_i) was calculated according to Stark and Banks (2003) using the following equation:

$$r_i = \ln(Nf/No)/T,$$

Where: Nf is the final number of insects

No is the initial number of insects

T is the change in time (number of days the experiment was run).

Positive values of *ri* indicate a growing population, *ri* = 0 indicates a stable population, and negative *ri* values indicate a population in decline and headed toward extinction. Spearman's rank order correlation analysis was conducted to establish the existence of relationships between larval vs. pupal mortalities and larval mortality vs. time taken before mortality in *S. recurvalis*.

Longevity and length of forewing and hind tibia between males and female *A. hemara* was compared using independent samples t-test. The significance of non-reproductive mortality was assessed by comparing natural mortalities in the control with mortalities in presence of parasitoid using paired t-test. The actual non-reproductive host mortality was identified using Abbott's formula (Abbott, 1925).

The number of eggs of *S. recurvalis* obtained in the choice and no-choice assays and the ratio of male to female F_1 parasitoids within each treatment of larval host plant, larval age or larval density was compared using a chi-square (χ^2)

goodness-of-fit test. Non-linear least-squares regression was used to determine the relationship between larval density and the number of hosts parasitized while a simple linear regression was used to determine the relationship between larval density and the rate of parasitism. Parasitism rate was calculated as the percentage of the number of parasitoid cocoons divided by the sum of pupae of the host and parasitoid cocoons.

The count numbers were $\log_{10}(x+1)$-transformed while the percentages were square-root transformed before analysis to obtain normally distributed datasets with similar variance among treatments. Tukey's test was used to separate means where significant differences occurred at $P < 0.05$.

CHAPTER FOUR. RESULTS

4.1 Assessing the occurrence and diversity of amaranth lepidopteran defoliators and stem weevils and their associated parasitoids in Arusha, Tanzania

4.1.1 Morphological characteristics of amaranth accessions

Amaranth accessions exhibited different morphological characteristics including leaf coloration, leaf shape, leaf size and growth habit among others. The susceptible accession had significantly broader (F = 37.9; df = 35,178; P < 0.001) and longer (F = 31.1; df =35,178; P < 0.001) leaves compared to the resistant accessions (Table 3.1). The smallest leaf sizes were recorded on accession VI036227 with width of 1.2 ± 0.1 cm and length of 3.2 ± 0.7 cm compared to 10.6 ± 0.6 and 19.3 ± 2.2 cm for the susceptible accession. The plant height and petiole lengths also differed significantly among the amaranth accessions. There was no significant difference in the number of branches across all the accessions. Leaf coloration and shape also varied among the accessions with accessions VI046233-A, VI033477 and VI056563 possessing red leaves compared to the green leaves in the susceptible accession (Table 3.1).

4.1.2 Composition and abundance of lepidopteran defoliators attacking amaranth in Tanzania

During the long rainy season between March and June 2016, a total of 630 lepidopteran larvae belonging to 5 families (Crambidae, Erebidae, Noctuidae, Scythrididae and Tortricidae), 7 sub-families (Arctiinae, Heliothinae, Noctuinae, Plusiinae, Spilomelinae, Scythridinae and Tortricinae) and 9 species were

recovered from 18 different accessions of amaranth (Figure 4.1). Of these, 80.45% were leaf-webbers, while 19.55% were leaf-worms. Among the leaf-webbers, 58.70% were *S. recurvalis* (Plate 4.1A), 37.94% *P. basalis*, 1.98% *Choristoneura* sp. (Lepidoptera: Tortricidae) (Plate 4.1B) and 1.38% *Eretmocera impactella* Walker (Lepidoptera: Scythrididae).

A **B**

Plate 4.1: Adult stages of amaranth leaf-webbers. A: *Spoladea recurvalis* (Mag. X2), B: *Choristoneura* sp. (Mag. X3).

The leaf-worms were composed of *S. exigua* (Plate 4.2A and 4.2B) (48.39%), *S. littoralis* (Plate 4.3) (39.52%), *Helicoverpa armigera* (Lepidoptera: Noctuidae) (2.42%), *Spilosoma* sp. (Lepidoptera: Erebidae) (1.61%), *Chrysodeixis* sp. (Lepidoptera: Noctuidae) (1.61%) and *Amyna axis* Guenee (Lepidoptera: Noctuidae) (6.45%) (Table 4.1). Between December 2016 and March 2017, a total of 1,424 lepidopteran larvae belonging to four families (Crambidae, Noctuidae, Scythrididae and Tortricidae), seven sub-families (Heliothinae, Noctuinae,

Plusiinae, Pyraustinae, Spilomelinae, Scythridinae and Tortricinae) and 14 species were recovered from 36 different accessions of amaranth (Figure 4.1). The most abundant species was *S. recurvalis* accounting for 47.17% and 69.74% in the short and long rainy seasons, respectively.

A B

Plate 4.2: Beet armyworm *Spodoptera exigua.* A: Larvae feeding on amaranth, B: Adult (Mag. X1.5)

A B

Plate 4.3: *Spodoptera littoralis.* A: Egg mass usually laid on the underside of amaranth leaves, B: Adult female (Mag. 1.5)

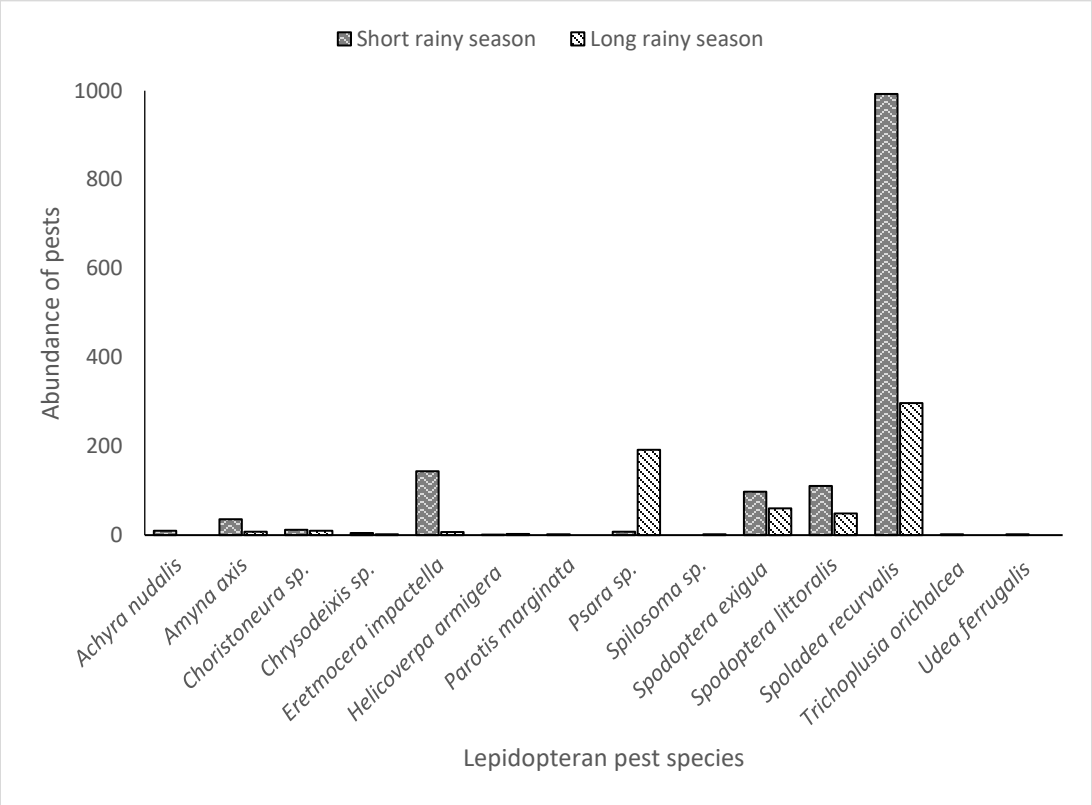

Figure 4.1: Composition and abundance of lepidopteran pests during long (2016) and short (2017) rainy seasons in Arusha, Tanzania

Leaf-webbers made up 82.23% of the total number of lepidopterans while leaf-worms were 17.77%. The leaf-webbers comprised of *S. recurvalis* (84.80%), *E. impactella* (12.30%), *Choristoneura* sp. (1.02%), *Achyra nudalis* Hübner (Lepidoptera: Crambidae) (0.85%), *P. basalis* (0.68%), *Udea ferrugalis* Hübner (Lepidoptera: Crambidae) (0.17%) (Plate 4.4) and *Parotis marginata* Hampson (Lepidoptera: Crambidae) (0.17%). The leaf-worms included *S. littoralis* (43.87%), *S. exigua* (38.74%), *Chrysodeixis* sp. (Lepidoptera: Noctuidae) (1.98%),

Trichoplusia orichalcea F. (Lepidoptera: Noctuidae) (0.79%), *H. armigera* (0.40%) and *A. axis* (14.23%) (Table 4.1).

A B

Plate 4.4: The rusty dot pearl moth *Udea ferrugalis.* A: Larvae of feeding on the underside of amaranth leaf, B: Adult moth (Mag. X3)

Table 4.1: Abundance (%) and composition of lepidopteran defoliators attacking amaranth accessions during the long (2016) and short (2017) rainy seasons in Arusha, Tanzania

Lepidopteran defoliators of amaranth			**Abundance (%)**	
Family	Sub-family	Species	Long rainy season (2016)	Short rainy season (2017)
Erebidae	Arctiinae	*Spilosoma* sp.	0.32	0.00
Crambidae	Spilomelinae	*Spoladea recurvalis*	47.14	69.74
		Udea ferrugalis	0.00	0.14
		Psara basalis	30.47	0.56
		Parotis marginata	0.00	0.14
	Pyraustinae	*Achyra nudalis*	0.00	0.70
Scythrididae	Scythridinae	*Eretmocera impactella*	1.11	10.11
Tortricidae	Tortricinae	*Choristoneura* sp.	1.59	0.84
Noctuidae	Noctuinae	*Spodoptera exigua*	9.52	6.88
		Spodoptera littoralis	7.78	7.80
	Plusiinae	*Chrysodeixis* sp.	0.32	0.35
		Trichoplusia orichalcea	0.00	0.14
	Heliothinae	*Helicoverpa armigera*	0.48	0.07
	Acontiinae	*Amyna axis*	1.27	2.53

The predominant pests during the long rainy season (2016) were *S. recurvalis* accounting for 47.14%, followed by *P. basalis* 30.48%. Within the first four weeks of the season, the predominant pest was *P. basalis*. The typical symptom of this pest is folded leaves in characteristic leaf shelters at the apical region thereby hindering apical development of the plant. *Spoladea recurvalis*

populations began to build up progressively from the second week, becoming the dominant pest from the fifth week, until the end of the season. Unlike *P. basalis* which exhibited a constant reduction in its proportion, *S. recurvalis* was on a constant rise throughout the season (Figure 4.2). During the short rainy season (2017), *P. basalis* was replaced by *E. impactella* (10.11%) as the second most dominant leaf-webber after *S. recurvalis* (69.73%). *Spoladea recurvalis* dominated from the sixth week until the end of the season while *E. impactella* declined from the sixth week (Figure 4.2). In both seasons, *S. recurvalis* was the most abundant pest with an overall abundance of 63.24%, followed by *P. basalis* at 9.80%.

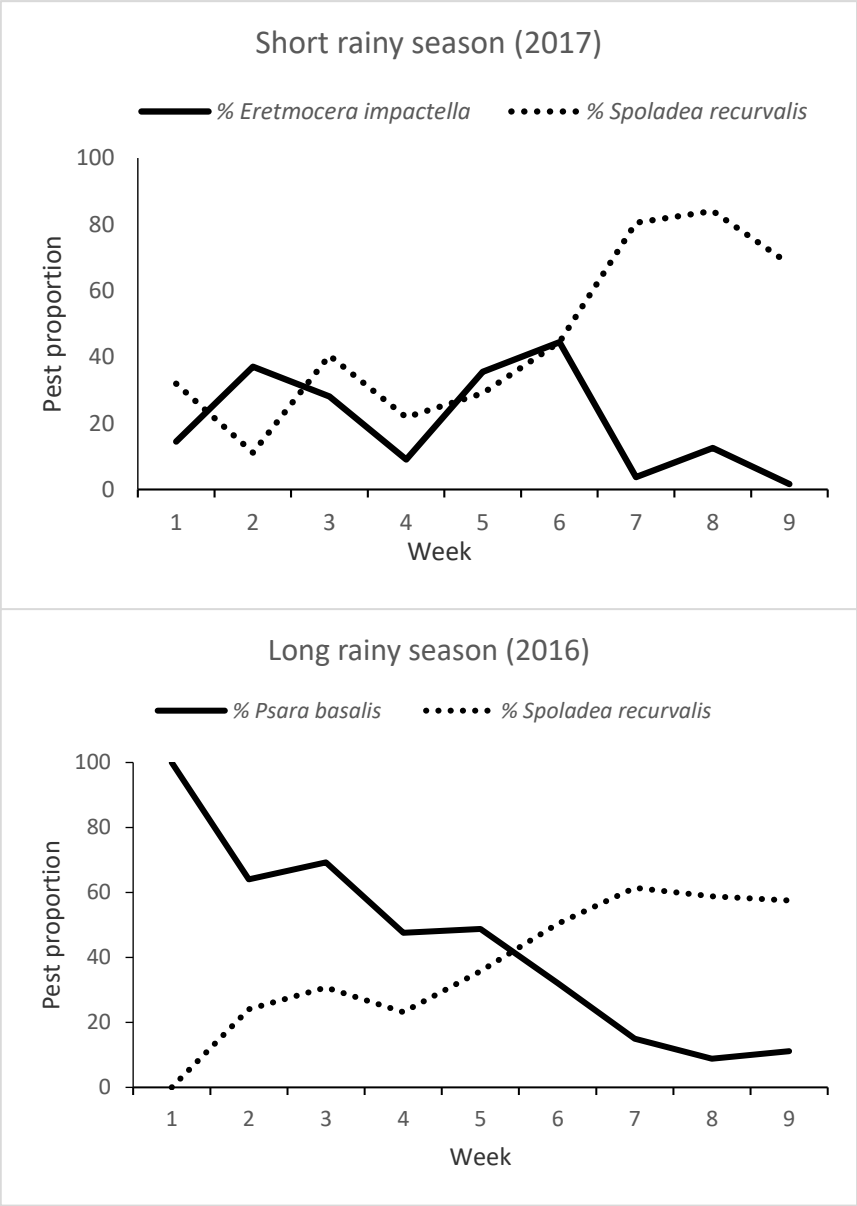

Figure 4.2: Weekly proportion (%) of the two most abundant lepidopteran pests during the long (2016) and short (2017) rainy seasons in Arusha, Tanzania

4.1.3 Composition and abundance of parasitoids of lepidopteran pests of amaranth in Tanzania

A total of 518 hymenopteran parasitoids from 14 species were recovered from the lepidopteran larvae feeding on amaranth during the two seasons. These were from the families Braconidae and Ichneumonidae and 10 sub-families (Braconidae: Agathidinae, Braconinae, Cardiochilinae and Microgastrinae; Ichneumonidae: Banchinae, Campopleginae, Cremastinae, Cryptinae, Mesochorinae and Metopiinae) (Table 4.2). Total parasitism of 26.35% and 24.72% was observed during the long and short rainy seasons, respectively. During the long rainy season in 2016, the most abundant parasitoid was the solitary endoparasitoid *Apanteles hemara* (Hymenoptera: Braconidae) (Plate 4.5) with 48.80% abundance and parasitism rate of 16.56% on *S. recurvalis* and *P. basalis*.

A **B**

Plate 4.5: *Apanteles hemara.* A: Adult female, B: White silken pupal cocoon (Mag. X30).

During the short rainy season in 2017, *A. hemara* remained the most abundant parasitoid accounting for 86.93% of the total individuals with a total parasitism rate of 30.60% on *S. recurvalis* and *P. basalis*. The second most abundant parasitoid during both seasons was *Atropha tricolor* Szepligeti (Hymenoptera: Ichneumonidae) (Plate 4.6) with parasitism rates of 4.70% and 2.0% during the long and short rainy seasons, respectively. *Spodoptera exigua* and *S. littoralis* were mainly parasitized by *Cotesia icipe* Fernandez-Triana and Fiaboe (Hymenoptera: Braconidae) during both seasons with parasitism rates of 18.33% and 5.10% on *S. exigua* and 2.04% and 1.80% on *S. littoralis* during the long and short rainy seasons, respectively.

Plate 4.6: *Atropha tricolor*, a parasitoid of *Spoladea recurvalis*. A: Pupal cocoons usually found within leaves glued together by the leaf-webbers, B: Adult female (Mag. X10)

Table 4.2: Composition and abundance (%) of parasitoids of lepidopteran pests attacking amaranth accessions during the long (2016) and short (2017) rainy seasons in Arusha, Tanzania

Parasitoids of lepidopteran pests of Amaranth				**Abundance**	
Family	**Sub-family**	**Species**	**Host pest**	**Long rainy season (2016)**	**Short rainy season (2017)**
Ichneumonidae	Banchinae	*Atropha tricolor*	*S. recurvalis*	13.86	5.68
	Campopleginae	*Diadegma* sp.	*S. recurvalis*	1.20	0.57
	Cremastinae	*Pristomerus* sp.	*S. recurvalis*	0.60	0.00
		Temelucha sp.	*S. recurvalis*	1.81	0.00
	Cryptinae	*Phygadeuontini* sp.	*S. recurvalis*	0.60	0.00
	Mesochorinae	*Mesochorus* sp.	*S. recurvalis*	0.60	0.00
	Metopiinae	*Triclistus bicolor*	*S. recurvalis*	5.42	0.00
Braconidae	Agathidinae	*Coccygidium luteum*	*S. littoralis/S. exigua*	2.41	2.56
		Braunsia occidentalis	*S. recurvalis*	6.02	0.00
	Braconinae	*Bracon* sp.	*S. recurvalis*	3.01	0.57
	Cardiochilinae	*Schoenlandella testacea*	*S. recurvalis*	2.41	0.85
	Microgastrinae	*Apanteles* sp.	*S. recurvalis/P. basalis*	48.80	86.93
		Cotesia icipe	*S. littoralis/S exigua*	11.45	1.99
		Cotesia sp.	*Choristoneura sp.*	1.81	0.85

4.1.4 Diversity indices of lepidopteran defoliators of amaranth and their associated parasitoids and parasitism rates

The Shannon Weiner diversity indices (H) for the lepidopteran defoliators during the long and short rainy seasons were H = 1.372 and H = 1.116, respectively, with the long rainy season (2016) recording significantly higher diversity of lepidopteran pests than the short rainy season (2017) (t = 5.056; *P* = 0.006). The Shannon Weiner diversity index of lepidopteran defoliators varied from 0.00 to 1.57 and 0.00 to 1.58 during the long and short rainy seasons, respectively. Except accession VI036227, all the others had higher diversity index compared to the susceptible check during the long rainy season (Table 4.3). Only VI033479, VI036227, VI044473, VI049698 and VI056563 had lower diversity index for the pests compared to the susceptible check during the short rainy season (Table 4.4). During the long rainy season, accessions VI044367 and VI036227 had the highest and lowest species richness of 8 and 0, respectively, whereas VI050609-B and four accessions (VI033479, VI036227, VI044473 and VI049698) had the highest and lowest species richness of 9 and 1, respectively, during the short rainy season (Table 4.3 and 4.4).

The diversity of the parasitoids differed significantly between the two seasons (t = 10.45; *P* = 0.039) with the long rainy season (2016) having higher parasitoid diversity (H =1.775) than the short rainy season (H = 0.596). Parasitoid diversity was highest (H = 1.61) on VI044473 whereas RVI00005, RVI00053, VI044367, VI044369 and VI044388 recorded higher Shannon diversity index than the susceptible check during the long rainy season (Table 4.3). Parasitism was

recorded in all the accessions except VI036227 (0.00%) with the highest on RVI00027 (45.45%) during the season. Parasitoid species richness in the same season was highest on VI033482, RVI00005 and VI044473 and lowest on VI036227.

During the short rainy season (2017), parasitoid diversity was highest on RVI00053 (H = 1.01) while 21 accessions had diversity H = 0.00 (Table 4.4). The susceptible check and VI056563 had the highest parasitoid richness whereas VI036227, VI048864-A, VI49504, VI049639, VI049698, VI054798, VI055128 and VI055135 did not record any parasitoids and consequently no cases of parasitism despite hosting the pests. Nonetheless, parasitism was recorded in 28 accessions with VI033479 recording the highest (66.67%) compared to the susceptible check (22.51%).

Table 4.3: Diversity indices of amaranth lepidopteran defoliators and their associated parasitoids and parasitism rates (%) per accession during the long rainy season (2016) in Arusha, Tanzania

Lepidopteran defoliators				Parasitoids			
Accession code	$H^{\$}$	Richness (Individuals)	Evenness	$H^{\$}$	Richness (Individuals)	Evenness	Parasitism (%)
VI033482*	0.67	7 (96)	0.34	1.12	6 (25)	0.63	26.04
RVI00002	1.12	5 (63)	0.70	0.41	2 (14)	0.59	22.22
RVI00005	1.21	4 (50)	0.88	1.65	6 (16)	0.92	32.00
RVI00027	1.04	3 (11)	0.94	0.50	2 (5)	0.72	45.45
RVI00053	1.33	5 (16)	0.83	1.33	5 (16)	0.83	29.63
VI033487	1.57	6 (19)	0.88	0.69	2 (2)	1.00	10.53
VI036225	1.07	4 (26)	0.77	1.07	4 (8)	0.77	30.77
VI036227	0.00	0 (0)	0.00	0.00	0 (0)	0.00	0.00
VI044367	1.48	8 (60)	0.71	1.17	4 (14)	0.84	23.33
VI044369	1.16	5 (52)	0.72	1.38	5 (14)	0.85	26.92
VI044388	1.07	4 (30)	0.77	1.42	5 (12)	0.88	40.00
VI044432	1.54	6 (20)	0.86	1.04	3 (4)	0.95	20.00
VI044437-A	1.26	7 (45)	0.65	0.90	4 (14)	0.65	31.11
VI044473	1.12	4 (33)	0.80	1.61	6 (10)	0.90	30.30
VI048076	1.56	6 (22)	0.87	0.69	2 (2)	1.00	9.09
VI049639	1.54	6 (22)	0.86	1.10	3 (3)	1.00	13.64
VI049698	0.80	3 (10)	0.73	0.64	2 (3)	0.92	30.00
VI054569	1.25	4 (17)	0.90	1.04	3 (4)	0.95	23.53

*Susceptible check, $H^{\$}$ = Shannon Weiner diversity index

Table 4.4: Diversity indices of amaranth lepidopteran defoliators and their associated parasitoids and parasitism rates (%) per accession during the short rainy season (2017) in Arusha, Tanzania

Lepidopteran defoliators				Parasitoids			
Accession code	H	Richness (Individuals)	Evenness	H	Richness (Individuals)	Evenness	Parasitism (%)
VI033482*	0.52	7 (542)	0.27	0.35	4 (122)	0.25	22.51
RVI00002	0.79	3 (18)	0.72	0.74	3 (10)	0.67	55.56
RVI00005	0.80	3 (7)	0.72	0.00	1 (3)	Na	42.86
RVI00027	0.72	3 (12)	0.66	0.56	2 (7)	0.81	58.33
RVI00053	1.04	5 (16)	0.64	1.01	3 (6)	0.92	37.50
VI033477	0.72	4 (104)	0.52	0.41	3 (44)	0.37	42.31
VI033479	0.00	1 (3)	Na	0.69	2 (2)	1.00	66.67
VI033487	0.64	4 (28)	0.46	0.00	1 (14)	Na	50.00
VI036225	1.58	5 (9)	0.98	0.00	1 (3)	0.00	33.33
VI036227	0.00	1 (2)	Na	0.00	0 (0)	1.00	0.00
VI044367	0.56	2 (4)	0.81	0.00	1 (2)	0.00	50.00
VI044369	1.07	5 (27)	0.67	0.50	2 (3)	0.32	11.11
VI044388	0.64	2 (3)	0.92	0.00	1 (1)	0.00	33.33
VI044432	1.07	4 (8)	0.77	0.00	1 (2)	0.00	25.00
VI044437-A	0.96	3 (7)	0.87	0.64	2 (3)	0.44	42.86
VI044473	0.00	1 (5)	Na	0.00	1 (1)	0.00	20.00
VI046233-A	0.75	3 (17)	0.69	0.00	1 (8)	0.00	47.06
VI047517-B	0.78	6 (40)	0.43	0.00	1 (8)	0.00	20.00
VI047555-B	0.81	5 (30)	0.50	0.30	2 (11)	0.17	36.67
VI048076	1.27	7 (34)	0.65	0.45	2 (6)	0.28	17.65
VI048864-A	1.17	4 (10)	0.84	0.00	0 (0)	1.00	0.00
VI048919	0.91	4 (53)	0.65	0.00	1 (3)	0.00	5.66
VI049242	0.85	3 (9)	0.77	0.69	2 (2)	0.50	22.22
VI049502	1.08	5 (18)	0.67	0.00	1 (2)	0.00	11.11

Table continues on next page

Continued from previous page

Lepidopteran defoliators				Parasitoids			
Accession code	**H$**	**Richness (Individuals)**	**Evenness**	**H$**	**Richness (Individuals)**	**Evenness**	**Parasitism (%)**
VI049504	0.90	3 (14)	0.82	0.00	0 (0)	1.00	0.00
VI049530	0.69	2 (2)	1.00	0.00	1 (1)	0.00	50.00
VI049639	1.21	4 (8)	0.88	0.00	0 (0)	1.00	0.00
VI049698	0.00	1 (2)	Na	0.00	0 (0)	1.00	0.00
VI050609-A	0.78	5 (120)	0.48	0.47	3 (24)	0.23	20.00
VI050609-B	1.58	9 (49)	0.72	0.41	2 (15)	0.24	30.61
VI054569	1.21	5 (20)	0.75	0.64	3 (10)	0.34	50.00
VI054798	0.83	3 (14)	0.76	0.00	0 (0)	1.00	0.00
VI055127	1.13	5 (14)	0.70	0.00	1 (1)	Na	7.14
VI055128	0.69	2 (2)	1.00	0.00	0 (0)	0.00	0.00
VI055135	1.39	4 (4)	1.00	0.00	0 (0)	0.00	0.00
VI056563	0.34	5 (169)	0.21	0.57	4 (38)	0.41	22.49

*Susceptible check, H$ = Shannon Weiner diversity index

4.1.5 Composition and abundance of amaranth stem weevils and their associated parasitoids in Tanzania

Adult amaranth stem weevils and their grubs (larvae) were found feeding on leaves and within stems, respectively, with a total of 165 and 110 adult weevils recovered during the long and short rainy seasons, respectively. The grubs found within the stems totalled962 and 3,726 during the long and short rainy seasons, respectively. Four species of amaranth stem weevils were encountered during the two seasons, namely *Cosmobaris* sp. (Curculionidae: Baridinae), *H. truncatulus* (Curculionidae: Lixinae), *Lixus* sp. (Curculionidae: Lixinae) and *Neocleonus* sp. (Curculionidae: Lixinae). The most abundant species was *H. truncatulus*, accounting for about 80% of the total weevils collected.

One parasitoid species was recovered from the larvae of the stem weevils. This belonged to the genus *Entedon*, family Eulophidae and order Hymenoptera. The parasitoid caused 0.50% parasitism on the amaranth stem weevils, becoming a first report in East Africa.

4.2 Evaluating amaranth accessions for resistance against lepidopteran defoliators and stem weevils

4.2.1 Susceptibility of amaranth accessions to lepidopteran defoliators and stem weevils under field conditions during the long rainy season of 2016

The incidence of lepidopteran defoliators across the amaranth accessions varied between 0.00 ± 0.00% and 20.74 ± 2.50%, with an overall mean of 8.68 ± 0.40%. Incidence of lepidopteran defoliators was significantly lower in all the tested accessions compared to the susceptible one, except in accessions RVI00002, RVI00053 and VI044367 (χ^2 = 172.76; df = 17, 4842; $P < 0.001$) (Table 4.5). The abundance of lepidopteran defoliators in all the tested accessions during the long rainy season was significantly lower (F = 10.14; df = 17, 4842; $P < 0.001$) than the susceptible one except for RVI00002. Notably, no leaf-webbers were found on VI036227 during the season and the accession had the least relative risk (RR) of 0.01 with reference to the susceptible check. RVI00002, RVI00005, RVI00053, VI044367 and VI044369 had significantly high ($P < 0.001$) abundance of lepidopteran defoliators (RR above 0.5) compared to VI036227, VI049698, RVI00027, VI054569, VI033487, VI044432, VI048076 and VI049639 (RR below 0.25). The mean abundance of lepidopteran defoliators on the assessed amaranth accessions ranged between 0.00 ± 0.00 and 0.36 ± 0.05.

Table 4.5: Leaf-webbers' incidence, abundance, and damage on various amaranth accessions under field conditions during the long rainy season of 2016 in Arusha, Tanzania

Gene bank code	Leaf-webber incidence	Relative Risk	Leaf-webber abundance	Relative Risk	Damage incidence by leaf-webbers	Relative Risk
VI033482*	20.74 ± 2.47a		0.36 ± 0.05a		50.00 ± 3.05ab	
RVI00002	16.67 ± 2.27ab	0.77	0.23 ± 0.04ab	0.66	54.81 ± 3.03a	1.21
RVI00005	10.74 ± 1.89bcd	0.47	0.19 ± 0.04b-e	0.53	47.41 ± 3.04abc	0.90
RVI00027	3.33 ± 1.09efg	0.14	0.04 ± 0.02hij	0.12	37.41 ± 2.95d	0.60
RVI00053	13.70 ± 2.10ab	0.61	0.20 ± 0.04bcd	0.57	51.11 ± 3.05ab	1.05
VI033487	6.30 ± 1.48def	0.27	0.07 ± 0.02ghi	0.21	46.30 ± 3.04a-d	0.86
VI036225	5.19 ± 1.35ef	0.22	0.10 ± 0.03e-i	0.28	24.07 ± 2.61ef	0.32
VI036227	0.00 ± 0.00g	0.01	0.00 ± 0.00j	0.01	5.56 ± 1.40g	0.06
VI044367	15.19 ± 2.19ab	0.69	0.22 ± 0.04bc	0.63	52.59 ± 3.04a	1.11
VI044369	12.59 ± 2.02bc	0.56	0.19 ± 0.04bcd	0.55	50.37 ± 3.05ab	1.01
VI044388	7.41 ± 1.60cde	0.32	0.11 ± 0.03d-h	0.32	22.96 ± 2.56ef	0.30
VI044432	6.67 ± 1.52def	0.28	0.07 ± 0.02ghi	0.22	24.81 ± 2.63ef	0.33
VI044437-A	11.11 ± 1.92bcd	0.48	0.17 ± 0.03b-f	0.47	42.59 ± 3.01bcd	0.74
VI044473	5.93 ± 1.44def	0.25	0.12 ± 0.03c-g	0.35	19.63 ± 2.42f	0.24
VI048076	6.30 ± 1.48def	0.27	0.08 ± 0.02f-i	0.24	40.74 ± 3.00cd	0.69
VI049639	7.04 ± 1.56cde	0.30	0.08 ± 0.02f-i	0.24	28.52 ± 2.75e	0.40
VI049698	2.59 ± 0.97fg	0.11	0.04 ± 0.01ij	0.11	24.81 ± 2.63ef	0.33
VI054569	4.81 ± 1.31ef	0.20	0.06 ± 0.02g-j	0.19	21.11 ± 2.49ef	0.27

*Susceptible check. Mean ± SE followed by the same letter within a column are not significantly different at P<0.05 (Tukey's test)

The incidence of damage by lepidopteran defoliators varied from 5.56 ± 1.40% to 54.81 ± 3.03% with an overall mean of 35.82 ± 0.69%. There were significant differences (χ^2 = 457.89; df = 17, 4842; P < 0.001) in damage incidence among the accessions with VI036227, VI044473, VI054569, VI044388, VI036225, VI044432, VI049698, VI049639, RVI00027, VI048076 and VI044437-A having lower incidences of damage compared to the susceptible check (Table 5). Accessions VI033487, RVI00005, VI044369, RVI00053, VI044367 and RVI00002 did not differ significantly (P < 0.001) in their incidence of damage compared to the susceptible check (Table 4.5).

Severity of damage caused by leaf-webbers differed significantly (χ^2 = 544.65; df = 17, 4842; P < 0.001) among the accessions with all but 4 (VI044367, VI044369, RVI00002 and RVI00053) having significantly lower severity compared to the susceptible check. Accession VI036227 had significantly lower severity of damage compared to all the other accessions with an odds ratio (OR) of 0.04.

The overall average incidence of amaranth stem weevils was 68.7 ± 2.0% during the long rainy season. The incidence of amaranth stem weevils was significantly different across the accessions with VI036227, VI036225, VI044473, VI044388, VI049698, VI049639 and RVI00027 having significantly lower pest incidence (RR 0 - 0.06) compared to the control (χ^2 = 141.11; df = 17, 522; P < 0.001). The incidence of stem weevils ranged from 0.0 ± 0.0% to 96.67 ± 3.33% with 11 accessions having incidence levels above 70% (Table 4.6). The abundance

of stem weevils varied between 0.00 ± 0.00 and 3.60 ± 0.63 with an overall average of 1.80 ± 0.09 throughout the season. Accessions VI036227, VI036225, VI049698, VI049639, RVI00027, VI044473, RVI00002, VI044432, VI054569, VI044437-A and VI044388 had significantly fewer (F = 10.16; df = 17, 517; P < 0.001) stem weevils (RR 0 - 0.55) compared to the susceptible check. There was high incidence of damage caused by the amaranth stem weevils averaging to 97.55 ± 0.88%. There was no significant difference (χ^2 = 7.39; df = 17, 517; P = 0.978) in the incidence of stem weevil damage across all accessions including the susceptible check. The incidence of damage by stem weevils ranged between 85.0 ± 8.19% and 100.0 ± 0.00%.

Table 4.6: Stem weevils' incidence, abundance, and damage on various amaranth accessions under field conditions during the long rainy season of 2016 in Arusha, Tanzania

Gene bank code	Stem weevil incidence	Relative Risk	Stem weevil abundance	Relative Risk	Stem weevil damage incidence	Relative Risk
VI033482*	96.67 ± 3.33a		3.23 ± 0.44ab		100.00 ± 0.00a	
RVI00002	70.00 ± 8.51a-d	0.08	1.40 ± 0.25d-g	0.43	100.00 ± 0.00a	1.00
RVI00005	80.00 ± 7.43abc	0.14	2.03 ± 0.38b-e	0.63	100.00 ± 0.00a	1.00
RVI00027	63.33 ± 8.95b-e	0.06	1.00 ± 0.19fgh	0.31	93.33 ± 4.63a	0.00
RVI00053	93.33 ± 4.63a	0.48	3.37 ± 0.53a	1.04	100.00 ± 0.00a	1.00
VI033487	80.00 ± 7.43abc	0.14	2.23 ± 0.35a-e	0.69	100.00 ± 0.00a	1.00
VI036225	36.67 ± 8.95e	0.02	0.53 ± 0.15hi	0.16	100.00 ± 0.00a	1.00
VI036227	0.00 ± 0.00f	0.00	0.00 ± 0.00i	0.01	85.00 ± 8.19a	0.00
VI044367	73.33 ± 8.21a-d	0.09	2.45 ± 0.50a-d	0.76	100.00 ± 0.00a	1.00
VI044369	93.33 ± 4.63a	0.48	3.60 ± 0.63a	1.11	100.00 ± 0.00a	1.00
VI044388	56.67 ± 9.20cde	0.05	1.79 ± 0.52c-f	0.55	100.00 ± 0.00a	1.00
VI044432	83.33 ± 6.92abc	0.17	1.60 ± 0.21d-g	0.49	100.00 ± 0.00a	1.00
VI044437-A	83.33 ± 6.92abc	0.17	1.69 ± 0.26c-f	0.52	93.10 ± 4.79a	0.00
VI044473	43.33 ± 9.20de	0.03	1.30 ± 0.40e-h	0.40	90.00 ± 5.57a	0.00
VI048076	80.00 ± 7.43abc	0.14	2.87 ± 0.47abc	0.89	100.00 ± 0.00a	1.00
VI049639	60.00 ± 9.10b-e	0.05	0.90 ± 0.18fgh	0.28	96.67 ± 3.33a	0.00
VI049698	56.67 ± 9.20cde	0.05	0.72 ± 0.15ghi	0.22	96.55 ± 3.45a	0.00
VI054569	86.67 ± 6.31ab	0.22	1.63 ± 0.23def	0.51	96.67 ± 3.33a	0.00

*Susceptible check. Mean ± SE followed by the same letter within a column are not significantly different at $P<0.05$ (Tukey's test)

4.2.2 Susceptibility of amaranth accessions to lepidopteran defoliators and stem weevils under field conditions during the short rainy season of 2017

The incidence of lepidopteran defoliators varied from 0.93 ± 0.05% to 46.30 ± 3.40% with an overall mean incidence of 7.09 ± 0.29%. The incidence was significantly lower (χ^2 = 531.38; df = 35, 7668; P < 0.001) than the susceptible check. Accessions VI033477, VI050609-B and VI056563 with RR above 0.22 had significantly higher incidence of lepidopteran defoliators than the accessions with RR below 0.11 (Table 4.7). VI036227, VI049530 and VI049698 had the least incidence of leaf-webbers with RR of 0.01. The overall mean abundance of lepidopteran defoliators across all accessions was 0.18 ± 0.02 larvae and ranged from 0.01 ± 0.01 to 2.51 ± 0.40 larvae. All the accessions had significantly lower (F = 22.08; df = 35, 7668; P < 0.001) pest abundance compared to the susceptible check. Accessions VI033477, VI050609-A and VI056563 also had significantly high pest abundance with RR above 0.19 compared to all other accessions which had RR below 0.10. Accessions VI036227, VI049530 and VI049698 also had the least pest abundance with RR of 0.00.

The damage by lepidopteran defoliators on all the accessions varied from 1.39 ± 0.98% to 88.89 ± 2.63%, with an overall mean of 67.72 ± 0.65%. Accessions RVI00005, VI049504, VI048076, VI046233-A, VI050609-B, VI054798, VI056563, RVI00053, VI033477 and VI050609-A had damage incidence that was not significantly different from the susceptible check, with RR ranging from 0.49 to 1.37 while all the other accessions (RR below 0.49) had

significantly lower incidence of damage compared to the susceptible check (χ^2 = 513.98; df = 35, 5098; $P < 0.001$) (Table 4.7). VI036227 had the least incidence of damage by leaf-webbers with a RR of 0.00 which was significantly lower than all other accessions.

Table 4.7: Comparative leaf-webbers' incidence, abundance, and damage on various amaranth accessions under field conditions during the short rainy season of 2017 in Arusha, Tanzania

Gene bank code	Leaf-webber incidence	Relative Risk	Leaf-webber abundance	Relative Risk	Damage incidence by leaf-webbers	Relative Risk
VI033482*	46.30 ± 3.40a		2.51 ± 0.40a		85.42 ± 2.95ab	
RVI00002	6.02 ± 1.62e-l	0.07	0.08 ± 0.02e	0.03	69.44 ± 3.85d-j	0.39
RVI00005	3.24 ± 1.21j-m	0.04	0.03 ± 0.01e	0.01	74.31 ± 3.65b-h	0.49
RVI00027	3.70 ± 1.29i-m	0.04	0.06 ± 0.02e	0.02	68.06 ± 3.9e-j	0.36
RVI00053	6.48 ± 1.68e-l	0.08	0.07 ± 0.02e	0.03	82.64 ± 3.17abcl	0.81
VI033477	16.67 ± 2.54bc	0.23	0.48 ± 0.12bcd	0.19	84.62 ± 3.03ab	0.94
VI033479	1.39 ± 0.80m	0.02	0.01 ± 0.01e	0.01	65.97 ± 3.96f-j	0.33
VI033487	9.72 ± 2.02c-h	0.12	0.13 ± 0.03e	0.05	71.53 ± 3.77c-i	0.43
VI036225	4.17 ± 1.36h-m	0.05	0.04 ± 0.01e	0.02	48.61 ± 4.18k	0.16
VI036227	0.93 ± 0.65m	0.01	0.01 ± 0.01e	0.00	1.39 ± 0.98l	0.00
VI044367	1.85 ± 0.92lm	0.02	0.02 ± 0.01e	0.01	58.33 ± 4.12jk	0.24
VI044369	8.33 ± 1.88e-j	0.11	0.13 ± 0.03e	0.05	65.28 ± 3.98g-j	0.32
VI044388	1.39 ± 0.80m	0.02	0.01 ± 0.01e	0.01	61.11 ± 4.08ijk	0.27
VI044432	3.24 ± 1.21j-m	0.04	0.04 ± 0.01e	0.01	60.42 ± 4.09ijk	0.26
VI044437-A	2.78 ± 1.12klm	0.03	0.03 ± 0.01e	0.01	72.22 ± 3.75c-i	0.44
VI044473	1.85 ± 0.92lm	0.02	0.02 ± 0.01e	0.01	62.50 ± 4.05hij	0.28
VI046233-A	6.02 ± 1.62e-l	0.07	0.08 ± 0.02e	0.03	77.78 ± 3.48b-f	0.60
VI047517-B	10.19 ± 2.06b-g	0.13	0.19 ± 0.04de	0.07	69.44 ± 3.85d-j	0.39
VI047555-B	8.80 ± 1.93d-i	0.11	0.14 ± 0.03e	0.06	69.44 ± 3.85d-j	0.39
VI048076	11.11 ± 2.14b-f	0.15	0.16 ± 0.03e	0.06	77.08 ± 3.51b-g	0.57
VI048864-A	3.24 ± 1.21j-m	0.04	0.05 ± 0.02e	0.02	63.89 ± 4.02hij	0.30

Table continues on next page

Continued from previous page

Gene bank code	Leaf-webber incidence	Relative Risk	Leaf-webber abundance	Relative Risk	Damage incidence by leaf-webbers	Relative Risk
VI048919	11.57 ± 2.18b-e	0.15	0.25 ± 0.06cde	0.10	67.36 ± 3.92e-j	0.35
VI049242	3.24 ± 1.21j-m	0.04	0.04 ± 0.02e	0.02	65.28 ± 3.98g-j	0.32
VI049502	6.48 ± 1.68e-l	0.08	0.08 ± 0.02e	0.03	65.97 ± 3.96f-j	0.33
VI049504	3.24 ± 1.21j-m	0.04	0.06 ± 0.03e	0.03	75.00 ± 3.62b-h	0.51
VI049530	0.93 ± 0.65m	0.01	0.01 ± 0.01e	0.00	62.50 ± 4.05hij	0.28
VI049639	3.70 ± 1.29i-m	0.04	0.04 ± 0.01e	0.01	58.33 ± 4.12jk	0.24
VI049698	0.93 ± 0.65m	0.01	0.01 ± 0.01e	0.00	65.97 ± 3.96f-j	0.33
VI050609-A	11.57 ± 2.18b-e	0.15	0.56 ± 0.37bc	0.22	88.89 ± 2.63a	1.37
VI050609-B	17.59 ± 2.60b	0.25	0.23 ± 0.04de	0.09	78.47 ± 3.44b-e	0.62
VI054569	7.41 ± 1.79e-k	0.09	0.09 ± 0.02e	0.04	70.14 ± 3.83d-j	0.40
VI054798	4.63 ± 1.43g-m	0.06	0.06 ± 0.02e	0.03	81.25 ± 3.26a-d	0.74
VI055127	5.09 ± 1.50f-m	0.06	0.06 ± 0.02e	0.03	57.64 ± 4.13jk	0.23
VI055128	1.39 ± 0.98m	0.02	0.01 ± 0.01e	0.01	68.75 ± 4.76d-j	0.38
VI055135	1.85 ± 0.92lm	0.02	0.02 ± 0.01e	0.01	61.11 ± 4.08ijk	0.27
VI056563	16.20 ± 2.51bcd	0.22	0.78 ± 0.32b	0.31	82.52 ± 3.19abc	0.81

*Susceptible check. Mean ± SE followed by the same letter within a column are not significantly different at P<0.05 (Tukey's test)

The mean incidence of amaranth stem weevils was 90.42 ± 1.0% on all the accessions during the short rainy season and ranged from 54.17 ± 10.39% to 100 ± 0.00% with 77.78% of the tested accessions having pest incidence levels above 80%. There were significant differences (χ^2 = 172.91; df = 35, 828; P < 0.001) in the incidence of amaranth stem weevils across the accessions with VI047517-B,

VI036227, VI048076, VI056563, and VI055128 having the least incidence and subsequently lower RRs compared to the susceptible check (Table 4.8). All other accessions had higher RRs compared to the susceptible check with 21 having significantly higher incidence of amaranth stem weevils compared to the susceptible check. The abundance of stem weevils on the different amaranth accessions ranged from 0.75 ± 0.18 to 9.42 ± 1.89 with an overall mean of 4.35 ± 0.14. Accessions VI047517-B, VI056563 and VI036227 had the least stem weevil abundance which were not significantly different from the susceptible check. The majority, 55.55% (20), of accessions had significantly higher (F = 8.93; df = 35, 820; $P < 0.001$) abundance of stem weevils compared to the susceptible check.

There was high incidence of damage caused by the amaranth stem weevils across all the accessions averaging 97.20 ± 0.56% during the season. However, there was no significant difference (χ^2 = 31.47; df = 35, 820; P = 0.64) in the incidence of amaranth stem weevil damage which ranged from 79.17 ± 8.47 to 100 ± 0.00 (Table 4.8). The weevils caused damage within amaranth stems with an overall mean of 9.20 ± 0.23 tunnels and ranging between 1.46 ± 0.29 and 17.54 ± 2.94. There were significant differences (F = 10.12; df = 35, 820; $P < 0.001$) in the number of tunnels (severity of weevil damage) with VI054798, VI049530, VI049698, VI044432 and VI054569 recording the highest whereas VI047517-B, VI056563 and VI036227 had the lowest (Table 4.8). There was a significant positive correlation between the number of stem weevils and number of tunnels (r = 0.96; df = 34; $P < 0.001$).

Table 4.8: Comparative stem weevils' incidence, abundance, and damage on various amaranth accessions under field conditions during the short rainy season of 2017, Arusha, Tanzania

Gene bank code	Stem weevil incidence	Relative Risk	Tunneling mines	Relative Risk	Stem weevil abundance	Relative Risk	Stem weevil damage incidence	Relative Risk
VI033482*	70.83 ± 9.48b-e		4.79 ± 0.62lmn		1.42 ± 0.28mno		87.5 ± 6.90a	
RVI00002	100.00 ± 0.00a	11.50	10.00 ± 1.27d-i	2.09	5.75 ± 0.85bef	4.06	100.00 ± 0.00a	4.60
RVI00005	95.83 ± 4.17a	5.50	8.63 ± 0.68e-k	1.80	4.08 ± 0.58f-k	2.88	100.00 ± 0.00a	4.60
RVI00027	100.00 ± 0.00a	11.50	10.17 ± 1.26d-h	2.12	5.88 ± 0.88b-f	4.15	100.00 ± 0.00a	4.60
RVI00053	95.83 ± 4.17a	5.50	9.58 ± 1.50d-j	2.00	4.29 ± 0.80f-j	3.03	100.00 ± 0.00a	4.60
VI033477	87.50 ± 6.90abc	2.50	5.21 ± 0.49k-n	1.09	2.29 ± 0.33j-n	1.62	100.00 ± 0.00a	4.60
VI033479	100.00 ± 0.00a	11.50	9.38 ± 0.99d-k	1.96	4.63 ± 0.60d-i	3.26	100.00 ± 0.00a	4.60
VI033487	95.83 ± 4.17a	5.50	10.79 ± 1.19c-g	2.25	5.33 ± 0.86b-g	3.76	100.00 ± 0.00a	4.60
VI036225	83.33 ± 7.77a-d	1.90	11.71 ± 1.82b-e	2.44	3.33 ± 0.88g-l	2.35	95.83 ± 4.17a	2.20
VI036227	58.33 ± 10.28de	0.59	3.50 ± 0.66mno	0.73	1.38 ± 0.31no	0.97	91.67 ± 5.76a	1.40
VI044367	100.00 ± 0.00a	11.50	11.13 ± 1.02c-f	2.32	5.29 ± 0.59c-g	3.74	100.00 ± 0.00a	4.60
VI044369	100.00 ± 0.00a	11.50	11.54 ± 1.33cde	2.41	6.04 ± 0.96b-f	4.26	100.00 ± 0.00a	4.60
VI044388	87.50 ± 6.90abc	2.50	7.54 ± 0.88e-l	1.57	3.08 ± 0.48g-m	2.18	100.00 ± 0.00a	4.60
VI044432	100.00 ± 0.00a	11.50	16.38 ± 1.84ab	3.42	8.17 ± 1.09ab	5.76	100.00 ± 0.00a	4.60
VI044437-A	91.67 ± 5.76ab	3.50	7.08 ± 0.68f-l	1.48	2.25 ± 0.30k-n	1.59	100.00 ± 0.00a	4.60
VI044473	79.17 ± 8.47a-e	1.50	5.96 ± 0.78j-m	1.24	2.08 ± 0.43l-o	1.47	95.83 ±4.17a	2.20
VI046233-A	91.67 ± 5.76ab	3.50	6.67 ± 1.14g-l	1.39	3.33 ± 0.88g-l	2.35	100.00 ± 0.00a	4.60
VI047517-B	54.17 ± 10.39e	0.50	1.46 ± 0.29o	0.30	0.75 ± 0.18o	0.53	79.17 ± 8.47a	0.60

Table continues next page

Continued from previous page

Gene bank code	Stem weevil incidence	Relative Risk	Tunneling mines	Relative Risk	Stem weevil abundance	Relative Risk	Stem weevil damage incidence	Relative Risk
VI047555-B	87.50 ± 6.9abc	2.50	10.13 ± 1.87d-i	2.11	5.04 ± 1.10c-g	3.56	91.67 ± 5.76a	1.40
VI048076	62.50 ± 10.09cde	0.70	6.50 ± 1.36h-l	1.36	2.25 ± 0.51k-n	1.59	83.33 ± 7.77a	0.76
VI048864-A	100.00 ± 0.00a	11.50	10.33 ± 1.13d-h	2.16	4.50 ± 0.58e-i	3.18	100.00 ± 0.00a	4.60
VI048919	95.83 ± 4.17a	5.50	6.08 ± 0.63i-m	1.27	2.83 ± 0.35h-n	2.00	100.00 ± 0.00a	4.60
VI049242	100.00 ± 0.00a	11.50	9.38 ± 1.07d-k	1.96	4.42 ± 0.59e-i	3.12	100.00 ± 0.00a	4.60
VI049502	100.00 ± 0.00a	11.50	9.50 ± 0.74d-j	1.98	4.58 ± 0.50d-i	3.24	100.00 ± 0.00a	4.60
VI049504	100.00 ± 0.00a	11.50	7.21 ± 0.89f-l	1.50	3.33 ± 0.39g-l	2.35	100.00 ± 0.00a	4.60
VI049530	100.00 ± 0.00a	11.50	13.17 ± 1.33a-d	2.75	7.17 ± 0.97a-d	5.06	100.00 ± 0.00a	4.60
VI049639	95.83 ± 4.17a	5.50	9.88 ± 1.16d-i	2.06	4.83 ± 0.73d-h	3.41	100.00 ± 0.00a	4.60
VI049698	100.00 ± 0.00a	11.50	14.88 ± 1.3abc	3.10	7.67 ± 0.95abc	5.41	100.00 ± 0.00a	4.60
VI050609-A	79.17 ± 8.47a-e	1.50	6.13 ± 0.99i-m	1.28	2.54 ± 0.68i-n	1.79	100.00 ± 0.00a	4.60
VI050609-B	83.33 ± 7.77a-d	1.90	7.21 ± 1.29f-l	1.50	3.38 ± 0.69g-l	2.38	91.67 ± 5.76a	1.40
VI054569	100.00 ± 0.00a	11.50	17.54 ± 2.94a	3.66	9.42 ± 1.89a	6.65	100.00 ± 0.00a	4.60
VI054798	100.00 ± 0.00a	11.50	12.88 ± 1.29a-d	2.69	6.92 ± 1.17a-e	4.88	100.00 ± 0.00a	4.60
VI055127	100.00 ± 0.00a	11.50	12.38 ± 1.23b-e	2.58	6.08 ± 0.85b-f	4.29	100.00 ± 0.00a	4.60
VI055128	100.00 ± 0.00a	7.50	12.56 ± 1.52a-e	2.62	5.63 ± 0.64b-g	3.97	100.00 ± 0.00a	3.00
VI055135	100.00 ± 0.00a	11.50	12.00 ± 0.81b-e	2.50	5.83 ± 0.43b-f	4.12	100.00 ± 0.00a	4.60
VI056563	62.50 ± 10.09cde	0.70	3.21 ± 0.79no	0.67	1.33 ± 0.33no	0.94	83.33 ± 7.77a	0.76

*Susceptible check. Mean ± SE followed by the same letter within a column are not significantly different at P<0.05 (Tukey's test)

4.3 Assessing the possible mechanisms underlying resistance in amaranth accessions through their effects on the biology of leaf-webbers infesting amaranths

4.3.1 Oviposition by *Spoladea recurvalis* in choice test

In oviposition choice tests, there were significant differences in the number of eggs oviposited by *S. recurvalis* on the tested accessions in set-up A (comprising 17 accessions) (χ^2 = 284.03; df = 17, 85; $P < 0.001$) and set-up B (χ^2 = 1056.40; df = 18, 93; $P < 0.001$) (Table 4.9). In set-up A, the susceptible accession had significantly higher number of eggs compared to all the other 17 accessions. The lowest number of eggs were recorded on accessions VI044432 and VI054569, which had a relative risk (RR) of 0.06 compared to the susceptible accession. In set-up B (comprising 18 accessions), the accession VI048919 (RR = 1.39) recorded significantly higher number of eggs compared to the susceptible accession, while accession VI050609-B (RR = 1.20) did not differ significantly from the susceptible accession in the number of eggs laid by *S. recurvalis*. The remaining 16 accessions had significantly lower number of eggs laid on them compared to the susceptible accession. The average number of eggs laid by *S. recurvalis* across all the accessions in both set-ups A and B was 7.80 ± 0.85 and ranged between 1.50 ± 0.56 in the least preferred accession to 40.20 ± 16.41 in the most preferred accession (Table 4.9).

Table 4.9: Mean number of eggs ± SE laid by *Spoladea recurvalis* on amaranth accessions in the choice tests

Set-up A			Set-up B		
Gene Bank Code	**Number of Eggs**	**Relative Risk**	**Gene Bank Code**	**Number of Eggs**	**Relative Risk**
VI033482 *	25.67 ± 7.62a		VI033482*	29.00 ± 7.95b	
VI033487	11.33 ± 4.57b	0.44	VI048919	40.20 ± 16.41a	1.39
VI044388	10.00 ± 2.92bc	0.39	VI050609-B	34.67 ± 9.47ab	1.2
VI036227	8.83 ± 3.93bcd	0.34	VI033477	15.00 ± 4.87c	0.52
RVI00027	7.33 ± 2.85be	0.29	VI049504	11.40 ± 6.21cd	0.39
RVI00005	6.17 ± 2.30cf	0.24	VI047517-B	9.83 ± 4.48cde	0.34
VI048076	6.17 ± 1.64cf	0.24	VI056563	9.83 ± 3.51cde	0.34
VI049639	4.33 ± 1.74def	0.17	VI055127	6.00 ± 3.04def	0.21
RVI00002	4.00 ± 2.08ef	0.16	VI046233-A	6.17 ± 3.82def	0.21
RVI00053	3.83 ± 1.70ef	0.15	VI049530	6.67 ± 3.48def	0.23
VI036225	3.67 ± 2.01ef	0.14	VI050609-A	6.67 ± 2.89def	0.23
VI044473	3.33 ± 1.54ef	0.13	VI047555-B	3.67 ± 1.87ef	0.13
VI044369	3.20 ± 2.03ef	0.12	VI055128	3.50 ± 2.05ef	0.12
VI044367	2.83 ± 0.83ef	0.11	VI054798	2.83 ± 2.46f	0.1
VI044437-A	2.40 ± 1.03ef	0.09	VI055135	2.50 ± 0.92f	0.09
VI049698	2.33 ± 0.56f	0.09	VI048864-A	2.33 ± 1.76f	0.08
VI044432	1.50 ± 0.56f	0.06	VI033479	2.17 ± 1.78f	0.07
VI054569	1.50 ± 0.85f	0.06	VI049242	2.17 ± 1.17f	0.07
			VI049502	1.50 ± 0.81f	0.05

* Susceptible accession. Means followed by same letter within a column are not significantly different at $P < 0.05$ (Tukey's test).

4.3.2 Oviposition by *Spoladea recurvalis* in no-choice test

In the no-choice test, where eight amaranth accessions were compared to the susceptible one, *S. recurvalis* laid significantly more eggs on the susceptible accession compared to the other accessions (χ^2 = 192.75; df = 7, 37; $P < 0.001$) (Table 4.10). The accession VI048076 recorded the least number of eggs (18.50 ± 6.63) (RR = 0.31) compared to the susceptible accession and also had significantly fewer eggs than all the other seven accessions. The accessions VI044437-A (RR = 0.49), VI047555-B (RR = 0.49) and RVI00053 (RR = 0.50) had fewer eggs compared to VI049698 (RR = 0.72). Accession VI036227 (RR = 0.67) had a higher number of eggs compared to accessions VI048076 and VI044437-A, but did not differ significantly from accessions RVI00053, VI047555-B, VI049698 and VI056563. The number of eggs laid by *S. recurvalis* in the no-choice assay (18.5 – 59.63) was significantly higher (χ^2 =1305.10; df = 1; $P < 0.001$) than those laid in the choice situation (2.33 – 21.58).

Table 4.10: Number of eggs (mean ± SE) laid by *S. recurvalis* on selected amaranth accessions in the no-choice and choice tests

Gene Bank Code	No Choice test	Relative Risk	Choice test	Relative Risk	χ^2	df	*p* Value
VI033482*	59.63 ± 10.49aA	-	21.58 ± 5.30aB	-	127.96	1	<0.001
RVI00053	30.00 ± 6.52cdA	0.50	3.83 ± 1.70cdB	0.18	183.54	1	<0.001
VI036227	40.20 ± 10.24bcA	0.67	8.83 ± 3.93bB	0.41	170.15	1	<0.001
VI044437-A	29.33 ± 10.24dA	0.49	2.40 ± 1.03cdB	0.11	282.65	1	<0.001
VI047555-B	29.40 ± 10.27cdA	0.49	3.67 ± 1.87cdB	0.17	181.96	1	<0.001
VI048076	18.50 ± 6.63eA	0.31	6.17 ± 1.64bcB	0.29	72.01	1	<0.001
VI049698	42.80 ± 18.14bA	0.72	2.33 ± 0.56dB	0.11	347.38	1	<0.001
VI056563	37.20 ± 5.54bcdA	0.62	9.83 ± 3.51bB	0.46	129.6	1	<0.001
	χ^2 = 192.75		χ^2 = 281.29				
	df = 7, 37		df = 7, 45				
	$P < 0.001$		$P < 0.001$				

*Susceptible accession. Means followed by the same upper-case letters within rows or lower-case letter within columns are not significantly different at $P < 0.05$ using Tukey's test (chi-square test).

4.3.3 Weight gain by larvae of *Spoladea recurvalis* after 48 h of feeding on the selected amaranth accessions

The weight gained in milligrams (mg) by larvae of *S. recurvalis* after feeding on the eight selected amaranth accessions for 48 h differed significantly (F = 6.13; df = 8, 99; $P < 0.001$) with accession VI036227 producing the lowest weight gain. Accessions RVI00053, VI033479, VI044437-A, VI047555-B, VI049698 and VI056563 were comparable with the susceptible accession in weight gain but were significantly higher than accession VI036227 (Figure 4.3). The average weight gain and percentage weight gain by *S. recurvalis* was 8.11 ± 0.68 mg and 181.0 ± 14.86%, respectively.

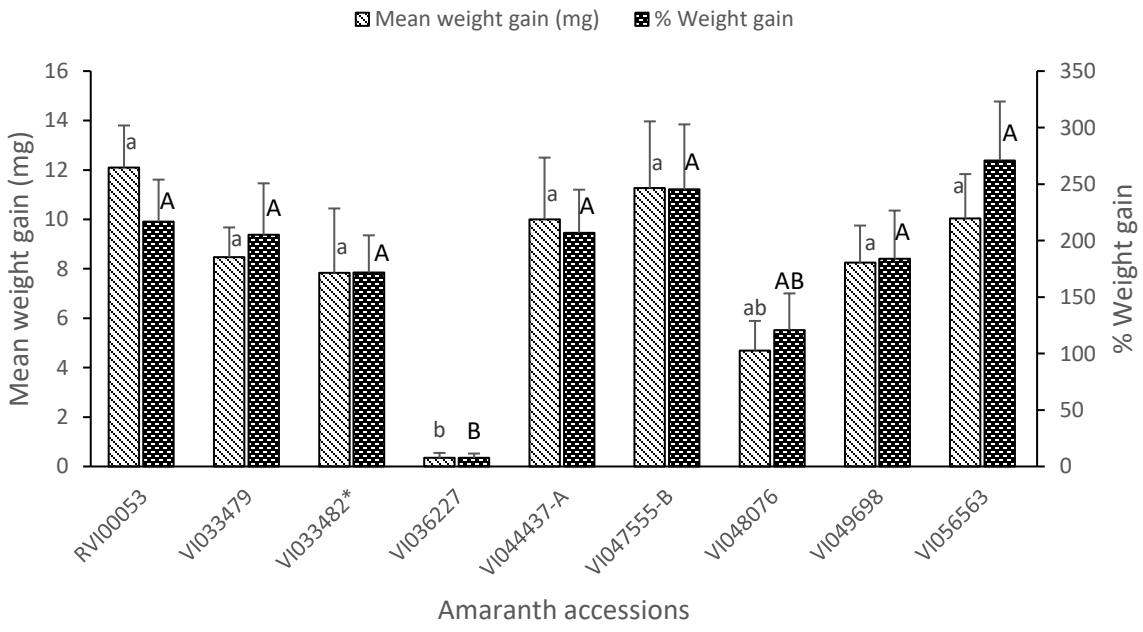

Figure 4.3: Weight gain (mg) and percentage weight gain (mean ± SE) by larvae of *Spoladea recurvalis* when fed on different amaranth accessions for 48 h.

4.3.4 Development time of *Spoladea recurvalis* on the selected amaranth accessions

The average larval development time was 13.5 ± 0.12 days across the eight selected accessions and ranged between 13.23 ± 0.3 and 14.0 ± 0.56 days on VI049698 and VI056563, respectively. Larval development on accession VI036227 did not advance beyond two days after exposure to the accession and hence development time on this accession could not be determined. When presented with leaves from accession VI026227, the larvae of *S. recurvalis* gnawed only a small portion of the leaf and in most cases did not even attempt to feed on the leaves. There were no significant differences in the larval (χ^2 = 1.07; df = 7, 228; P = 0.994), pupal (χ^2 = 3.35; df = 7, 112; P = 0.851), and total (χ^2 = 1.04; df = 7, 112; P = 0.994) development times of *S. recurvalis* across the tested accessions (Table 4.11). The mean pupal development time across all the accessions was 6.36 ± 0.13 days and ranged between 5.86 ± 0.17 and 7.45 ± 0.69 days on VI044437-A and VI033482, respectively. The mean total development time of *S. recurvalis* was 19.09 ± 0.15 days across the tested accessions, ranging between 18.60 ± 0.45 and 20.0 ± 0.80 days.

Table 4.11: Developmental time (mean ± SE) (days) and larval mortality of *Spoladea recurvalis* fed on selected amaranth accessions

Gene Bank Code	Larval development time	Pupal development time	Total development time	Early stage larval mortality (%)
VI033482 *	13.43 ± 0.30a	7.45 ± 0.69a	20.00 ± 0.8a	8.0 ± 3.27bc
RVI00053	13.45 ± 0.37a	6.60 ± 0.34a	18.60 ± 0.45a	4.0 ± 0.51c
VI033479	13.24 ± 0.23a	6.11 ± 0.35a	18.67 ± 0.44a	8.0 ± 3.27bc
VI036227	NA	NA	NA	100.0 ± 0.0a
VI044437-A	13.29 ± 0.29a	5.86 ± 0.17a	18.82 ± 0.28a	14.0 ± 5.21bc
VI047555-B	13.77 ± 0.36a	6.16 ± 0.21a	19.11 ± 0.24a	24.0 ± 6.53bc
VI048076	13.77 ± 0.43a	6.20 ± 0.43a	19.00 ± 0.45a	22.0 ± 7.57bc
VI049698	13.23 ± 0.30a	6.43 ± 0.39a	19.24 ± 0.36a	12.0 ± 6.11bc
VI056563	14.00 ± 0.56a	6.63 ± 0.42a	19.75 ± 0.53a	34.0 ± 11.57b
	$\chi^2 = 1.066$	$\chi^2 = 3.348$	$\chi^2 = 1.042$	$F = 12.22$
	df = 7, 228	df = 7, 112	df = 7, 112	df = 8, 81
	$P = 0.994$	$P = 0.851$	$P = 0.994$	$P < 0.001$

*Susceptible accession. Means followed by same letter within a column are not significantly different at $P < 0.05$ (Tukey's test).

4.3.5 Mortality rates and instantaneous rate of increase of *Spoladea recurvalis* on selected amaranth accessions

Early stage larval mortality, within the first 36 h, was observed in all the accessions with an overall mean mortality of 25.11 ± 4.56% and ranged between 4.0 ± 0.51 and 100.0 ± 0.0% across the accessions. There were significant differences ($F = 12.22$; df = 8, 81; $P < 0.001$) in early stage larval mortalities that occurred on the different accessions with VI036227 leading to significantly higher

mortalities compared to all the other accessions including the susceptible accession (Table 4.11). The accession VI056563 led to significantly higher early stage mortality compared to RVI00053. The lowest early stage mortalities were recorded on accessions RVI00053, VI033479 and the susceptible check VI033482. The total larval mortalities across the different accessions ranged between 24.0 ± 4.99% and 100 ± 0.0% with an average of 47.56 ± 3.19% (Figure 4.4). There were significant differences (F = 5.91; df = 8, 81; P < 0.001) in the larval mortalities when *S. recurvalis* was fed on different amaranth accessions. The accession VI036227 led to 100 ± 0.0 %, larval mortality, which was significantly higher than larval mortality in all the other accessions except VI056563, which led to 64.00 ± 9.8% larval mortality. The lowest larval mortality was recorded on accession RVI00053, which had a mean mortality of 24.0 ± 4.99% (Figure 4.4). There was a significant negative linear correlation (r = −0.428; P < 0.001) between the number of days before larval mortality occurred and the rate of larval mortality.

Pupal mortality also differed significantly (F = 2.92; df = 7, 68; P = 0.01) among the accessions tested. Accession VI044437-A had significantly lower pupal mortalities than RVI00053, VI033479 and the susceptible accession VI033482 (Figure 4.4). The average pupal mortality was 50.15 ± 4.03%. There was no correlation between larval and pupal mortalities (r = 0.064; P = 0.58).

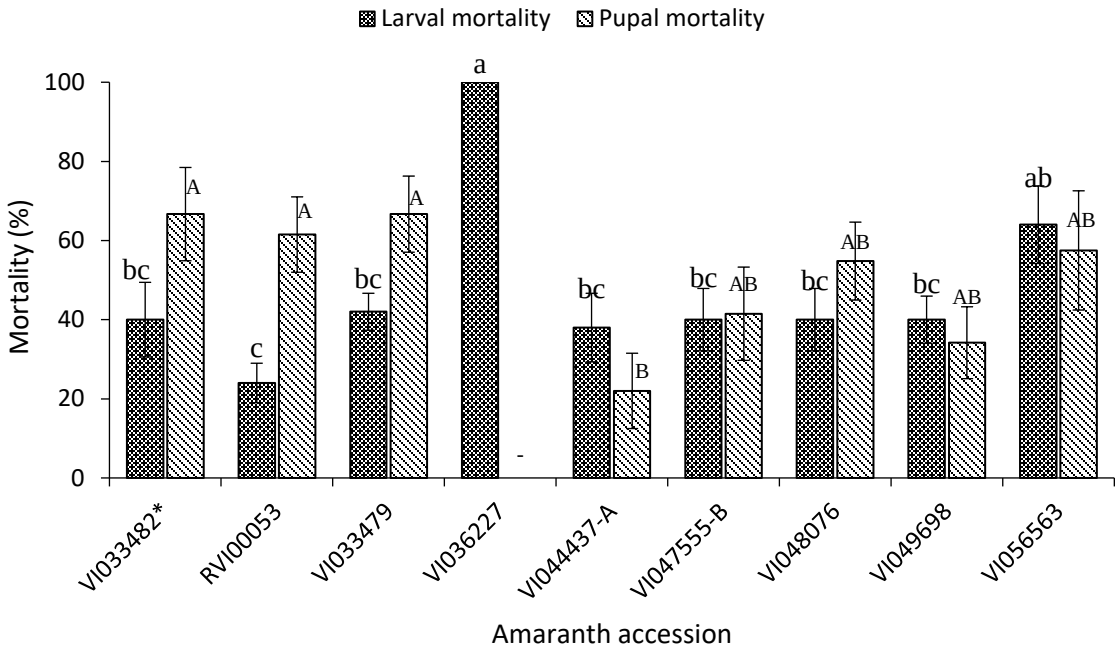

Figure 4.4: Total larval and pupal mortalities (mean ± SE) of *Spoladea recurvalis* recorded on selected accessions of amaranth. Bars indicating larval and pupal mortalities with similar small letters and capital letters, respectively, do not differs significantly at P < 0.05 (Tukey's test).

The instantaneous rate of increase (r_i) when the larvae of *S. recurvalis* were exposed to the different amaranth accessions were negative (Figure 4.5). The r_i also did not differ significantly among all the tested accessions.

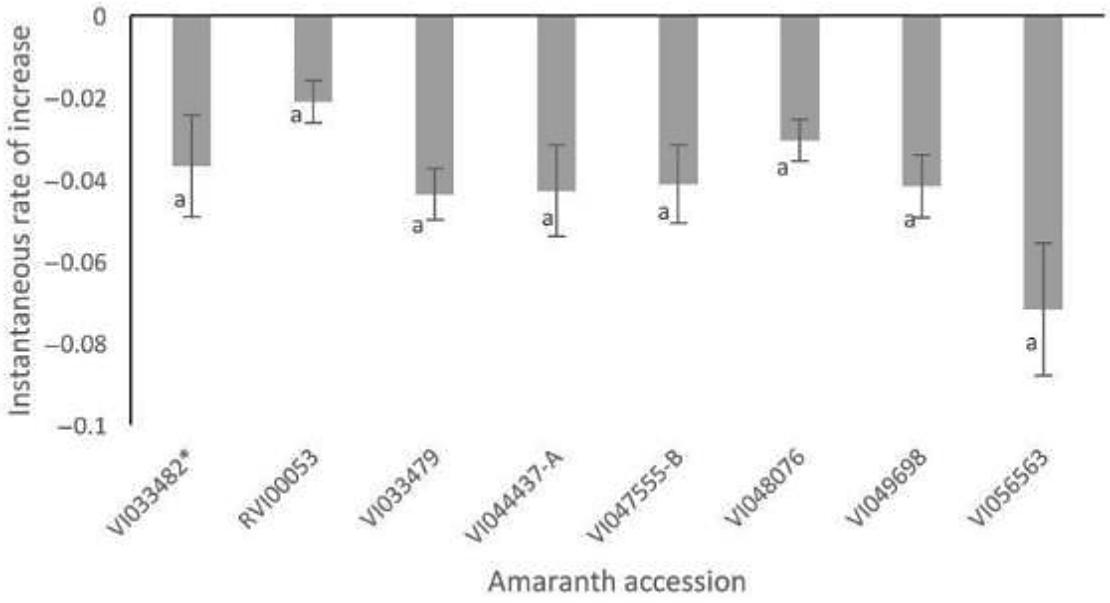

Figure 4.5: The mean instantaneous rate of population increase (r_i) among larvae of *Spoladea recurvalis* when exposed to different amaranth accessions. Positive values of r_i indicate a growing population, $r_i = 0$ indicates a stable population, and negative r_i values indicate a population in decline and headed toward extinction

4.3.6 Adult longevity, fecundity, egg viability and sex ratios of *Spoladea recurvalis*

There were significant differences in the adult longevity among the amaranth accessions (χ^2 = 92.51; df = 7, 380; P < 0.001) with accession VI047555-B producing adults with the shortest longevity (8.7 ± 0.61 days), whereas adults from accession VI048076 had the highest longevity (14.25 ± 0.82 days) (Table 4.12). Adults obtained from accessions VI056563 and VI048076 had significantly higher longevity compared to adults obtained from the susceptible accession VI033482 and the accessions VI047555-B, VI044437-A and VI033479. Accession

VI047555-B also produced adults that had a shorter longevity compared to those from accessions VI044437-A, VI033479, RVI00053, and VI049698. The viability of eggs laid by F_1 females of *S. recurvalis* that were reared on the different amaranth accessions did not differ significantly (F = 0.89; df = 7, 32; P = 0.527). Fecundity of the F_1 females obtained from the various amaranth accessions differed significantly (F = 6.07; df = 7,14; P = 0.002) with accessions VI049698 and the susceptible accession VI033482 leading to the production of more eggs compared to accessions VI033479, VI044437-A and VI048076 (Table 4.12). There was no significant difference (F = 0.74; df = 7, 25; P = 0.638) in the proportions of F_1 females obtained from the amaranth accessions tested.

Table 4.12: *Spoladea recurvalis* adult longevity, egg viability (%), fecundity and proportion of F_1 females (mean ± SE) reared on selected amaranth accessions

Gene Bank Code	Adult longevity (days)	Odds ratio	Egg viability (%)	Fecundity/female at 4-d-old	Proportion of females (%)
VI047555-B	8.70 ± 0.61d	0.9	91.25 ± 1.93a	13.06 ± 1.20ab	51.79 ± 7.03a
VI033482*	9.69 ± 0.85cd		95.49 ± 1.50a	22.67 ± 3.67a	59.17 ± 8.86a
VI044437-A	10.85 ± 0.52c	1.12	97.79 ± 3.13a	11.09 ± 0.80b	44.97 ± 8.01a
VI033479	10.85 ± 0.85c	1.12	97.62 ± 1.61a	10.97 ± 1.71b	45.49 ± 4.51a
RVI00053	11.00 ± 0.79bc	1.14	97.53 ± 1.51a	13.40 ± 1.74ab	66.91 ± 8.09a
VI049698	11.22 ± 0.49bc	1.16	96.25 ± 2.33a	23.75 ± 3.50a	46.03 ± 6.31a
VI056563	12.63 ± 0.82ab	1.3	95.90 ± 4.10a	14.67 ± 5.67ab	49.77 ± 5.83a
VI048076	14.25 ± 0.82a	1.47	94.66 ± 2.59a	8.50 ± 0.74b	56.76 ± 13.44a
	$P < 0.001$		$P = 0.527$	$P = 0.002$	$P = 0.638$
	df = 7, 380		df = 7, 32	df = 7,14	df = 7,25
	$\chi^2 = 92.51$		$F = 0.89$	$F = 6.07$	$F = 0.74$

*Susceptible accession. Means followed by the same letter within a column are not significantly different at $P < 0.05$ (Tukey's test).

4.4 Evaluating selected pest resistant amaranth accessions for water stress tolerance

4.4.1 Effect of water stress on phenological parameters of amaranth

There were significant interactions between soil water level and amaranth accessions for the plant height (F = 16.93; df = 14,24; P < 0.001), number of leaves (F = 4.20; df = 14,24; P < 0.001), branches (F = 7.11; df = 14,24; P < 0.001) and leaf area (F = 5.77; df = 14,24; P < 0.001). Accession VI033482 did not show significant difference in plant height at 40, 60 and 90% water holding capacity (WHC), and was significantly shorter than all the other accessions irrespective of the WHC (Figure 4.6). Whereas accessions VI033479, VI048076, and VI056563 differed in height between 40% and 60% WHC, no significant difference was observed between 60 and 90% WHC. Accessions RVI00053, VI044437-A and VI047555-B were significantly taller in the control group (90% WHC) than either at 40 or 60% treatments. The control (90% WHC) had significantly taller plants compared to the severely stressed plants (40% WHC) in all accessions except VI033482 and VI049698.

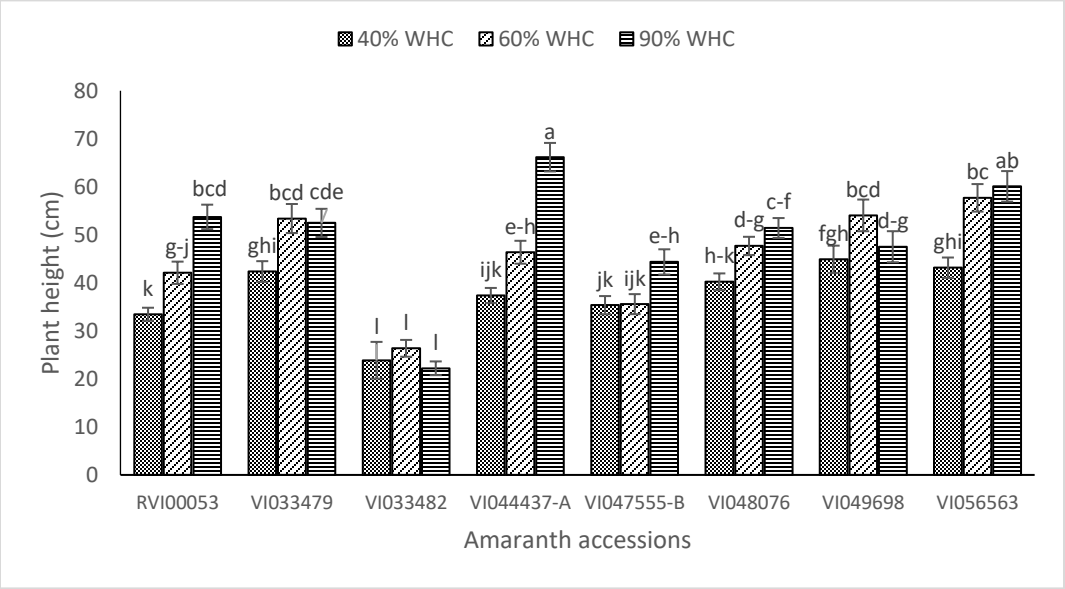

Figure 4.6: Mean (±SE) plant height of amaranth accessions when subjected to three soil water levels (40, 60 and 90% water holding capacity (WHC)). Means with the same letter labels are not significantly different at *P* < 0.05 (Tukey's test).

There was no significant effect of soil water level on the number of leaves per plant in all the accessions except VI044437-A and VI048076 in which the controls had more leaves than the 40 and 60% WHC treatments (Figure 4.7). Irrespective of the water treatment, accession VI044437-A had significantly higher number of leaves compared to VI033482, VI049698 and VI056563. The control treatment of accession VI044437-A produced more leaves compared to all other accessions and treatments.

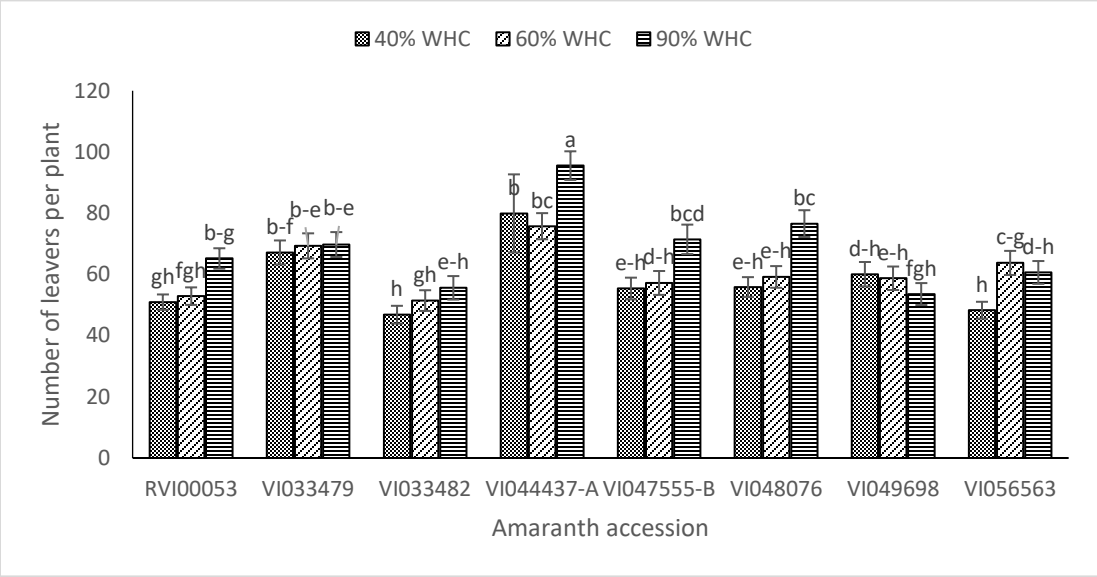

Figure 4.7: Mean (±SE) number of leaves per plant on amaranth accessions at different soil water levels (40, 60 and 90% water holding capacity (WHC)). Means with the same letter labels are not significantly different at *P* < 0.05 (Tukey's test).

The number of branches on individual plants of accessions VI033479, VI033482, VI047555-B, VI049698 and VI056563 did not differ significantly across the three water treatments (Figure 4.8). At 90% WHC, accession RVI00053 had significantly higher number of branches compared to 40% WHC. All the water treatments on accession VI033479 had significantly higher number of branches compared to VI033482 irrespective of water treatment and more branches than RVI00053, VI047555-B, VI048076 at 40% and 60% WHC. Both 60% and 90% WHC treatments on accession VI044437-A produced significantly higher number of branches compared to all other accessions irrespective of the water treatment they received.

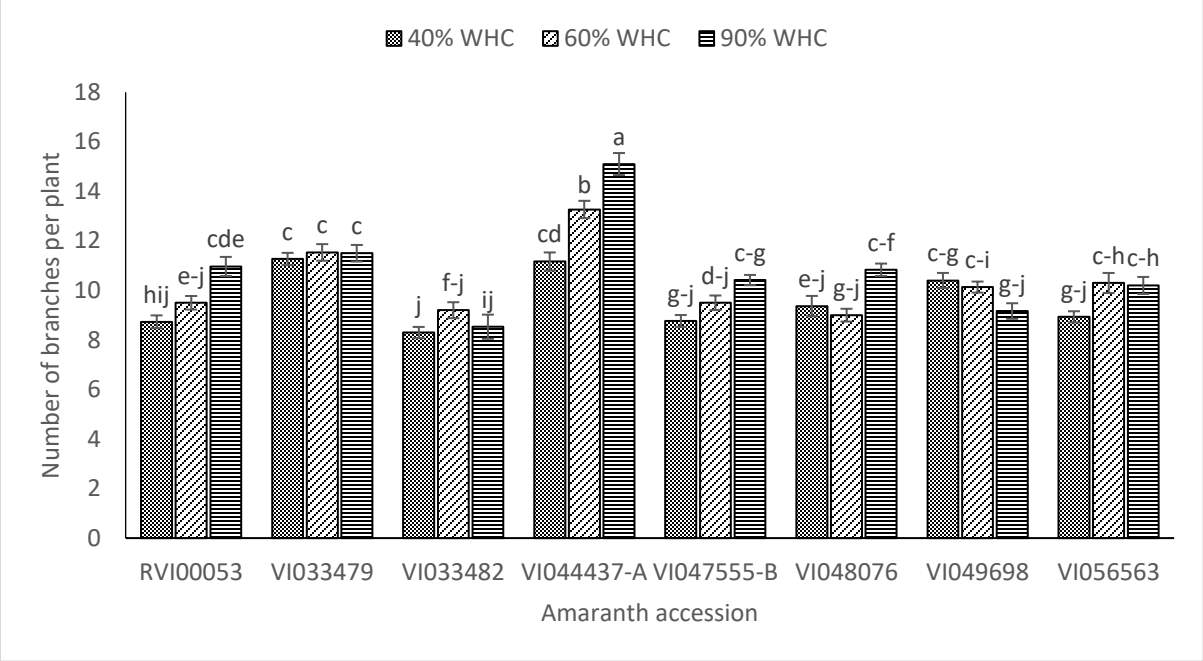

Figure 4.8: Mean (±SE) number of branches on amaranth accessions at different soil water levels (40%, 60% and 90% water holding capacity (WHC)). Means with the same letter labels are not significantly different at $P < 0.05$ (Tukey's test).

The relative growth rate (RGR) of accessions VI033479, VI049698 and VI056563 were not significantly affected by the three soil water levels (Figure 4.9). The control treatments in accessions RVI00053, VI044437-A, VI047555-B and VI048076 had significantly higher RGR compared to either 40% or 60% WHC treatments which did not differ significantly within these accessions. At 40% WHC, accession VI047555-B had significantly higher RGR than VI033482, VI044437-A, VI048076 and VI0049698. The RGR did not differ significantly between 40% and 60% WHC treatments for each accession. The control of VI047555-B had a higher RGR compared to all other accessions irrespective of

water treatment except the control of RVI00053 and 60% WHC treatment of VI033479. The least RGR was recorded in the 40% WHC treatment of VI044437-A.

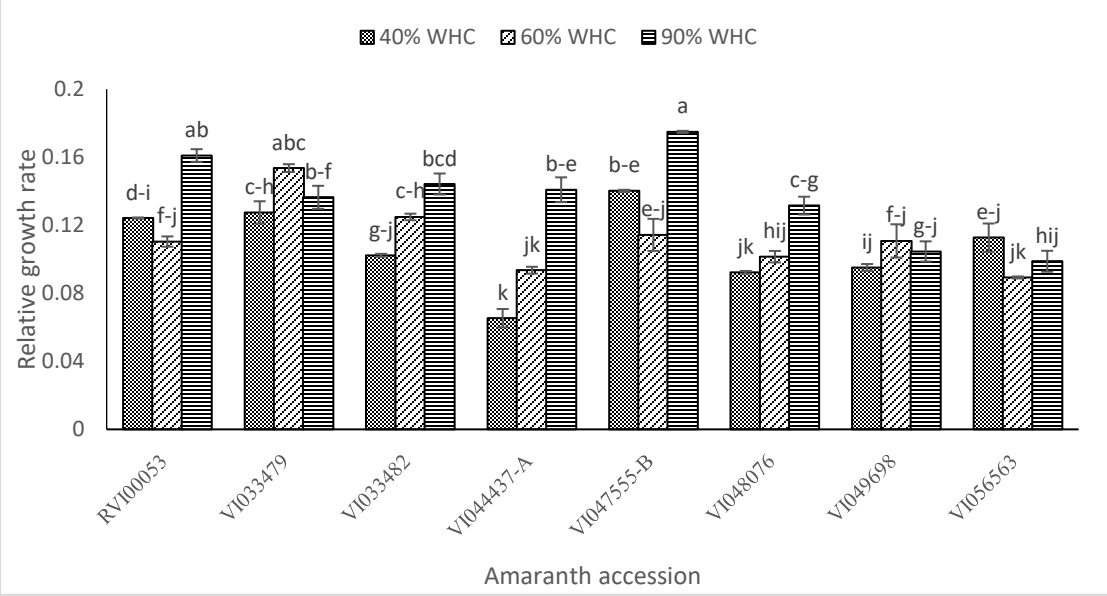

Figure 4.9: Relative growth rate of amaranth accessions at three soil water levels (40, 60 and 90% water holding capacity (WHC)). Means with the same letter labels are not significantly different at *P* < 0.05 (Tukey's test).

Leaf area of the accessions RVI00053, VI033479, VI044437-A, VI048076, VI049698 and VI056563 were not significantly affected by water treatments (Figure 4.10). VI033482 had significantly larger leaves at 40% WHC than at both 60 and 90% WHC. Furthermore, the leaf area of VI033482 at 40% WHC was significantly larger than that of all other accessions irrespective of water treatment. The leaf area of VI047555-B was significantly larger at 60% WHC than in the

control. The leaf area of VI049698 at 40 and 60% WHC was comparable to that of VI033479 at 40, 60 and 90% WHC, however, the former accession at 90% WHC was significantly smaller than the latter at 40% WHC.

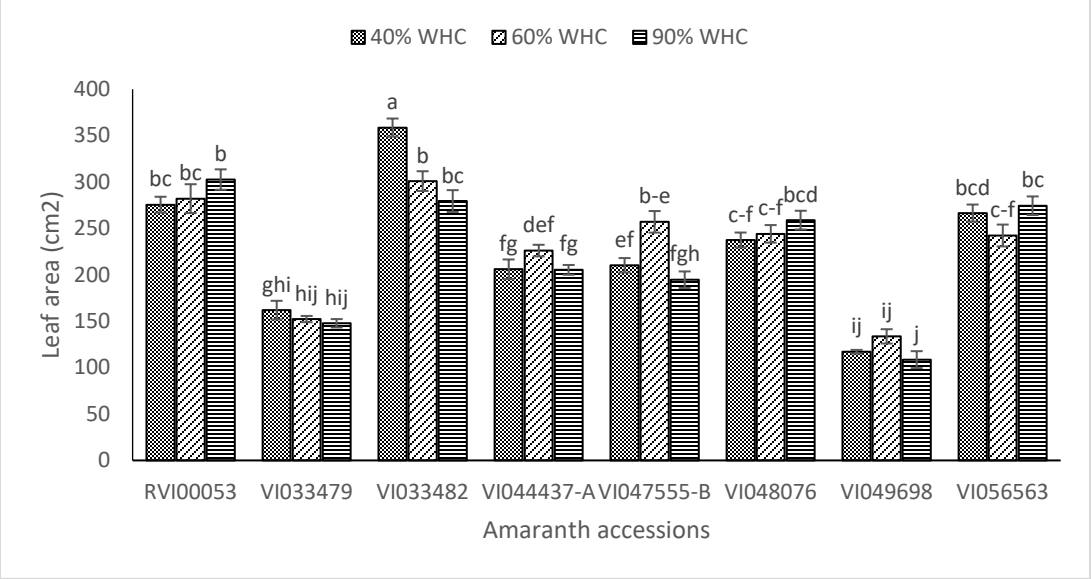

Figure 4.10: Leaf area in cm^2 (mean ± SE) of amaranth accessions at different soil water levels (40, 60 and 90% water holding capacity (WHC)). Means with the same letter labels are not significantly different at *P* < 0.05 (Tukey's test).

The total dry mass of all accessions except RVI00053 and VI044437-A were not significantly affected by soil water level (Figure 4.11). The total dry mass of VI044437-A in the control was significantly greater than all the other accessions irrespective of water treatment except in the controls of RVI00053 and VI048076.

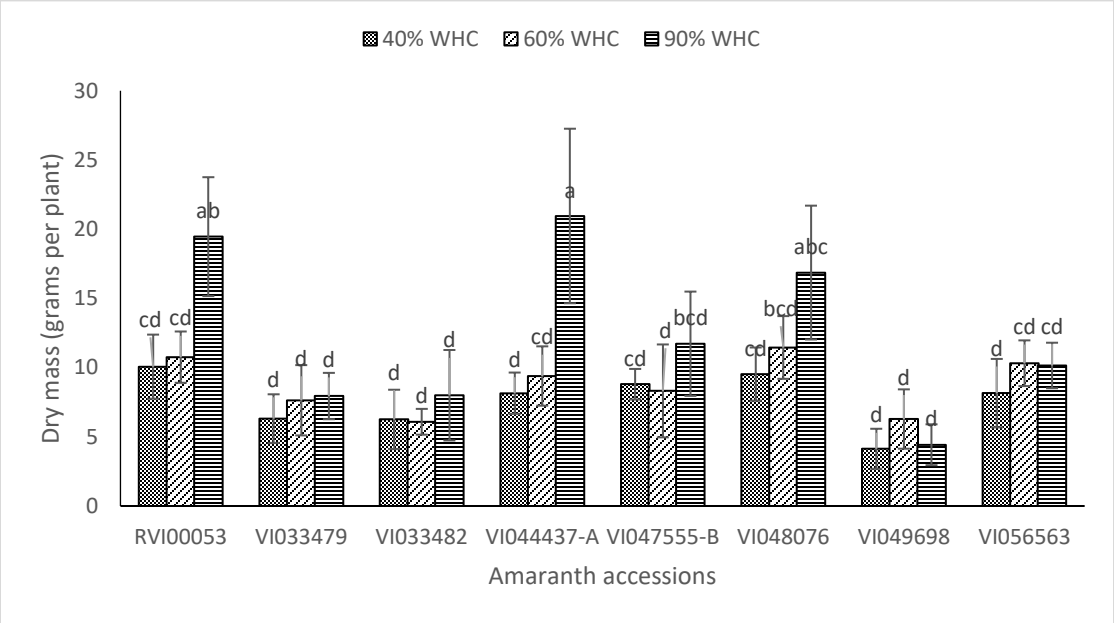

Figure 4.11: Mean (±SE) dry mass of amaranth accessions at three soil water levels (40, 60 and 90% water holding capacity (WHC)). Means with the same letter labels are not significantly different at $P < 0.05$ (Tukey's test).

4.4.2 Effect of water stress on biomass partitioning in amaranth accessions

There was a significant effect of accession (F = 17.30; df = 7,71; P < 0.001) and soil water level (F = 5.97; df = 2,71; P = 0.004) on leaf weight ratio (LWR) (Table 4.13). The LWR was significantly lower in accession VI048076 compared to RVI00053, VI033479, VI033482 and VI049698. Accession VI033482 had the highest LWR and was significantly different from all the tested accessions. The LWR was significantly higher in the control compared to the 60% WHC treatment but was comparable to the 40% WHC treatment. The root to shoot ratio (RSR) was not significantly affected by accession (F = 2.06; df = 7,71; P = 0.059) but there was significant effect of soil water level on RSR (F = 6.73; df =

2,71; P = 0.002). The RSR was significantly lower in the controls compared to either 60% or 40% WHC treatments.

The leaf area ratio (LAR) was significantly affected by amaranth accessions (F = 7.46; df = 7,71; P < 0.001) but not water treatments (F = 0.75; df = 2,71; P = 0.476) (Table 4.13). Accession VI033482 had the highest LAR and was significantly greater than all other accessions except VI049698. Accessions VI044437-A, RVI00053 and VI048076 had significantly lower LAR compared to VI049698. The root mass ratio (RMR) was significantly affected by both accession (F = 2.21; df = 7,71; P = 0.044) and water treatment (F = 7.31; df = 2,71; P = 0.001). The RMR of accession VI044437-A was significantly higher than that of VI033482, however, both accessions differed significantly from the other six in RMR. Specific leaf area (SLA) was only affected by accession (F = 8.01; df = 7,71; P < 0.001) but not water treatments (F = 1.79; df = 2,71; P = 0.170) (Table 4.13). VI033482 had significantly higher SLA compared to all the other accessions except VI049698 and VI056563.

Table 4.13: Leaf weight ratio, Root to shoot ratio, Leaf area ratio, Root mass ratio and Specific leaf area of selected amaranth accessions at different soil water levels

Amaranth accession	Leaf weight ratio	Root to shoot ratio	Leaf area ratio	Root mass ratio	Specific leaf area
RVI00053	0.66±0.02bc	0.15±0.01a	14.36±1.61c	0.13±0.01ab	19.23±1.23b
VI033479	0.68±0.02b	0.14±0.01a	17.37±1.96bc	0.12±0.01ab	18.06±1.85b
VI033482	0.78±0.01a	0.12±0.01a	35.95±4.66a	0.11±0.01b	34.83±3.17a
VI044437-A	0.62±0.01bcd	0.18±0.03a	13.04±1.83c	0.15±0.02a	17.27±1.43b
VI047555-B	0.63±0.02bcd	0.15±0.01a	17.90±1.97bc	0.13±0.01ab	23.02±1.71b
VI048076	0.58±0.03d	0.14±0.01a	15.39±2.10c	0.12±0.01ab	21.56±1.89b
VI049698	0.66±0.02bc	0.17±0.01a	29.68±6.62ab	0.14±0.01ab	25.55±4.60ab
VI056563	0.60±0.03cd	0.16±0.01a	20.63±2.88bc	0.14±0.01ab	25.87±2.40ab
Soil water level (SWL)					
40% WHC	0.65±0.02ab	0.16±0.01a	22.12±2.76a	0.13±0.01a	25.01±1.80a
60% WHC	0.63±0.02b	0.17±0.01a	18.98±1.73a	0.14±0.01a	22.07±1.35a
90% WHC	0.67±0.02a	0.13±0.01b	20.52±2.65a	0.11±0.00b	22.41±1.75a
Significance					
Accession×SWL	$P = 0.344$	$P = 0.102$	$P = 0.661$	$P = 0.113$	$P = 0.702$
Accession	$P < 0.001$	$P = 0.059$	$P < 0.001$	$P = 0.044$	$P < 0.001$
SWL	$P = 0.004$	$P = 0.002$	$P = 0.476$	$P = 0.001$	$P = 0.170$

Means followed by the same letter(s) within a column are not significantly different at $P < 0.05$ (Tukey's test).

4.5 Assessing the effects of selected pest resistant amaranth accessions on the performance of *Apanteles hemara*

4.5.1 Parasitism rate and developmental time of *Apanteles hemara* on *Spoladea recurvalis* fed on different amaranth accessions

The parasitism rate of *A. hemara* on *S. recurvalis* raised on the different amaranth accessions ranged between 34.3 ± 11.5 and 91.7 ± 6.5% across the accessions. There were significant differences (F = 5.4; df = 7, 44; $P < 0.001$) in the levels of parasitism across the accessions, with accession VI056563 recording lower parasitism rate of 34.3 ± 11.5 compared to RVI00053, VI044437-A, VI047555-B, VI048076, VI033479, VI049698 and the susceptible accession VI033482 (Table 4.14). Parasitism rates did not differ significantly between the susceptible check and all the other moderately resistant accessions.

Egg and larval developmental time (from the time the parasitoid lays an egg into the host larva until it pupates) of *A. hemara* within the larvae of *S. recurvalis* differed significantly (F = 3.2; df = 7, 52; P = 0.007) among the resistant amaranth accessions with accession RVI00053 having shorter developmental time compared to VI044437-A. The parasitoid's egg and larval development time did not differ significantly between the resistant accessions compared to the susceptible check (Table 4.14). Similarly, the pupal development time differed significantly (F = 2.0; df = 7, 46; P = 0.042) among the resistant amaranth accessions with accession RVI00053 recording shorter pupal development time of 3.4 ± 0.4 days compared to VI033479 with 4.7 ± 0.2 days. The pupal development time did not differ significantly between the resistant

accessions and the susceptible check. The parasitoid's total developmental time was significantly shorter (F = 3.8; df = 7, 44; P = 0.002) on accession RVI00053 compared to accessions VI033479, VI044437-A, VI048076 and VI049698. The total development time also did not differ significantly between the resistant accessions and the susceptible check.

Table 4.14: Parasitism rates and developmental time (mean ± SE) of *Apanteles hemara* on moderately resistant amaranth accessions

		Parasitiod development		
Amaranth accession code	**Parasitism rate (%)**	**Larval period (days)**	**Pupal period (days)**	**Total development time (days)**
VI033482*	76.0 ± 9.8a	7.2 ± 0.3ab	3.9 ± 0.3ab	11.1 ± 0.5ab
RVI00053	83.2 ± 7.7a	6.0 ± 0.5b	3.4 ± 0.4b	9.4 ± 0.7b
VI033479	87.5 ± 4.9a	7.2 ± 0.4ab	4.7 ± 0.2a	11.9 ± 0.2a
VI044437-A	81.9 ± 6.8a	7.9 ± 0.2a	4.2 ± 0.2ab	11.9 ± 0.3b
VI047555-B	91.7 ± 6.5a	7.4 ± 0.3ab	4.0 ± 0.3ab	11.3 ± 0.2ab
VI048076	91.4 ± 4.0a	6.7 ± 0.3ab	4.3 ± 0.2ab	11.4 ± 0.3b
VI049698	85.5 ± 6.0a	7.0 ± 0.4ab	4.3 ± 0.3ab	11.3 ± 0.4b
VI056563	34.3 ± 11.5b	6.5 ± 0.4ab	4.7 ± 0.3ab	10.8 ± 0.2ab
P-value	< 0.001	0.007	0.042	0.002
F value	5.4	3.2	2.0	3.8
df	7,44	7,52	7,46	7,44

*Susceptible accession. Means followed by the same letter(s) within a column are not significantly different at $P < 0.05$ (Tukey's test).

4.5.2 Sex ratio of *Apanteles hemara* attacking *Spoladea recurvalis* fed on different accessions of amaranth

There were significant differences (F = 2.5; df = 6, 71; P = 0.031) in the proportion of females among the F_1 parasitoids obtained from the various amaranth accessions tested. The accessions RVI00053 and VI048076 had significantly higher proportions of female parasitoids compared to the susceptible check VI033482, whereas accessions VI033479, VI044437-A, VI047555-B and VI049698 did not show significant differences in the proportion of F_1 females compared to the susceptible check (Figure 4.12). There were no significant differences in the female to male sex ratios among the F_1 parasitoids obtained from accessions RVI00053 (t = 0.6; df = 10; P = 0.576), VI033479 (t = 1.2; df = 8; P = 0.252), VI044437-A (t = 1.9; df = 13; P = 0.086), VI047555-B (t = 0.7; df = 4; P = 0.553), VI048076 (t = 0.4; df = 20; P = 0.676) and VI049698 (t = 0.3; df = 9; P = 0.777) whereas the susceptible check VI033482 led to male biased sex ratios among the F_1 parasitoids (t = 6.2; df = 5; P = 0.002).

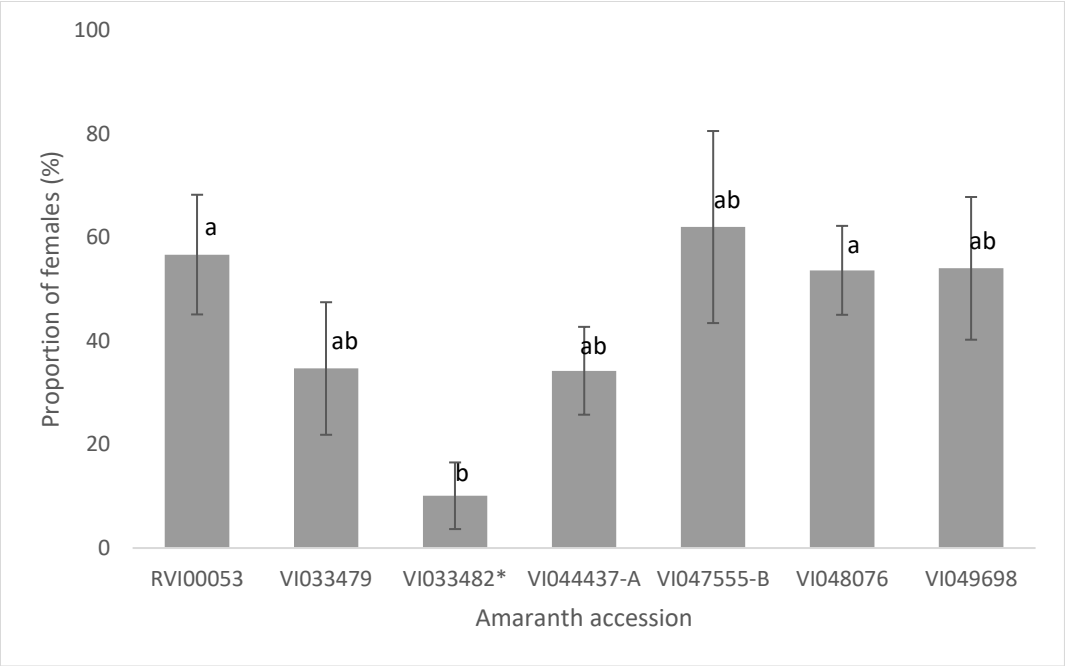

Figure 4.12: Proportion (%) of females in F_1 parasitoids of *Apanteles hemara* across moderately resistant and a susceptible amaranth accession.

4.5.3 Parasitoid's pupal mortality and non-reproductive host larval and pupal mortalities on different accessions of amaranth

The parasitoid's pupal mortality did not differ significantly (F = 0.4; df = 7, 44; P = 0.870) among the amaranth accessions and ranged between 40.0 ± 18.8 and 66.0 ± 18.9%. The parasitoid induced an average non-reproductive mortality of 32.1 ± 2.8% on *S. recurvalis* larvae across all the accessions. Significant non-reproductive host larval mortality due to the activity of the parasitoid was recorded in the accessions RVI00053 (t = 3.9; df = 5; P = 0.011), VI033479 (t = 5.2; df = 6; P = 0.002), VI044437-A (t = 6.8; df = 8; P < 0.001), VI047555-B (t = 4.9; df = 5; P = 0.004), VI048076 (t = 9.8; df = 9; P < 0.001), VI049698 (t = 3.9; df = 5; P =

0.011), VI056563 (t = 4.8; df = 6; P = 0.003) and the susceptible check VI033482 (t = 9.2; df = 7; P < 0.001) (Table 4.15). There were also significant differences in the non-reproductive larval mortalities (F = 2.4; df = 7, 51; P = 0.031) induced by the parasitoid among the resistant accessions. The accession VI047555-B recorded significantly lower non-reproductive mortalities compared to VI033479. There was, however, no significant difference in the non-reproductive mortality between the susceptible check and all the resistant accessions. Significant non-reproductive host pupal mortality was recorded on accessions VI033479 (t = 4.1; df = 4; P = 0.015), VI044437-A (t =2.9; df =4; P = 0.043), VI048076 (t = 4.6; df =3; P = 0.019), VI048076 (t = 4.0; df = 5; P = 0.010) and the susceptible accession VI033482 (t = 3.5; df = 4; P = 0.025). Non-reproductive pupal mortality was not significant in accessions VI049698 (t = 2.4; df = 3; P = 0.092), VI047555-B (t= 1; df = 3; P = 0.5) and RVI00053 (t = 2.3; df = 3; P = 0.101). Non-reproductive host pupal mortality did not differ significantly among the tested accessions (F = 0.9; df = 7,26; P = 0.513) (Table 4.15).

Table 4.15: Non-reproductive host larval and pupal mortality (%) caused by *Apanteles hemara* (mean ± SE) on selected moderately resistant amaranth accessions

Amaranth accession code	Non-reproductive host larval mortality	Non-reproductive host pupal mortality
VI033482*	25.8 ± 2.6ab	18.1 ± 4.9a
RVI00053	39.8 ± 14.2ab	20.6 ± 2.4a
VI033479	55.3 ± 11.2a	35.3 ± 8.4a
VI044437-A	28.6 ± 5.1ab	28.7 ± 11.1a
VI047555-B	15.0 ± 3.4b	16.6 ± 0.0a
VI048076	37.5 ± 5.8ab	27.7 ± 5.9a
VI049698	23.9 ± 6.7ab	16.7 ± 6.4a
VI056563	28.0 ± 6.9ab	21.2 ± 4.7a
P-value	0.031	0.513
F	2.4	0.9
df	7,51	7,26

*Susceptible accession. Means followed by the same letter(s) within a column are not significantly different at $P < 0.05$ (Tukey's test).

4.5.4 Length of fore wing and hind tibia of F_1 *Apanteles hemara* reared from *Spoladea recurvalis* fed on different accessions of amaranth

The female F_1 parasitoids reared from *S. recurvalis* reared from amaranth accession RVI00053 had significantly shorter ($F = 5.6$; df = 7, 74; $P < 0.001$) fore wing length compared to those of females obtained from accessions VI033479, VI044437-A, VI047555-B, VI048076 and VI056563 (Table 4.16). The fore wing length of F_1 female parasitoids obtained from the susceptible check was not significantly different from those obtained from the resistant amaranth accessions.

The fore wing of F_1 parasitoid females from hosts feeding on accession VI033479 were significantly (t = 3.6; df = 20; P = 0.002) longer compared to the fore wing of their male counterparts. The forewing length of F_1 male parasitoids obtained from hosts feeding on the different accessions did not show significant differences (F = 1.2; df = 7, 56; P = 0.314). The hind tibia length of the F_1 female parasitoids also showed significant differences (F = 4.4; df = 7, 82; P < 0.001) across hosts feeding on the accessions, with hosts from accession RVI00053 producing shorter hind tibia compared to those from accessions VI033479, VI044437-A and VI047555-B. The hind tibia from F1 female arising from accessions VI033479, VI044437-A, VI047555-B, VI048076, VI049698 and VI056563 were significantly longer that hind tibia of their F_1 male counterparts (Table 4.16). The hind tibial length of males differed significantly (F = 2.6; df =7, 61; P = 0.023) across the tested accessions with accession VI056563 producing males with the shortest hind tibia (Table 4.17).

Table 4.16: Fore wing length (mean ± SE) of F_1 progeny of *Apanteles hemara* reared on *Spoladea recurvalis* fed on selected moderately resistant amaranth accessions

Amaranth accession code	Female fore wing (mm)	Male fore wing (mm)	t	df	*P*-value
VI033482*	2.62±0.16abcA	2.71±0.08aA	0.6	3	0.587
RVI00053	2.43±0.07cA	2.58±0.06aA	1.5	16	0.153
VI033479	2.77±0.04aA	2.56±0.03aB	3.6	20	0.002
VI044437-A	2.70±0.04abA	2.57±0.06aA	1.9	23	0.065
VI047555-B	2.64±0.05abA	2.54±0.02aA	1.8	19	0.090
VI048076	2.65±0.04abA	2.61±0.03aA	0.8	24	0.422
VI049698	2.51±0.04bcA	2.49±0.03aA	0.4	16	0.733
VI056563	2.72±0.05abA	2.49±0.10aA	2.3	9	0.045
P-value	<0.001	0.314			
F value	5.579	1.208			
df	7,74	7,56			

*Susceptible accession. Means followed by the same lower-case letter(s) within a column and same uppercase letter(s) within a row are not significantly different at $P < 0.05$ (Tukey's test, t-test).

Table 4.17: Hind tibia length (mean ± SE) of F_1 progeny of *Apanteles hemara* reared on *Spoladea recurvalis* fed on selected moderately resistant amaranth accessions

Amaranth accession code	Female hind tibia (mm)	Male hind tibia (mm)	t	df	*P*-value
VI033482*	0.75±0.01abA	0.72±0.04abA	0.6	4	0.554
RVI00053	0.72±0.01bA	0.73±0.03aA	0.4	16	0.705
VI033479	0.80±0.02aA	0.72±0.02abB	3.0	19	0.008
VI044437-A	0.83±0.01aA	0.72±0.02aB	4.9	25	<0.001
VI047555-B	0.79±0.01aA	0.67±0.02bcB	5.5	18	<0.001
VI048076	0.77±0.02abA	0.73±0.01aB	2.2	28	0.041
VI049698	0.78±0.01abA	0.67±0.02abcB	5.1	23	<0.001
VI056563	0.76±0.01abA	0.63±0.03cB	4.9	10	0.001
P-value	<0.001	0.023			
F value	4.388	2.554			
df	7,82	7,61			

*Susceptible accession. Means followed by the same lower-case letter(s) within a column and same uppercase letter(s) within a row are not significantly different at $P < 0.05$ (Tukey's test, t-test).

4.5.5 Adult longevity of progeny of *Apanteles hemara* reared on *Spoladea recurvalis* fed on different amaranth accessions

Significant differences ($F = 9.2$; df = 7, 218; $P < 0.001$) were recorded in adult longevity of the F_1 parasitoids obtained from hosts reared on the different accessions with accession RVI00053 producing adults with more prolonged longevity compared to those from accessions VI044437-A, VI048076, VI049698, VI056563, VI047555-B and the susceptible check VI033482 (Table 4.18). The adults from hosts fed on accessions VI048076 and VI033479 also produced

parasitoids with significantly longer adult longevity compared to the susceptible check. There were significant differences (F = 7.2; df = 7, 92; P < 0.001) in the longevity of female parasitoids obtained from the different amaranth accessions with accession RVI00053 producing females with prolonged longevity compared to accessions VI044437-A, VI048076, VI049698, VI056563, VI047555-B and the susceptible check VI033482. The average female longevity of F_1 parasitoids obtained from all the amaranth accessions was 16.0 ± 0.9 days. The male longevity also differed significantly (F = 3.3; df = 6,117; P = 0.005) across the amaranth accessions with accession RVI00053 producing males with prolonged longevity than accessions VI044437-A, VI047555-B and the susceptible check VI033482. The average male longevity of F_1 parasitoids obtained from all the amaranth accessions was 11.1 ± 0.6 days. The overall female longevity of F_1 parasitoids obtained from all the accessions was significantly longer (t = 4.7; df = 224; P < 0.001) than the male longevity. Females produced from the following accessions had significantly longer adult longevity compared to their male counterparts: VI044437-A (t = 2.2; df = 36; P = 0.034), VI049698 (t = 2.2; df = 21; P = 0.038), RVI00053 (t = 2.5; df = 36; P = 0.019) and VI033479 (t = 3.1; df = 28; P = 0.004).

Table 4.18: Adult longevity (mean ± SE) of F_1 progenies of *Apanteles hemara* obtained from *Spoladea recurvalis* fed on selected moderately resistant amaranth accessions

Amaranth accession code	Longevity of both males and females (days)	Female longevity (days)	Male longevity (days)	t	df	*P*-value
VI033482*	8.3 ± 1.2d	7.4 ± 1.9cA	8.6 ± 1.5bA	0.5	28	0.654
RVI00053	20.3 ± 1.6a	23.6 ± 2.2aA	16.2 ± 2.0aB	2.5	36	0.019
VI033479	15.1 ± 1.9ab	22.6 ± 3.1abA	11.4 ± 2.0abB	3.1	28	0.004
VI044437-A	9.6 ± 0.4cd	10.9 ± 0.8cA	9.0 ± 0.5bB	2.2	36	0.034
VI047555-B	10.0 ± 1.3bcd	12.3 ± 1.6cA	7.8 ± 1.3bA	2.2	6	0.073
VI048076	14.0 ± 1.0bc	15.0 ± 1.5bcA	12.7 ± 1.4abA	1.1	53	0.265
VI049698	11.2 ± 0.7bcd	12.4 ± 0.9cA	9.7 ± 0.8abB	2.2	21	0.038
VI056563	13.8 ± 1.9bcd	16.0 ± 0.0abc	-			
P-value	<0.001	<0.001	0.005			
F value	9.2	7.2	3.3			
df	7,218	7,92	6,117			

*Susceptible accession. Means followed by the same lower-case letter(s) within a column and same uppercase letter(s) within a row are not significantly different at $P < 0.05$ (Tukey's test, t-test).

4.6 Effect of host's density and age on the performance of the larval endoparasitoid *Apanteles hemara*

4.6.1 Effect of host density on parasitism rates, pupal survival and sex ratio of *Apanteles hemara*

Spoladea recurvalis larval density significantly (F = 9.27; df = 3, 19; P = 0.001) affected parasitism by *A. hemara* such that higher parasitism was recorded in low larval densities. The cohort of 10 host larvae led to significantly higher parasitism rates compared to 20, 30 and 40 larval densities. There was no significant difference in parasitism between 20 and 30 larval densities whereas the highest larval density led to the least parasitism (Table 4.19). There was a significant negative linear correlation between host-larval density and percent parasitism by *A. hemara* ($y = -0.44x + 54.68$; $R^2 = 0.57$; F = 27.54; df = 1,21; P < 0.001) (Figure 4.13), denoting a decreasing percentage parasitism with increasing host density. On the contrary, a higher number of larvae were parasitized at higher host density with cohorts of 30 and 40 larvae recording significantly higher parasitization than the cohort of 10 larvae (F = 5.70; df = 3,19; P = 0.006) (Table 4.19). A regression model of the number of hosts supplied to the parasitoid against the number of larvae parasitized resulted into a curve showing a gradual increase in the number of larvae parasitized up to the density of 30 hosts supplied and then a slight decrease at the highest density of 40 hosts ($y = -0.42 + 0.77x + -0.01x^2$, R^2 = 0.47, F = 8.99; df = 2,20; P = 0.002) (Figure 4.14).

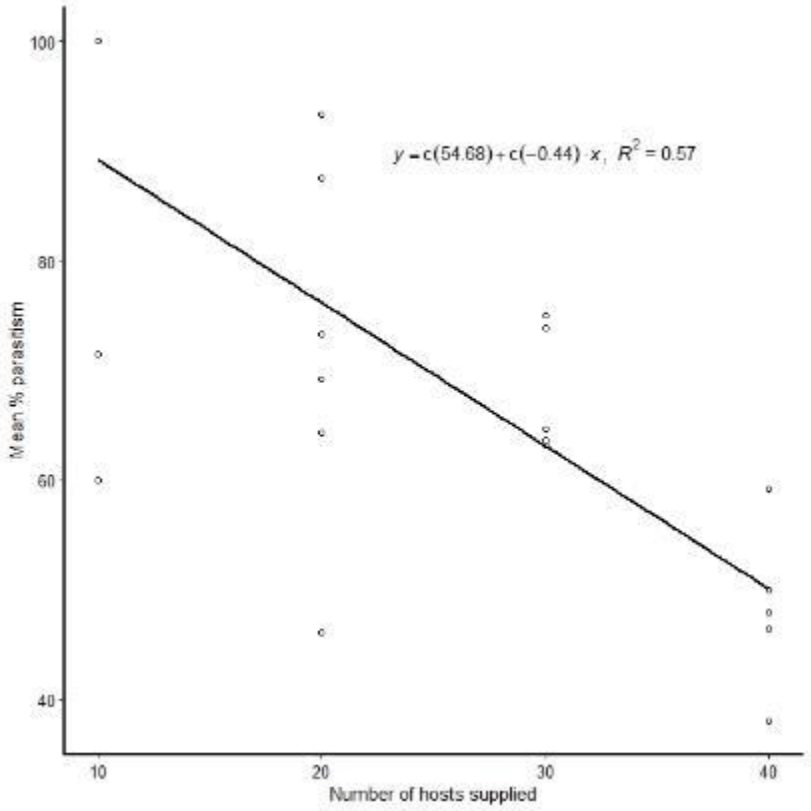

Figure 4.13: Correlation plot between host larval density of *Spoladea recurvalis* and percent parasitism rates by *Apanteles hemara*

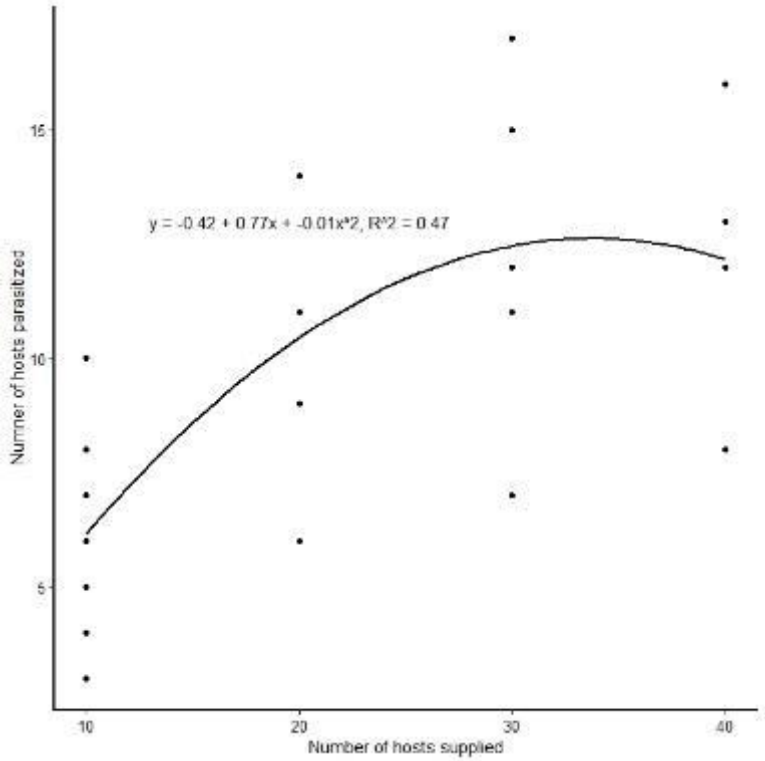

Figure 4.14: Functional response curve fit by non-linear least-squares regression of *Apanteles hemara* parasitizing its host *Spoladea recurvalis* at densities of 10, 20, 30 and 40 larvae.

The host larval mortality (number of dead larvae) was significantly lower in the cohorts of 10 and 20 larvae compared to the cohorts of 30 and 40 larvae (F = 22.97; df = 3,19; $P < 0.001$) (Table 4.19). Similarly, host pupal mortality was significantly higher in the cohort of 40 larvae compared to cohorts of 10, 20 and 30 larvae (F = 10.61; df = 3,14; P = 0.001). The parasitoid's pupal survival was significantly affected by the host-larval density with the least larval density (10 larvae) producing more pupae giving rise to adults compared to 20 and 40 larval densities (F = 3.76; df = 3,19; P = 0.028). There was no significant difference in

pupal viability at 20, 30 and 40 larval densities. Host larval density did not have a significant effect on the parasitoid's F_1 sex ratio (F = 2.41; df = 3,13; P = 0.113). For all densities tested, the sex ratio was balanced; 10 larvae (χ^2 = 0; df = 1; P = 1), 20 larvae (χ^2 = 2.27; df = 1; P = 0.132), 30 larvae (χ^2 = 3.5; df = 1; P = 0.061), 40 larvae (χ^2 = 0.11; df = 1; P = 0.739) (Table 4.19).

Table 4.19: Mean ± SE of parasitism rates, number of hosts parasitized, pupal survival and sex ratio of *Apanteles hemara* and host larval and pupal mortality at different host densities

Number of larvae	Parasitism (%)	Number of hosts parasitized	Host larval mortality	Host pupal mortality	Parasitoid pupal survival (%)	Female proportion	χ^2	df	*P*-value
10	90.20±6.45a	6.14±0.91b	3.29±0.75b	1.5±0.5b	63.55±9.65a	47.52±6.55a	0	1	1
20	72.31±6.91b	10.50±1.28ab	5.67±0.49b	2.0±0.26b	37.01±7.15b	39.67±4.87a	2.27	1	0.132
30	68.08±2.62b	12.40±1.72a	12.0±2.0a	3.2±0.37b	43.21±5.23ab	66.96±8.73a	3.5	1	0.061
40	48.36±3.40c	12.20±1.28a	15.0±1.22a	6.0±0.95a	27.82±7.77b	47.22±12.11a	0.11	1	0.739
F-value	9.27	5.70	22.97	10.61	3.76	2.41			
df	3,19	3,19	3,19	3,14	3,19	3,13			
P-value	0.001	0.006	< 0.001	0.001	0.028	0.113			

Mean ± SE followed by the same alphabet within a column are not significantly different at $P < 0.05$ (Tukey's test).

4.6.2 Effect of host densities on the development time and adult longevity of *Apanteles hemara*

The development times were comparable across the four larval densities tested, including the larval period (χ^2 =3.26; df = 3; P = 0.353), pupal period (χ^2 = 0.98; df = 3; P = 0.807), and total development time (χ^2 = 1.52; df = 3; P = 0.679) (Table 4.20). Similarly, no significant differences were observed in the longevity of either female (χ^2 = 0.19; df = 3; P = 0.98) or male (χ^2 = 3.64; df = 3; P = 0.303) wasps obtained at the different larval densities.

Table 4.20: Developmental time and adult longevity (days) (mean ± SE) of *Apanteles hemara* at different densities of host *Spoladea recurvalis*

Number of larvae	Larval development time	Pupal development time	Total development time	Longevity	Female longevity	Male longevity
10	7.70±0.20a	3.50±0.13a	11.13±0.32a	12.24±0.50a	12.18±0.86a	12.29±0.62a
20	8.34±0.14a	3.65±0.15a	11.75±0.29a	13.50±0.61a	12.60±0.85a	14.25±0.84a
30	8.63±0.13a	3.61±0.11a	12.18±0.25a	12.24±0.48a	12.67±0.69a	11.55±0.53a
40	7.91±0.22a	4.06±0.17a	11.33±0.35a	12.30±0.56a	12.22±0.81a	12.36±0.80a
χ^2	3.26	0.98	1.52	2.06	0.19	3.64
df	3	3	3	3	3	3
P-value	0.353	0.807	0.679	0.56	0.98	0.303

Mean ± SE followed by the same alphabet within a column are not significantly different at $P < 0.05$ (Tukey's test)

4.6.3 Influence of *Spoladea recurvalis* larval age on parasitism, pupal viability and sex ratio of *Apanteles hemara*

Apanteles hemara was able to successfully parasitize *S. recurvalis* larvae of all the age groups tested below seven days while older larvae beyond seven days escaped parasitism. The rate of parasitism was significantly higher in 1-2-day-old *S. recurvalis* larvae compared to 3-4-day-old larvae but did not differ between 3-4-day-old larvae and 5-6-day-old larvae (F = 4.0; df = 2,15; P = 0.04) (Table 4.21). The viability of pupae of *A. hemara* was significantly higher among 5-6-day-old larvae compared to both 1-2-day-old and 3-4-day-old larvae (F = 6.4; df = 2,15; P = 0.01). The proportion of female parasitoids obtained from all the three larval age groups did not differ significantly (F = 0.644; df = 2,15; P = 0.539). However, within each larval age group, there were significantly more males than females from 5-6-day-old larvae (χ^2 = 6.7; df = 1; P = 0.01) while there were balanced sex ratios from both 1-2-day-old (χ^2 = 1.68; df = 1; P = 0.194) and 3-4-day-old larvae (χ^2 = 0.00; df = 1; P = 1.00).

Table 4.21: Mean ± SE of parasitism, pupal survival and sex ratio (%) of *Apanteles hemara* at different host-larval age groups

Host-larval age	Parasitism (%)	Pupal survival	Female proportion	χ^2	df	*P*-value
1-2-days	79.17±7.22a	49.29±9.85b	60.00±16.96a	1.68	1	0.194
3-4-days	52.64±5.29b	43.75±8.29b	43.75±14.06a	0.00	1	1.00
5-6-days	61.82±6.64ab	80.51±5.17a	37.08±3.95a	6.70	1	0.010
F-value	4.0	6.4	0.644			
df	2,15	2,15	2,15			
P-value	0.04	0.01	0.539			

Mean ± SE followed by the same alphabet within a column are not significantly different at $P < 0.05$ (Tukey's test).

4.6.4 Non-reproductive *Spoladea recurvalis* larval and pupal mortality caused by *Apanteles hemara* when subjected to larvae of different age groups

Significant non-reproductive larval mortality caused by presence of *A. hemara* was observed among 1-2-day-old larvae ($W = 0$; $P = 0.027$), 3-4-day-old larvae ($W = 2$; $P = 0.004$) and 5-6-day-old larvae ($W = 3.5$; $P = 0.025$). The non-reproductive larval mortality of *S. recurvalis* was significantly higher among 1-2-day-old larvae compared to 3-4-day-old larvae and 5-6-day-old larvae ($F = 5.37$; df = 2,16; $P = 0017$) (Figure 4.15). There was no significant difference in non-reproductive larval mortality between 3-4-day-old larvae and 5-6-day-old larvae. Similarly, non-reproductive pupal mortality did not differ significantly across the three larval age groups ($F = 0.67$; df = 2, 13; $P = 0.528$).

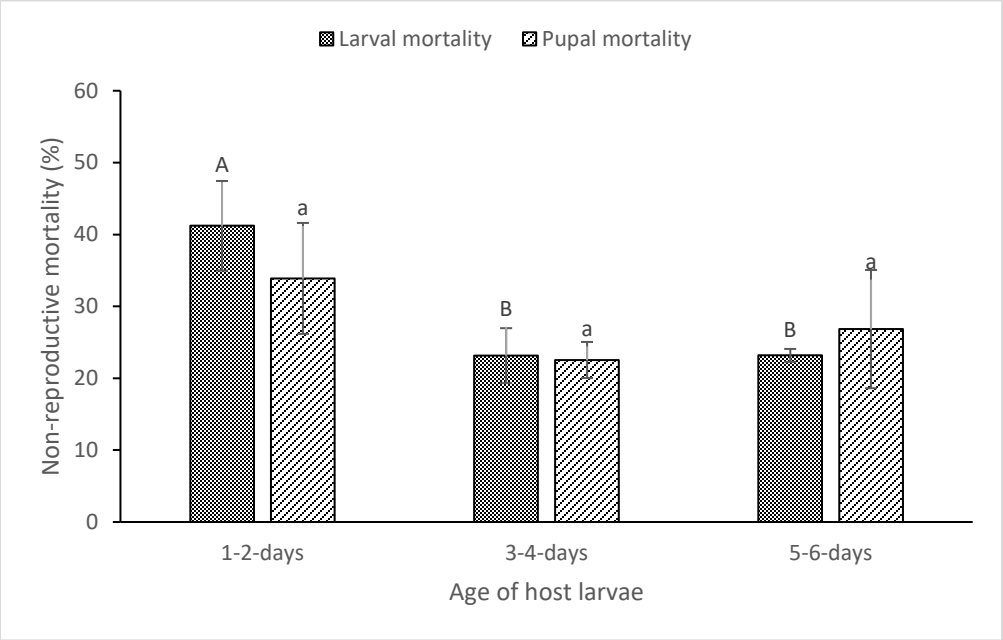

Figure 4.15: Non-reproductive larval and pupal mortality (mean ± SE) by *Apanteles hemara* at different age groups of *Spoladea recurvalis*

4.6.5 Effect of host larval age of *Spoladea recurvalis* on the development time and adult longevity of *Apanteles hemara*

The parasitoid's larval development time was significantly influenced by the age of *S. recurvalis* -larva. Larval development time was prolonged in the younger age groups compared to the older ones. 1-2-day-old host larvae resulted in longer development time compared to 3-4-day-old larvae while 5-6-day-old larvae had the shortest larval development time (χ^2 = 23.53; df = 2; P < 0.001) (Table 4.22). On the contrary, the pupal (cocoon) development time was not significantly influenced by the age of their host-larvae (χ^2 = 0.96; df = 2; P = 0.619). Subsequently, the whole development cycle of the parasitoid was 1.57 and 2.48 days shorter when 3-4-day-old and 5-6-day-old larvae were parasitized,

respectively, compared to that of 1-2-day-old parasitized larvae (F = 8.20; df = 2; P = 0.017). The total development time was also significantly shorter when 5-6-day-old larvae were parasitized compared to that of 3-4-day-old larvae. No significant differences were observed in the longevity of both male (χ^2 = 1.45; df = 2; P = 0.483) and female wasps (χ^2 = 0.003; df = 2; P = 0.998) when larvae of all the tested age groups were parasitized by their mothers.

Table 4.22: Developmental time and adult longevity (days) (mean ± SE) of *Apanteles hemara* at different age groups of *Spoladea recurvalis*

Host-larval age	Larval period (days)	Pupal period (days)	Total development time (days)	Longevity (days)	Male longevity	Female longevity
1-2-days	8.70±0.12a	4.45±0.36a	12.77±0.41a	11.75±0.71a	11.22±1.12a	12.18±0.94a
3-4-days	7.71±0.13b	3.85±0.29a	11.20±0.30b	12.42±0.76a	12.70±1.18a	12.11±0.99a
5-6-days	6.19±0.10c	4.08±0.16a	10.29±0.18c	12.61±0.43a	12.86±0.55a	12.10±0.69a
χ^2	23.53	0.96	8.2	0.77	1.45	0.003
df	2	2	2	2	2	2
P-value	<0.001	0.619	0.017	0.682	0.483	0.998

Mean ± SE followed by the same alphabet within a column are not significantly different at $P < 0.05$ (Tukey's test)

4.6.6 Fore wing and hind tibia length of progeny of *Apanteles hemara* developing from *Spoladea recurvalis* larvae of different age groups

Host larval age significantly influenced the morphometric features of the F_1 parasitoids of *A. hemara*. The fore wing length of female wasps obtained when 3-4-day-old *S. recurvalis* larvae were parasitized was significantly longer compared

to those obtained when 1-2-day-old and 5-6-day-old larvae were parasitized (F = 4.88; df = 2,63; P = 0.011) (Table 4.23). No significant difference was observed in the fore wing length of male wasps across the tested age groups (F = 2.44; df = 2,82; P = 0.094). The fore wing length of female wasps obtained when 3-4-day-old larvae were parasitized was significantly longer than that of their male counterparts (t = 3.75; df = 41; P = 0.001) while there was no significant difference in the fore wing length between male and female wasps when 1-2-day-old (t = 1.12; df = 76; P = 0.268) and 5-6-day-old larvae (t = 1.84; df = 30; P = 0.075) were parasitized.

Table 4.23: Fore wing length (mm) of male and female (mean ± SE) F_1 progeny of *Apanteles hemara* obtained from different *S. recurvalis* age groups

Host-larval age	Female fore wing (mm)	Male fore wing (mm)	t	df	*P*-value
1-2-days	2.81 ± 0.02bA	2.78 ± 0.02aA	1.12	76	0.268
3-4-days	2.92 ± 0.03aA	2.77 ± 0.03aB	3.75	41	0.001
5-6-days	2.80 ± 0.05bA	2.69 ± 0.04aA	1.84	30	0.075
F-value	4.88	2.44			
df	2,63	2,82			
P-value	0.011	0.094			

Means followed by the same lower-case letter within a column and same upper-case letter within a row are not significantly different at $P < 0.05$ (Tukey's test, t-test)

The length of the hind tibia of both female (F = 0.1; df = 2,61; P = 0.909) and male (F = 2.38; df = 2,76; P = 0.099) wasps obtained when larvae from the three age groups were parasitized did not differ significantly. However, within each of the three age groups, the length of hind tibia of females were significantly longer than those of their male counterparts (Table 4.24).

Table 4.24: Hind tibia length (mm) of male and female (mean ± SE) F_1 progeny of *Apanteles hemara* obtained from different host age groups

Host-larval age	Female hind tibia (mm)	Male hind tibia (mm)	t	df	*P*-value
1-2-days	0.84 ± 0.01a	0.76 ± 0.01a	5.59	70	<0.001
3-4-days	0.84 ± 0.01a	0.77 ± 0.02a	3.1	40	0.004
5-6-days	0.83 ± 0.02a	0.73 ± 0.02a	4.04	29	<0.001
F-value	0.1	2.38			
df	2,61	2,76			
P-value	0.909	0.099			

Means followed by the same lower-case letter within a column and same uppercase letter within a row are not significantly different at $P < 0.05$ (Tukey's test, t-test)

CHAPTER FIVE. DISCUSSION

5.1 Occurrence and diversity of amaranth lepidopteran defoliators and stem weevils and their associated parasitoids in Arusha, Tanzania

There is a broad diversity of insect pests that have been reported on amaranth in several parts of the world (Clarke-Harris *et al*., 1998; James *et al*., 2010; García *et al*., 2011; Aderolu *et al*., 2013; Mureithi *et al*., 2017). This is contrary to the popular belief that amaranth and other AIVs are seldom attacked by pests (Dinssa *et al*., 2016). Clarke-Harris and Fleischer (2003), James *et al*. (2010), Aderolu *et al*. (2013) and Mureithi *et al*. (2017) indicate that Lepidopteran pests are the most damaging to cultivated amaranths. The findings from the current study revealed a similarly high diversity of the lepidopteran pests attacking amaranths in Tanzania. With 14 different lepidopteran species recorded from amaranth during two seasons, the leaf-webber *S. recurvalis* was the most predominant. These results concur with the reports from India (Batra and Bhattacherjee, 1960; Pande, 1972; Arivudainambi *et al*., 2010), Jamaica (Clarke-Harris *et al*., 1998; Clarke-Harris *et al*., 2004), Nigeria (Aderolu *et al*., 2013), and Kenya (Mureithi *et al*., 2017), where *S. recurvalis* has been reported to be the most destructive pest of amaranth.

During both seasons, *S. recurvalis* occurrences were preceded by different species of leaf-webber pests; *P. basalis* which folds apical leaves of amaranths into characteristic leaf shelters and *E. impactella* which webs leaves that are near the soil. These also inflict substantial amount of damage to amaranth and in cases

where *P. basalis* infestations occurred, apical growth was hindered. The other pests of economic importance in amaranth production in the region were the leaf-worms, *S. littoralis* and *S. exigua,* which are known to be polyphagous in nature and extremely voracious feeders. It is apparent that the pests of amaranths occur as a complex array of species that contribute to substantial foliage loss. Similar observations were made by Aderolu *et al*. (2013) in Nigeria where 17 species of lepidopteran defoliators were reported to infest and damage amaranth. In East Africa, this is the first extensive study to document such a broad diversity of lepidopteran defoliators of amaranths.

Associated with the lepidopteran defoliators was a rich diversity of 14 indigenous parasitoid species from the families Braconidae and Ichneumonidae, which had varying parasitism levels per accession in the two seasons. Indigenous parasitoids, if conserved optimally, can play an important role in keeping the pest populations under check. Othim *et al*. (2017), through laboratory trials, reported parasitism rates of up to 90% by an indigenous parasitoid *Apanteles hemara* Nixon (Hymenoptera: Braconidae) on the amaranth leaf-webbers *S. recurvalis* and *U. ferrugalis*. Open field parasitism rates ranging from 11 to 62% caused by *Apanteles* sp. on *S. recurvalis* have been reported in parts of India (Narayanan *et al*., 1957; Bhattacherjee and Ramdas, 1964; Peter and Balasubramanian, 1984; Arivudainambi *et al*., 2010; Kedar and Kumaranag, 2013). In addition, *A. hemara* has been reported from various countries across Africa, Asia, Europe and Oceania (Kedar and Kumaranag, 2013; Madl and van Achterberg, 2014; Yu *et al*., 2016;

Fernandez-Triana *et al*., 2017). However, the performance (parasitism, development and reproduction) of a parasitoid has been reported to be differentially affected by its host plant (Turlings and Benrey, 1998). The variations in the levels of parasitism recorded in our study suggest an effect of the different accessions on the parasitoids. The variation in the number of lepidopteran hosts and interspecific competition may also affect parasitism levels. With the rich diversity of parasitoids reported from this study, these can be incorporated in conservation and/or augmentative biological control of the lepidopteran defoliators of amaranth.

The Eulophid wasp *Entedon* sp. was also found on amaranth stem weevils causing low levels of parasitism on the immature stages during both seasons. The first case of parasitism on amaranth stem weevils was reported in South Africa two decades ago by Louw *et al*. (1995). This study becomes the second to report such parasitism in Africa and the first in East Africa. Due to the dearth of information regarding this parasitoid, additional studies are recommended to assess the biology and performance of *Entedon* sp. with an aim of integrating it with HPR in an Integrated Pest Management (IPM) package for amaranth pests.

Stem weevils belonging to four different species were observed to cause damage to amaranth alongside the lepidopteran defoliators. According to Torres-Saldaña *et al*. (2004), Tara *et al*. (2009), García *et al*. (2011), Aderolu *et al*. (2013), Kagali *et al*. (2013), and Mureithi *et al*. (2017), the amaranth stem weevil, *H. truncatulus* is classified among the major pests that can cause significant

amounts of damage to the crop. Our study also showed high abundance of *H. truncatulus* in Tanzania compared to other species of stem weevils.

Several species of lepidopteran defoliators and stem weevils of amaranth predominated by the leaf-webber *S. recurvalis* and the stem weevil *H. truncatulus* were found to cause high levels of damage to the crop in Tanzania. The populations of *S. recurvalis* on amaranth gradually increased as the populations of other leaf-webber species declined over time. Extremely high incidence and abundance of amaranth stem weevils in the open fields stresses the need for an alternative management strategy that would work in synergy with the identified resistant accessions. There is also a rich diversity of indigenous parasitoids of both lepidopteran defoliators and amaranth stem weevils which have a potential to offer significant control for these pests and synergize the resistant accessions. This study, as far as we know, is the first to report on the incidence of amaranth stem weevil parasitoids in East Africa.

5.2 Evaluation of amaranth accessions for resistance against leaf-webbers and stem weevils

Amaranth accessions tested differed significantly in the incidence (infestation), abundance and damage caused by lepidopteran defoliators, compared to the susceptible accession. The level of pest incidence or abundance on any given accession portrays its level of non-preference by or resistance to the pest. Several accessions exhibited non-preference to amaranth lepidopteran defoliators with VI036227, VI049698, RVI00027, VI054569, VI033487, VI044432, VI048076, VI049639 and VI036225 showing high to moderately high levels of non-preference during the long rainy season. Accessions VI036227, VI049530 and VI049698 were the least preferred during the short rainy season and 22 others showed moderately high levels of resistance. During both seasons, accessions VI036227 and VI049698 were highly resistant to lepidopteran defoliators. Low pest abundance in the resistant accessions could be due to antixenosis or antibiosis traits.

Antixenosis involves behavioural factors that compel the pest to avoid the plant for feeding or laying its eggs while antibiosis involves adverse effects that the crop may have on the pest because of chemicals (secondary metabolites) or structures the plant possesses (Kogan and Ortman, 1978; Kishore Kumar *et al*., 2007). Further studies are thus recommended to explore these (antixenosis and antibiosis) resistance traits and the dynamics involved in host-pest interactions among the resistant amaranth accessions. In addition, the possibilities of

transferring these resistance traits into susceptible locally grown varieties of amaranth by methods such as introgression also need further study, especially in instances where the susceptible varieties are the most preferred by consumers.

Extremely high infestation of stem weevils and their corresponding damage was recorded during both seasons with infestation rates of up to 100% on several accessions. This is concurrent with the findings of Torres-Saldaña *et al*. (2004) and García *et al*. (2011) in Mexico and Tara *et al*. (2009) in India, who reported infestation rates of up to 100%, 92% and 82.3%, respectively on amaranth by the stem weevils. Whereas Torres-Saldaña *et al*. (2004) did not find significant effect of stem weevil abundance and tunnelling on grain yield reduction and biomass production, Phogat *et al*. (1994) and García *et al*. (2011) have demonstrated that substantial losses in grain yields occur due to stem weevil infestations. The high level of infestation and tunnelling damage by the stem weevils in our study points to the importance of these pests in amaranth production, particularly grain amaranths. However, whether this heavy presence of stem weevil grubs causes a reduction in the yield of leaves is still not clear. Further studies are recommended to show whether presence of stem weevil grubs affect yield of leaves of resistant accessions and enhance other negative attributes such as lodging. Since the stem weevil pests cause damage both to the foliage (as adults) and within the stems and roots (as grubs), sustainable management strategies are of utmost need. Accessions VI047517-B, VI036227 and VI056563 had the least stem weevil infestations (below 62.5%) and consequently the least tunnels as a

result of weevil feeding during both seasons suggesting that they possess low levels of resistance against the stem weevil pests. Whether this resistance is due to antixenosis, antibiosis, or tolerance is still unclear and further studies are recommended to unravel the mechanisms involved.

Thus, this study identified two highly resistant amaranth accessions against lepidopteran defoliators and 24 moderately resistant accessions. Three accessions with low levels of resistance against stem weevils were also identified. Accession VI036227 had the highest resistance to the complex of defoliators and weevils. In addition to the accession with the highest resistance to the complex of defoliators and weevils, VI036227, the 24 moderately resistant accessions are also recommended for advancement for release to farmers.

5.3 Assessing the possible mechanisms underlying resistance in amaranth accessions through their effects on the biology of leaf-webbers infesting amaranths

Amaranth accessions possessed different morphological and physical characteristics such as leaf coloration, shape and sizes. The accession VI036227 had significantly smaller leaves compared to the susceptible accession while accessions VI046233-A, VI033477 and VI056563 had red leaves compared to the green leaves of the susceptible accession. Pest preference for a plant variety has been attributed to the plants' physical, morphological and chemical features (Gatehouse, 2002; Jared *et al*., 2016). Physical features like petiole length, breadth

of leaf, pigmentation and presence of trichomes have been reported to affect insect pest preference in several crops including amaranths (Jiang *et al*., 2000; War *et al*., 2012; Akaneme and Ani, 2013). Hillier *et al*. (2004) also related pest abundance to the density of plant foliage. In addition, morphological characteristics play an important role in determining farmer and consumer preferences for a variety over others (Dinssa *et al*., 2016). Therefore, whereas accession VI036227 exhibited high resistance, the tiny leaves it possessed may become a hindrance to its acceptance by farmers in certain regions. Similarly, the red coloration of accession VI056563 may inhibit its acceptability in certain regions. Further studies are recommended to assess consumer and farmer preferences for selected resistant amaranth accessions.

When offered a choice of host plants for oviposition, *S. recurvalis* exhibited varying levels of preference for the different accessions for oviposition. The accessions VI050609-B and VI048919 did not show antixenosis for oviposition as they had significantly higher number of eggs compared to the susceptible control VI033482. Majority of the accessions (32 out of the 35 tested) exhibited oviposition deterrence, having <50% of eggs recorded in the susceptible control. Accessions VI044432, VI049502 and VI054569 exhibited high levels of antixenosis with <2 eggs compared to 21 eggs in the susceptible control. The choice by an insect to oviposit on a particular host plant and not on the other is usually determined by factors such as plant volatiles, plant anatomy, host nutrition, mobility of immature webworms, presence of natural enemies and competitors,

among others (Martínez *et al*., 2013). In the case of *S. recurvalis* on the different accessions of amaranth, it is still premature to predict with certainty which one of these factors played a significant role in antixenosis, but this study predicts that plant volatiles might be the most important. Further research is therefore recommended to determine which of these factors are key in the expression of antixenosis against *S. recurvalis*.

Spoladea recurvalis was observed to lay more eggs on the susceptible accession in no choice oviposition test compared to the selected resistant accessions. This further reiterates the expression of antixenosis for oviposition at varying levels against *S. recurvalis* in these resistant accessions with VI048076 having reduced number of eggs. Significantly fewer eggs were also laid per female in the choice than the no-choice oviposition tests on all the accessions. According to Grovida (2015), *S. recurvalis* is largely restricted to plants in the family Amaranthaceae and can be said to be a specialist. In seeking oviposition sites, specialists are usually under pressure to find suitable hosts and prioritize hosts that will offer quality nutrition for their offspring (Jaenike, 1990; Martínez *et al*., 2013). There is, therefore, a likely trade-off by *S. recurvalis* between the number of eggs and time spent by the female in seeking for a suitable host in the choice conditions compared to no-choice situations. In addition, competition between the female conspecifics for suitable host in the choice assay may also lead to reduced number of eggs. Thus, in practice, it would be more beneficial for a farmer to grow more than one variety/line/species of amaranth in a mixed cropping system so as

to reduce the pest burden or to interplant susceptible varieties, or avoid monocultures altogether.

Larval, pupal and total developmental time did not differ significantly among the tested accessions where development was successfully completed. This is probably due to similarity in the nutrient composition and quantities or composition of secondary compounds among the amaranth accessions (Mardani-Talaei *et al*., 2012). Shorter developmental time of an insect pest on a host is usually an indicator of a more suitable host crop (Mardani-Talaei *et al*., 2012). Jeyasankar and Gokilamani (2016) reported mean larval, pupal and total development times of *S. recurvalis* to be 13 ± 3.0, 10 ± 2.0 and 25.5 ± 5.5 days, respectively on an amaranth variety, which is similar to our values of 13.5 ± 0.12, 6.36 ± 0.13 and 19.09 ± 0.15 days for larval, pupal and total developmental times, respectively. Similar developmental times of *S. recurvalis* were also recorded by Seham *et al*. (2006) on sugar beet *Beta vulgaris* and on an identified plant species at 25 ± 2 °C (Bhattacherjee and Ramdas, 1964). The slight variations in the developmental times of *S. recurvalis* may be due to differences in experimental conditions and host plants used or to differences in genetic populations of *S. recurvalis*.

A high level of resistance was observed on accession VI036227 on which larval development of *S. recurvalis* could not proceed beyond the first instar. This could be a result of expression of antixenosis by the accession in which the plant produces feeding deterrents (volatiles) that prevent the larvae of *S. recurvalis* from

feeding and resulting in death due to starvation. There is also a possibility of antibiosis where the plant possesses highly potent secondary metabolites that kill the pest larvae upon feeding on it. Secondary metabolites belonging to the group of phenolic acids were shown to have negative effects on insects by acting as deterrents or being toxic to non-adapted insects by inducing toxic oxidative stress on herbivores (Summers and Felton, 1994; Simmonds, 2003; Niveyro *et al*., 2013). Hence, further studies are recommended to elucidate the bases of resistance of this accession in comparison to other resistant accessions with a special focus on the analysis of secondary metabolites and their role in pest resistance.

Larval mortality was highest on accession VI036227 (100%) and higher on accession VI056563 compared to RVI00053. High larval mortality on accession VI056563 is therefore an indication that it is unsuitable for the development of *S. recurvalis* in comparison to RVI00053. Negative r_i values on all the accessions also indicate a decline in larval populations on these accessions. High larval mortality rates could be due to sub-optimal nutritional quality in the accession or presence of secondary metabolites that do not promote development of *S. recurvalis.* Early stage larval mortality was highest on accession VI036227 followed by VI056563 and was least on RVI00053. Voracious feeding by larvae of *S. recurvalis* usually begins after the second instar, when larvae can feed on entire foliage leaving only leaf veins intact (James *et al*., 2010; Grovida, 2015; Othim *et al*., 2017). Low mortality rates during the early stages of larval development would therefore result in greater damage inflicted on the plant as the

larvae grow and feed. High early stage larval mortalities as observed on accession VI036227 is of critical importance and very desirable in the selection of resistant accessions because negligible damage is caused by larvae at this stage. Nevertheless, there was no significant difference in oviposition choice between this accession and the susceptible one, making it a 'dead-end' trap crop for *S. recurvalis*. On the contrary, accessions RVI00053, VI033479 and the susceptible check VI033482, which had low early stage mortalities, provide increased opportunities for the pest to cause extensive foliage damage as it matures. Pupal mortalities were higher on accessions RVI00053 and VI033479 compared to VI044437-A and were not correlated to larval mortalities suggesting that different compounds are responsible for mortality in the larvae and pupae of *S. recurvalis*. High larval and pupal mortalities have a significant role in reducing the populations of the pest in the subsequent generations and therefore accessions that lead to greater mortalities are highly desirable.

Apart from accession VI036227, which led to a significantly low weight gain when larvae of *S. recurvalis* were fed on it, weight gain from the other accessions did not differ significantly. The minimal weight gain on accession VI036227 (7.57% compared to >120% in other accessions) further reiterates the presence of either a feeding deterrent or a highly toxic secondary metabolite against larvae of *S. recurvalis.* Weight gain in the remaining accessions did not differ significantly, indicating that feeding by *S. recurvalis* larvae on the accessions was not deterred and suggests a lack of antixenosis for feeding in the accessions.

Significant differences in the longevity of adults of *S. recurvalis* raised on the different accessions were noted in our study, with accession VI047555-B producing adults with the shortest longevity. Shortened adult longevity is usually an indication of a less suitable host plant and is mainly attributed to low nutritional quality of that host plant (Liu *et al*., 2004). Differences in the adult longevity of *S. recurvalis* were also reported between desert horsepurslane *Trianthema portulacastrum* L. (5.68 ± 0.7 days) and *Amaranthus* sp. (4.99 ± 0.3 days) (Hsu and Srinivasan, 2012) in Taiwan. Pande (1972), also reported short adult longevity of between 3.5 and 6 days in males and females, respectively on *T. portulacastrum*. In contrast, Shirai (2006) reported extended adult longevity of 18.8±7.6 days and 15.1 ± 6.9 days in females and males of *S. recurvalis,* respectively when fed on spinach leaves (*Spinacia oleracea* L.) and Seham *et al*. (2006) reported longevity of 28.46 ± 1.88 and 26.08 ± 1.83 in females and males of *S. recurvalis,* respectively, when fed on sugar beet (*Beta vulgaris* L.). Although this broad variation in *S. recurvalis* adult longevity can be due to the differences in experimental conditions as in Seham *et al*. (2006) at 18.6 ± 2 °C and 70 ± 5% RH, the host plant on which the pest develops could play a big role (Hsu and Srinivasan, 2012). Other studies involving lepidopteran pests when reared on different host plants including *Helicoverpa armigera* Hübner (Liu *et al*., 2004) have also shown differences in adult longevity. Thus, shortened adult longevity can be attributed to expression of antibiosis by the host plant or inadequate nutrition in the host plant.

The accession VI036227 exhibited exemplary antibiotic traits by causing 100% pest mortality. However, it possessed very undesirable morphological/agronomic traits mainly tiny leaves (more than 6 times smaller than susceptible accession), slow germination and prostate growth habit. In East Africa, vegetable leaf yield is of high importance to both farmers and breeders (Dinssa *et al*., 2016), posing the challenge of acceptability of this resistant accession by farmers and consumers. The other seven accessions had traits such as moderate mortality rates on accession VI056563 and elicited lower rates of oviposition compared to the susceptible accession. They also possess better morphological/agronomic traits compared to VI036227, including erect growth habit and large/broad leaves, which may result in high vegetable leaf yields and might easily be accepted by local farmers and breeders. Whether the desirable antibiotic trait of accession VI036227 can be transferred to confer resistance to locally cultivated varieties and other accessions of amaranth is still unclear and is strongly recommended for future studies. Further studies are also recommended to assess farmers' and consumers' preferences and acceptance/willingness to cultivate and consume these pest resistant accessions. The yield potential, storability, drought tolerance and nutritive attributes of these different resistant varieties under various agro-ecological conditions also warrant further research.

The assessed amaranth accessions expressed both antixenotic and antibiotic resistance traits against *S. recurvalis.* Antixenosis traits exhibited through non-preference for oviposition were highly expressed in several accessions including

VI044432, VI049502, VI054569 and VI048076. Larval development was completely hindered on accession VI036227, resulting in 100% larval mortality and points to presence of potent antibiosis. In addition, VI036227 showed no antixenosis, suggesting further potential as a 'dead-end' trap crop. Early stage larval mortality, total larval and pupal mortality as well as adult longevity were moderate on accessions VI048076, VI056563 and VI047555-B suggesting moderate level of antibiosis. Host plant resistance (HPR) to insect pests forms the core of many IPM programs (Cortesero *et al*., 2000; Pappas *et al*., 2017) but is seldom exploited for pest management among TLVs. This is despite the fact that HPR is not only compatible with environmental concerns and other pest management strategies, but also significantly reduces pest control expenses (Liu *et al*., 2004), since the pest management solution is inherent in the crop. The accessions expressing adverse effects on the biology of *S. recurvalis* are thus recommended for evaluation for an IPM package for the management of the pest.

5.4 Evaluating selected pest resistant amaranth accessions for water stress tolerance

Water stress significantly reduced the shoot growth in all the accessions except VI033482 and VI049698. Several studies have shown that drought stress can affect the growth of plant organs in many ways which may result in the alteration of their morphological features (French and Turner, 1991; Spollen *et al*., 1993; Liu and Stützel, 2004). The negative effects on plant height for the six

accessions is consistent with the effects of water deficit on vegetable amaranth, *Amaranthus* sp., (Liu and Stützel, 2002a), palmer amaranth *Amaranthus palmeri* (Moran and Showler, 2005), spider plant *Gynandropsis gynandra* (Masinde *et al*., 2005), orchid tree *Bauhinia faberi* var. *microphylla* Duane (Li *et al*., 2008), maize, *Zea mays* (Shahrabian and Soleymani, 2011), *A. cruentus*, *A. hypochondriacus*, Ethiopian kale *Brassica carinata* Braun, African nightshade *Solanum scabrum* and *Solanum villosum* Mill. (Luoh *et al*., 2014). In most cases, a reduction in shoot growth under drought stress is compensated by an increase in root elongation. According to Liu and Stützel (2004), conservative shoot growth during drought could be advantageous to the crop if root growth is promoted as this will ensure the survival of the crop.

Root elongation and growth is another strategy used by plants in response to water stress. As drought conditions occur and the surface soil dries up, the roots of a plant extend to the deeper moist soils where it will be able to extract more available water from the soil (Martin and Thorstenson, 1988; Luoh *et al*., 2014). This leads to higher root to shoot dry mass ratio in drought stressed plants because growth is concentrated in the roots instead of the shoot (Malik *et al*., 1979; Turner, 1996; Liu and Stützel, 2004; Luoh *et al*., 2014). The results of this study showed a significant increase in the root to shoot dry mass ratio at lower soil water levels of 40 and 60% WHC, suggesting that root growth was promoted among the amaranth accessions in drought conditions. Nevertheless, sustained shoot growth during drought, especially among leafy vegetables, could also be of advantage as the crop

may have greater productivity and marketability (Liu and Stützel, 2004). Therefore, accessions VI033482 and VI049698 can be said to have greater adaptability to drought stress conditions as they can sustain both their root and shoot growth at low soil water levels and may result to greater productivity and marketability.

The leaf area of all accessions except VI033482 and VI047555-B were not significantly affected by soil water levels. A reduction of the leaf area as a result of the inhibition of cell expansion is one of the mechanisms used by plants in response to drought conditions to control water loss through transpiration and prevent dehydration of leaf tissue (Blum, 1996; Luoh *et al*., 2014). This alteration (reduction) of the leaf area has been observed in several studies with different species/genotypes of amaranth (Liu and Stützel, 2002a, b; Liu and Stützel, 2004, Slabbert and Krüger, 2014) and other plants (Masinde *et al*., 2005; Li *et al*., 2008; Shahrabian and Soleymani, 2011) in response to drought stress. Since the leaf area of accessions RVI00053, VI033479, VI044437-A, VI048076, VI049698 and VI056563 were not affected by drought stress, while that of VI033482 and VI047555-B were broader under severe and moderate drought stress, respectively, compared to the control, we deduce that these pest resistant accessions can also perform well in drought conditions without any negative effects in their marketable and nutritional yield. This is further supported by our results on the specific leaf area (SLA) which was not significantly affected by soil water level but differed significantly between the amaranth accessions. According to Garnier

et al. (2001), the SLA is a measure of the crop's leaf expansion against nutrient conservation taking into account the total leaf dry weight (leaf thickness). Taiz and Zeiger (2002), observed that drought vulnerable plants tend to have higher SLA values than drought tolerant plants. This reduction of SLA in drought tolerant plants could be their strategy to improve water use efficiency as thicker leaves tend to have more chlorophyll and proteins per unit leaf area, thus, greater photosynthetic capacity compared to thinner leaves (Wright *et al*., 1994; Craufurd *et al*., 1999; Liu and Stützel, 2004). The differences among accessions in their SLA suggest that VI033482 is more susceptible to drought than the other accessions most likely as a result of its extremely large leaf area.

Water stress significantly reduced the relative growth rate (RGR) of accessions RVI00053, VI033482, VI044437-A, VI047555-B and VI048076 but not VI033479, VI049698 and VI056563. Relative growth rate is a measure of the biomass production per unit of current biomass over an established period of time (Radosevich *et al*., 1997; Horak and Loughin, 2000). Hence, the variations observed in the RGR among amaranth accessions can be attributed to a difference in the strategies used by the accessions in response to water stress. First, reduced RGR could be as a result of restricted leaf formation as a response to water stress leading to low number of leaves per plant as observed in VI044437-A, VI047555-B and VI048076 (Gorai *et al*., 2010). Accessions VI033479, VI049698 and VI056563 on the other hand had comparable RGR between water stress conditions and the controls which is suggestive of their capabilities to produce biomass in

drought conditions. This is further supported by the results of the total dry mass (DM) which did not differ significantly between the water stress treatments and the controls in these accessions.

Leaf weight ratio (LWR) of many crops is often reduced as a result of drought stress as a strategy to conserve water (Erice *et al*., 2010). The findings of this study show a significant effect of water stress on LWR with 90% WHC recording higher LWR compared to 60% WHC. This modification of the leaf DM with regard to total DM (LWR) has also been observed in alfalfa as a mechanism to cope with drought (Erice *et al*., 2010). In amaranth, this reduction in LWR could be, mainly, due to a reduction of the SLA in certain accessions resulting in reduction of leaf biomass. This is also supported by the increased root to shoot ratios and increased RMR at 60% and 40% WHC compared to the control. Thus, these amaranth accessions alter their biomass allocation pattern from the leaves to other plant parts particularly the roots in order to cope with water stress.

5.5 The effect of selected resistant accessions on the performance of indigenous parasitoids of amaranth leaf-webbers

Parasitism rates by *A. hemara* varied among the amaranth accessions on which *S. recurvalis* was tested. Differences in parasitism rates have been reported in various Braconidae. For instance, *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) caused significantly higher parasitism on larvae of maize stem borer *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) that were fed on *Sorghum bicolor* L. (40.3%) than those fed on *Sorghum arundinaceum* (Desv.) Stapf (13.6%) (Sétamou *et al*., 2005). Idris and Grafius (1996) also showed that *Diadegma insulare* Cresson (Hymenoptera: Ichneumonidae) achieved significantly higher parasitism rate of 91.5% on *Plutella xylostella* L. (Lepidoptera: Plutellidae) fed on wild mustard *Brassica kaber* D. C. Wheeler compared to cabbage *B. oleracea* var. *capitata* L., where the level of parasitism was 76.5%. Benrey *et al*. (1997) reported a positive correlation between a host plant's attractiveness through release of certain volatile cues and parasitism of *Cotesia glomerata* L. (Hymenoptera: Braconidae). We hypothesise that this variance in parasitism depending on amaranth accessions could be due to the differences in attractiveness of the plants through the release of volatile blends or due to structural, morphological, and biochemical features of the plants such as the red leaf coloration of accession VI056563 on which the least parasitism rates were recorded (Benrey *et al*., 1997). Further studies are recommended to identify potential specific volatiles and/or morphological and biochemical features of

accessions that aid or deter parasitism. In addition, the immune response of a host larva feeding on an unsuitable host plant may be reduced and in turn enhance parasitism success (Gols and Harvey, 2009; Karimzadeh and Wright, 2008). This might have been the case in all the moderately resistant accessions in which parasitism rates were high except accession VI056563.

Amaranth accessions on which *S. recurvalis* larvae were fed significantly affected developmental time of *A. hemara*. The fastest development from egg to adult occurred on the accession RVI00053 and the slowest on VI033479 and VI044437-A. Several reports exist where the development time of koinobiontic parasitoids is shown to vary depending on the host plant species/variety/cultivar and consequently reflect host plant quality for the herbivore involved (Sétamou *et al*., 2005; Gols *et al*., 2008a; Gols *et al*., 2008b; Kahuthia-Gathu *et al*., 2008; Sarfraz *et al*., 2012). For example, *C. flavipes* parasitizing *C. partellus* took 18.4 and 20.6 days to complete its development on *S. bicolor* and *S. arundinaceum* respectively, (Sétamou *et al*., 2005). Similarly, *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae) and *D. semiclausum* parasitizing *P. xylostella* took significantly longer to complete their development on *B. oleracea* var. *acephala* (14.2 days *C. plutellae*; 15.9 days *D. semiclausum*) than on *Brassica juncea* Czern (12.2 days *C. plutellae*; 14.0 days *D. semiclausum*) (Kahuthia-Gathu *et al*., 2008). Extended development time in koinobiontic parasitoids is often assumed to be a result of the host-herbivore's suboptimal nutrition (Godfray, 1994; Harvey and Strand, 2003; Othim *et al*., 2017), which is directly influenced by the host plant

nutritional quality. It has also been reported in several studies that allelochemicals, such as glucosinolates which is common in Brassicaceae, found in the host diet can negatively affect growth and development of their parasitoids (Harvey and Strand, 2003; Harvey *et al*., 2007a; Harvey *et al*., 2007b). Since such allelochemicals have not yet been identified in amaranths, further studies are recommended to identify the allelochemicals present among pest resistant amaranth accessions and establish their mechanisms of action. According to the 'slow growth-high mortality hypothesis' (Benrey *et al*., 1997), prolonged development time by *A. hemara* on accessions VI033479 and VI044437-A may open an extended window of the parasitoid's vulnerability to a wide range of natural enemies such as hyperparasitoids and other abiotic stresses (Sarfraz *et al*., 2009; Harvey and Gols, 2011b).

Female parasitoids are the ones responsible for attacking the pests and also responsible for building up populations hence are desired in biological control programs (Chow and Heinz, 2005; Ode and Hardy, 2008; Othim *et al*., 2017). Higher proportions of female parasitoids were obtained from *S. recurvalis* larvae reared on accessions RVI00053 and VI048076 compared to the susceptible check VI033482. Furthermore, the ratio of male to female parasitoids obtained from all the resistant accessions was balanced except on VI033482 where the sex ratio was male biased. Female and male biased sex ratios have been reported in *A. hemara* on *S. recurvalis* and *Udea ferrugalis* Hübner (Lepidoptera: Crambidae), respectively when reared on a similar host plant (Othim *et al*., 2017). Eben *et al*.

(2000) reported that the citrus fruit species on which the fruit fly *Anastepha ludens* Loew (Diptera: Tephritidae) was fed affected the sex ratio and proportion of female progeny of the parasitoid *Diachasmimorpha longicaudata* Ashmead (Hymenoptera: braconidae) with *Citrus paradisi* Macfaden producing higher female proportions than *Citrus aurantium* L. However, Ode *et al*. (2004), assessing *Heracleum sphondylium* L. and *H. mantegazzianum* Sommier and Levier, and Sétamou *et al*. (2005), assessing *S. bicolor* and *S. arundinaceum*, found that the different host plant varieties did not affect the sex ratios of *Copidosoma sosares* (Walker) (Hymenoptera: Encyrtidae) and *C. flavipes*, respectively. Parasitoids have a flexibility in their sex allocation which is normally reflected in sex-ratio shifts in response to various environmental conditions including herbivore host size and quality among others (Godfray, 1994; King, 2002; Ode and Heinz, 2002; Ode *et al*., 2004; Colinet *et al*., 2005; Shuker *et al*., 2005; Lebreton *et al*., 2009; Bügler *et al*., 2013). Many studies have reported that the quality of a parasitoid's herbivore host is directly influenced by the host plant on which it feeds (Sétamou *et al*., 2005; Gols *et al*., 2008a; Gols *et al*., 2008b; Harvey and Gols, 2011a; Sarfraz *et al*., 2012). The nutritional characteristics of a herbivore's food plant can also affect the sex ratio of parasitoids, either by influencing decisions concerning sex allocation or by differentially affecting the survival of the sexes (Fox *et al*., 1990; Fox *et al*., 1996; Turlings and Benrey, 1998). Our results suggest that the moderately resistant amaranth accessions

improved the allocation and/or survival of the female sex compared to the susceptible accession.

In addition to parasitism, the parasitoid *A. hemara* caused significant non-reproductive larval mortalities of *S. recurvalis* fed on the different amaranth accessions. Non-reproductive mortalities are usually caused by host-feeding or stinging (ovipositor probing followed by host rejection) behaviour of a parasitoid (Bellows and Fisher, 1999; Foba *et al*., 2015; Othim *et al*., 2017). Othim *et al*. (2017), reported significant non-reproductive larval mortalities caused by *A. hemara* on the amaranth leaf-webbers *S. recurvalis* and *Udea ferrugalis*. Dannon *et al*. (2012), also reported host-feeding by the koinobiontic endoparasitoid *Apanteles taragamae* Viereck (Hymenoptera: Braconidae) on legume pod borer *Maruca vitrata* F. (Lepidoptera: Crambidae). Host-feeding has been shown to be of reproductive importance to synovigenic parasitoids as it aids egg maturation but does not have reproductive importance in proovigenic parasitoids (Bellows and Fisher, 1999; Byeon *et al*., 2009; Dannon *et al*., 2012). Being a koinobiontic parasitoid, *A. hemara* is likely to cause non-reproductive host killing through host stinging than host feeding (Bellows and Fisher, 1999; Godfray, 1994).

The size of an emerging parasitoid is an important correlate of fitness because it often affects an individual's reproductive success through variations in fecundity, longevity, dispersal, searching efficiency and host handling strategies (Visser, 1994; Kazmer and Luck, 1995; Turlings and Benrey, 1998; Eben *et al*., 2000). Amaranth accessions had a significant effect on the sizes (fore wing and

hind tibia lengths) of *A. hemara* F1 progenies obtained from *S. recurvalis* larvae reared on them. Previous studies have shown that parasitoids emerging from hosts reared on different host plants differed significantly in their body sizes and longevities. For example, when *P. xylostella* was fed on kale *B. oleracea* var. *acephala* and *B. oleracea* var. *capitata*, it produced *C. plutellae* parasitoids that had significantly longer hind tibia and fore wings compared to those from *P. xylostella* that was fed on *B. juncea* (Kahuthia-Gathu *et al*., 2008). Similarly, the parasitoid *D. longicaudata* that developed from Mexican fruit fly *Anastrepha ludens* Loew (Diptera: Tephritidae) fed on *Citrus aurantium* had significantly longer hind tibia than those from hosts reared on *C. paradisi* (Eben *et al*., 2000). In the present study, the smaller size of *A. hemara* obtained from accession RVI00053 may result in lower fecundity, thus a lower rate of population increase. In addition, reduced wing area can adversely affect the dispersal and foraging efficiency of these parasitoids (Sarfraz *et al*., 2009).

Adult longevity of both male and female F1 progenies of *A. hemara* varied among the tested amaranth accessions. Accessions RVI00053, VI033479 and VI048076 had significantly extended adult longevity compared to the susceptible accession. Just like size, a parasitoid's longevity is an important fitness correlate that affects reproductive success. A variation in adult longevity was observed on the parasitoid *Patrocloides montanus* Cresson (Hymenoptera, Ichneumonidae) that developed from cabbage looper *Trichoplusia ni* Hübner (Lepidoptera, Noctuidae) with those fed on *B. oleracea var. capitata* living longer than those fed on black

mustard *Brassica nigra* L. Koch (Fox *et al*., 1996). Extended female longevity not only enables the parasitoids to regenerate more fertilized eggs but also seek more hosts to parasitize. Thus, higher parasitism success can be obtained with females having prolonged longevity than those with short lifespan.

The performance of *A. hemara* was not adversely affected by most of the moderately resistant amaranth accessions compared to the susceptible accession. Except for VI056563 which had significantly lower parasitism rates and smaller male parasitoid size than on the susceptible accession, all other moderately resistant accessions tested did not affect parasitism rates of *A. hemara*. The moderately resistant accession RVI00053 produced F1 parasitoids that possess desirable fitness parameters including shortened developmental time, higher female proportions and prolonged male and female longevity but had smaller sized parasitoids. These moderately resistant accessions, apart from VI056563, can thus be used in combination with the endoparasitoid *A. hemara* to manage the leaf-webber *S. recurvalis* in amaranth in the context of IPM.

5.6 The effect of host's age and density on the performance of *Apanteles hemara*

5.6.1 Effect of host density on parasitism and other biological parameters of *Apanteles hemara*

The response elicited by a parasitoid at varying host density is an important attribute in considering an agent for biological control (Berryman, 1999). This is because of the fact that host density has been reported in several studies to affect the performance of a parasitoid (Harbison *et al*., 2001, Islam *et al*., 2006, Zanuncio *et al*., 2013, de Pedro *et al*., 2017, Harbi *et al*., 2018). In this study, treatments with low host larval density resulted in significantly higher rates of parasitism by *A. hemara* compared to those with high larval densities. A similar trend was reported by Harbi *et al*. (2018) in which higher parasitism rates by *Diachasmimorpha longicaudata* Ashmead (Hymenoptera: Braconidae) were recorded at lower densities of Mediterranean fruit fly *Ceratitis capitata* Wiedeman (Diptera: Tephritidae). and Zanuncio *et al*. (2013) where parasitism by *Campoletis flavicincta* (Ashmead; Hymenoptera: Ichneumonidae) decreased with increasing density of *Spodoptera frugiperda* (Smith; Lepidoptera: Noctuidae). These results are, however, contrary to the findings of Dannon *et al*. (2010), who used similar host densities as in the present study, in which the percent parasitism of legume pod borer *Maruca vitrata* Fabricius (Lepidoptera: Pyralidae) larvae by *Apanteles taragamae* Viereck (Hymenoptera: Braconidae) increased with larval density.

However, the trend observed by Dannon *et al*. (2010) with increasing parasitism rates following increasing host densities is atypical, considering the trend in most solitary endoparasitoids (Montanya *et al*., 2000, Harbison *et al*., 2001, Kitthawee *et al*., 2004, Islam *et al*., 2006, Zanuncio *et al*., 2013, de Pedro *et al*., 2017, Harbi *et al*., 2018). The authors themselves stressed this out and indicated that their functional response study was conducted using a simplified experimental arena due to preliminary observation that the female parasitoids were escaping experimental units few minutes after release, without parasitizing host larvae (Dannon *et al*., 2010). The results in the present study follow the most common trend found in solitary endoparasitoids where higher parasitism rates are expected at lower host densities, and decrease linearly with increasing densities. This decrease in parasitism rate might not be as a result of decreased activity but could rather be a reflection of the fact that the calculation of parasitism rate itself includes an element of host density. This could further contribute to findings of Fernández-arhex and Corley (2003), who reviewed 32 functional response studies on parasitoids and concluded that there is no clear relationship between the parasitoids' functional response curves and their actual success in the field. We therefore recommend further robust studies on the best use of functional responses in parasitoids compared to their use in predators.

Nevertheless, when considering functional response as the relationship between number of hosts attacked by a parasitoid as a function of prey density (Holling, 1959; Fernández-arhex and Corley, 2003; Zanuncio *et al*., 2013), the

data from this study corresponds to type II functional response. This means that the number of hosts parasitized by *A. hemara* increases with its host *S. recurvalis* density but gradually decelerates to a constant regardless of host density resulting into an asymptotic curve (Fernández-arhex and Corley, 2003). This study suggests that this approach reflects a more realistic scenario and is more practical than the use of rate since this could reveal the *satiation* level which, in the case of parasitoids, could be the maximum daily oviposition potential resulting from the depletion of the eggs in the parasitoid's ovaries at higher host densities (Berryman, 1999, Hassel, 2000, Wajnberg *et al*., 2008, Zanuncio *et al*., 2013, Harbi *et al*., 2018). Zanuncio *et al*. (2013) also suggested that host defences against natural enemies could be more efficient when the hosts are present at higher densities. Thus, determining the optimum number of eggs oviposited by female *A. hemara* per unit time would be useful for informing successful biological control programs, specifically parasitoid to host ratios to be applied. For example, Othim *et al*. (2017) obtained 94.67% and 44.55% parasitism using a cohort of five parasitoids and a single parasitoid, respectively.

It is important to note that, in the present study, functional response was assessed by analysis of parasitism both as the percentage of hosts parasitized and as absolute number of parasitized hosts, which was unlike results of other studies (Islam *et al*., 2006, Luna *et al*., 2007). For instance, studies by Harbison *et al*. (2001) and Islam *et al*. (2006) used only the absolute numbers of hosts attacked and emerged parasitoids, respectively, to assess functional response. On the other

hand, Dannon *et al*. (2010) and de Pedro *et al*. (2017) used only proportion/percent parasitism to assess functional response. In consonance with the present study, Luna *et al*. (2007), Zanuncio *et al*. (2013) and Harbi *et al*. (2018) assessed functional response using both percentage of hosts parasitized and absolute number of hosts parasitized and found that at higher host densities, the number of parasitized hosts increased, but the parasitism percentage declined. Complete contrast in results from the two different approaches used in the assessment of functional response calls for careful verification of the methods of assessment given that percentages contrast the actual numbers parasitized. Contrasting both approaches in the present study demonstrated that the lower parasitism rates reported at higher host density does not translate directly into lower absolute number of hosts parasitized. Rather, the number of parasitized hosts increased with host densities until satiation level from where a plateau was obtained. The approach based on number of hosts parasitized can be used to compare performance of different parasitoid species on the same host, or the same parasitoid on different host species. Furthermore, it can be used to generate models to simulate the impacts of *A. hemara* on the populations of *S. recurvalis* in open field or screen house conditions, and guide on how much parasitoids can be released (calibration of release) in a biological control program for effective management of the pest (Tonnang *et al*., 2009).

There were more viable parasitoid cocoons when the parasitoid encountered fewer host larvae than when higher densities of larvae were

encountered. This might suggest that the parasitoid *A. hemara,* can choose to lay only fit/mature eggs at low host density while at higher host densities, even unfit/immature eggs could be laid. Zanuncio *et al*. (2013) did not observe significant difference in the percentage of *Campoletis flavicincta* (Hymenoptera: Ichneumonidae) pupae that did not emerge into adults at different densities of fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Similarly, no significant difference in the pupal viability of *Allorhogas pyralophagus* Marsh (Hymenoptera: Braconidae) were found at varying densities of Mexican rice borer *Eoreuma loftini* Dyar (Lepidoptera: Pyralidae) (Harbison *et al*., 2001). It is probable that other factors such as sex of eggs laid by the parasitoid and environmental conditions can also play a role in determining successful adult emergence, hence further studies are recommended to explore such factors.

The immature developmental time, sex ratio and the longevity of both male and female adults of the parasitoid did not vary across the host densities. A parasitoid's developmental time, sex ratio and adult longevity are often reflective of its host's nutritional quality (Harvey, 2000; Othim *et al*., 2017). Since the host larvae supplied to the parasitoids were of the same age and fed on the same host plant, similarity in development time, sex ratio and adult longevity was expected. Zanuncio *et al*. (2013) also reported no significant difference in the sex ratio of *C. flavicincta* at different densities of *S. frugiperda* caterpillars.

5.6.2 Influence of *Spoladea recurvalis* larval age on some biological parameters of *Apanteles hemara*

Parasitism rates were significantly higher in 1-2-day-old *S. recurvalis* larvae compared to 3-4-day-old larvae. Morphological and physical characteristics such as size, texture, and movement responses elicited by a host are able to affect a parasitoid's attempts and capabilities to oviposit (Godfray, 1994; Lauro *et al*., 2005; Othim *et al*., 2017). As the host larvae get older, they usually display stronger physical defence to the ovipositing female parasitoid compared to younger and often smaller ones which can be easily handled and consequently higher parasitization of the younger larvae (Brodeur *et al*., 1996; Shi *et al*., 2002). Much older larvae are also larger relative to the size of the parasitoid, therefore, other than increasing the handling time, they also present a risk to the parasitoid due to their developed mechanisms of defence. According to our observation, the younger larvae of *S. recurvalis* also tended to aggregate while feeding which could reduce the host searching time and result in higher parasitism rates. Similar findings were reported by Shi *et al*. (2002) where significantly higher parasitism by *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae) was observed on the second and third larval instars of diamondback moth *Plutella xylostella* L. (Lepidoptera: Plutellidae) compared to fourth instar larvae. On carob moth *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae), *Apanteles myeloenta* Wilkinson (Hymenoptera: Braconidae) parasitized more second instar than first

instar larvae, although, parasitism was least among third instar larvae (Farahani and Goldansaz, 2013).

In contrast to parasitism, the pupal survival rate was significantly lower in the younger larvae compared to the older ones. Successful development of immature parasitoids has been shown to be affected by the quality of host on which they feed (Godfray, 1994). Several studies have also established that larger or older larvae have higher nutritional quality than smaller or younger ones (Godfray, 1994; Harvey, 2000; Harvey and Strand, 2002). It is hypothesised that koinobiontic parasitoids attacking low quality (small) hosts delay their development to allow their hosts increase in size and supply their nutritional needs (Mackauer and Sequeira, 1993; Shi *et al*., 2002). However, once *S. recurvalis* larva is parasitized, its feeding rate is reduced to mere gnawing of the leaves and it undergoes a substantial retardation in growth (size) (Othim *et al*., 2017). As such, the host becomes unable to increase the nutrient intake to meet the threshold requirement for the development of the parasitoid. The immature survival of *A. hemara* was, thus, higher on the older larvae because these larvae had accumulated enough nutrients to meet the minimum threshold requirements for the developing parasitoid.

The developmental period of the parasitoid was longest in 1-2-day-old *S. recurvalis* larvae and least in 5-6-day-old larvae. Quality nutrition is a key determinant of the developmental period of a parasitoid and is directly influenced by its host (Harvey, 2000; Othim *et al*., 2017). Extended or longer developmental

time in koinobiontic parasitoids is often interpreted to be a result of the host's suboptimum nutrition (Godfray, 1994; Harvey and Strand, 2003; Othim *et al*., 2017). Most studies have also presented nutritional richness in terms of host size and age (Godfray, 1994; Harvey, 2000). The trend observed in our study where there is prolonged development time in younger larvae can be explained using a model proposed by Mackauer and Sequeira (1993). The model postulates that parasitoids attacking hosts of low-quality exhibit a lag phase in their development to allow the host to acquire sufficient nutrients for their development. This trend has been observed in several studies involving parasitoids of lepidoptera and other orders. For example, *A. myeloenta* had prolonged developmental time in the first instar larvae of *E. ceratoniae* compared to the second and third instars (Farahani and Goldansaz, 2013). Similarly, *Meteorus pulchicornis* Wesmael (Hymenoptera: Braconidae) took longer to develop in first instar larvae of cabbage moth *Mamestra brassicae* L. (Lepidoptera: Noctuidae) compared to the second and subsequent instars (Malcicka and Harvey, 2014).

Apart from developmental time, host size (quality) has also been presented in many studies as a determinant of a parasitoid's fitness (size) (Godfray, 1994; Visser, 1994; Harvey, 2000; Ode and Heinz, 2002). Fitness of *A. hemara* in terms of size of F1 offspring was significantly influenced by the age of its host larva. However, this effect of host age on female size of *A. hemara* was not linear as the intermediate age group gave larger females compared to the age groups in either extremity. Several cases are reported in which parasitoid size is a non-linear or

increasing function of host age (Harvey *et al*., 1994; Harvey *et al*., 1999; Harvey, 2000; Harvey and Strand, 2002; Harvey *et al*., 2004; Harvey, 2005). Specifically, Harvey *et al*. (2004) demonstrated that the size of *Microplitis demolitor* Wilkinson (Hymenoptera: Braconidae) did not have a linear correlation with larval age of soybean looper *Pseudoplusia includens* Walker (Lepidoptera: Noctuidae). While our results on parasitoid fitness can be interpreted superficially to suggest that the intermediate age group produced more fit parasitoids, the non-linear relationship between host age and parasitoid size makes it difficult to draw conclusions about the fitness benefits of body size (Harvey *et al*., 2004). Furthermore, that interpretation would not take into consideration the costs related to the developmental time.

The results show that there was significant non-reproductive host larval mortality caused by *A. hemara* which was significantly higher among 1-2-day old larvae than either 3-4-day or 5-6-day old larvae. This mortality is often caused by host feeding or host stinging behaviour of the parasitoid and is an important contributor to pest suppression (Byeon *et al*., 2009, Akutse *et al*., 2015, Foba *et al*., 2015, Othim *et al*., 2017). Othim *et al*. (2017) established that *A. hemara* can attempt oviposition more than once on a single host larva suggesting a possibility of super-parasitism by this parasitoid on *S. recurvalis*. The high non-reproductive mortality among younger host larvae can be explained by multiple visits to the same host by *A. hemara* that implies repeated host stinging in which the parasitoid causes physical injuries by inserting its ovipositor into the host several times

(Keinan *et al*., 2012). The young hosts are also less mobile and not capable of providing for themselves a strong physical defence making them more vulnerable and accessible to the parasitoid (Shi *et al*., 2002). The occurrence of significant non-reproductive mortality has been reported to be a frequent phenomenon in ectoparasitoids while only a few endoparasitoids have this ability (Bernardo *et al*., 2006, Tran and Takagi, 2006, Mafi and Ohbayashi, 2010, Akutse *et al*., 2015, Muchemi *et al*., 2018a,b,c). For instance, significant non-reproductive mortality has been reported in the ectoparasitoid *Diglyphus isaea* Walker (Hymenoptera: Eulophidae) parasitizing *Liriomyza* sp. (Diptera: Agromyzidae) (Akutse *et al*., 2015, Muchemi *et al*., 2018b). Foba *et al*., (2015) and Muchemi *et al*., (2018c) reported insignificant non-reprodutive mortality by the endoparasitoids *Phaedrotoma scabriventris* Nixon (Hymenoptera: Braconidae), *Opius dissitus* Muesebeck (Hymenoptera: Braconidae) and *Halticoptera arduine* Walker (Hymenoptera: Pteromalidae) on *Liriomyza* sp. However, in congruence with our findings on *A. hemara*, significant non-reproductive mortality has been reported in the endoparasitoids *Copidosoma koehleri* Blanchard (Hymenoptera: Encyrtidae) parasitizing potato tuber moth, *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae) (Keinan *et al*., 2012), *D. longicaudata* parasitizing *C. capitata* (Harbi *et al*., 2018), *Aganaspis daci* (Weld; Hymenoptera: Figitidae) parasitizing *C. capitata* (de Pedro *et al*., 2017) and *Chrysocharis flacilla* Walker (Hymenoptera: Eulophidae) parasitizing *Liriomyza* sp. (Diptera: Agromyzidae) (Muchemi *et al*., 2018a,b).

Thus, *A. hemara* exhibited a type II functional response in which parasitism rates reduced with increasing host density. A contrasting result was also found between the two approaches used to assess the effect of host density on parasitism rate and call for careful comparison between literatures on functional response of parasitoids. The results from host density on number of parasitized hosts can be used in generating mathematical models to establish densities required for biological control. More viable pupae were also observed at low host densities than at high densities. However, host density had no effect on development time, sex ratio and adult longevity of the parasitoid. Higher non-reproductive mortality at low host density due to super-parasitism or host stinging was also observed. These suggest that *A. hemara* is a potential biological control agent of *S. recurvalis* for seasonal augmentative release in amaranth fields. The age of the host larvae variously influenced parasitism rates, immature mortality, non-reproductive mortality and size (fitness) of the female adult progeny. The development time of the parasitoid was prolonged in younger host larvae compared to the older larvae. For mass rearing of *A. hemara*, the shorter development time and low immature mortality achieved in the older hosts make them more favourable than the younger hosts in rearing.

CHAPTER SIX. CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

- Several species of lepidopteran defoliators and stem weevils of amaranth predominated by the leaf-webber *S. recurvalis* and the stem weevil *H. truncatulus* were found to cause high levels of damage to amaranth in Tanzania. The populations of *S. recurvalis* on amaranth gradually increased as the populations of other leaf-webber species declined with time.
- There is a rich diversity of indigenous parasitoids of both lepidopteran defoliators and amaranth stem weevils which have a potential to offer significant control for these pests, especially *Apanteles hemara* and *Atropha tricolor*. This study is the first to report on the incidence of amaranth stem weevil parasitoids in east Africa.
- This study identified two highly resistant amaranth accessions, VI036227 and VI049698, against lepidopteran defoliators and 24 moderately resistant accessions to lepidopteran defoliators attacking amaranth. Three accessions (VI047517-B, VI036227 and VI056563) with low levels of resistance against stem weevils were also identified. VI036227 had the highest resistance to the complex of defoliators and weevils.
- The assessed amaranth accessions expressed both antixenotic and antibiotic resistance traits against *S. recurvalis.* Antixenosis traits exhibited through non-preference for oviposition were highly expressed in several accessions

including VI044432, VI049502, VI054569 and VI048076. Larval development was completely hindered on accession VI036227, resulting in 100% larval mortality and points to presence of potent antibiosis. Early stage larval mortality, total larval and pupal mortalities as well as adult longevity were moderate on accessions VI048076, VI056563 and VI047555-B suggesting moderate level of antibiosis.

- The selected amaranth accessions including RVI00053, VI033479, VI044437-A, VI048076 and VI049698 were identified to be drought tolerant. They displayed several modifications of their morphological features and in biomass partitioning in response to drought stress. Key among the modifications was the reduction in shoot growth and an increase in root elongation leading to a higher root to shoot ratio. These accessions can thus tolerate dry conditions and produce good yields.
- The performance of *A. hemara* was not adversely affected by most of the moderately resistant amaranth accessions compared to the susceptible accession. Except VI056563 which had significantly lower parasitism rates and smaller male parasitoid's size than on the susceptible accession, all other moderately resistant accessions tested did not affect parasitism rates of *A. hemara*. The moderately resistant accession RVI00053 produced F1 parasitoids that possess desirable fitness parameters including shortened developmental time, higher female proportions and prolonged male and female longevity but had smaller sized parasitoids.

- *Apanteles hemara* exhibited a type II functional response in which parasitism reduced with increasing host density. More viable cocoons were also observed at low host densities than at high densities. However, host density had no effect on development time, sex ratio and adult longevity of the parasitoid.
- The age of the host larvae variously influenced parasitism rates, immature mortality, non-reproductive mortality and size (fitness) of the female adult progeny. The development time of the parasitoid was prolonged in younger host larvae compared to the older larvae.

6.2 Recommendations

- The diverse parasitoid species recovered from the open field trials are recommended for harnessing to complement/synergize HPR.
- In addition to the accession with the highest resistance to the complex of leaf defoliators and stem weevils, VI036227, the 24 moderately resistant accessions are also recommended for advancement for release to farmers. The accessions expressing adverse effects on the biology of *S. recurvalis* are thus recommended for evaluation for an IPM package for the management of the pest.
- This study recommends the use of accession VI036227 as a 'dead-end' trap crop in managing leaf-webbers attacking amaranth. This is because it led to 100% larval mortality while it showed no antixenosis for oviposition.

- The selected accessions expressing water stress tolerance traits are recommended for cultivation in regions experiencing drought conditions across Africa and around the world.
- The moderately resistant accessions, apart from VI056563, are recommended for use in combination with the endoparasitoid *A. hemara* to manage the leaf-webber *S. recurvalis* in amaranth in the context of IPM.
- Given its type II functional response, it is recommended that *A. hemara* is utilized as a potential biological control agent of *S. recurvalis* for seasonal augmentative release in amaranth crops. For mass rearing of *A. hemara*, the shorter development time and low immature mortality achieved in the older hosts make them more favourable than the younger hosts hence highly recommended for laboratory mass rearing.

6.3 Recommendations for further studies

- This study has reported the diversity and dynamics of pests attacking amaranth and their natural enemies in two seasons, however, further studies are warranted to assess changes in pests and natural enemies' diversity over a longer period.
- The rich diversity of lepidopteran parasitoids reported from this study are recommended for further studies to assess their individual performance on selected accessions and the possibility of having them incorporated in

conservation and/or augmentative biological control of the lepidopteran defoliators of amaranth

- This being the first report of the stem weevil parasitoid in East Africa, further studies are recommended to assess the biology and performance of *Entedon* sp. with an aim of integrating it with HPR in an Integrated Pest Management (IPM) package for amaranth pests.
- Further studies are recommended to elucidate the transferability of the water stress tolerance traits to other high yielding varieties and lines in breeding programs.

REFERENCES

Abang, A., Srinivasan, R., Kekeunou, S., Hanna, R., Chagomoka, T., Chang, J. and Bilong, C. B. (2014). Identification of okra (*Abelmoschus* spp.) accessions resistant to aphid (*Aphis gossypii* Glover) in Cameroon. *African Entomology*, *22*(2), 273-284. doi: https://doi.org/10.4001/003.022.0201

Abang, A., Srinivasan, R., Kekeunou, S., Yeboah, M., Hanna, R., Lin, M.-Y., . . . Bilong Bilong, C. (2016). Relationship of phenotypic structures and allelochemical compounds of okra (*Abelmoschus* spp.) to resistance against *Aphis gossypii* Glover. *International Journal of Pest Management, 62*(1), 55-63. doi: https://doi.org/10.1080/09670874.2015.1095372

Abbott, W. (1925). A method of computing the effectiveness of an insecticide. *Journal of economic entomology*, *18*(2), 265-267.

Adebooye, O. and Opabode, J. (2004). Status of conservation of the indigenous leaf vegetables and fruits of Africa. *African Journal of Biotechnology, 3*(12), 700-705.

Aderolu, I., Omooloye, A. and Okelana, F. (2013). Occurrence, abundance and control of the major insect pests associated with amaranths in Ibadan, Nigeria. *Entomology, Ornithology and Herpetology, 2*(112), 2161-0983.1000112.

Akaneme, F. and Ani, G. (2013). Morphological assessment of genetic variability among accessions of *Amaranthus hybridus*. *World Applied Sciences Journal*, *28*(4), 568-577.

Akutse, K., Van den Berg, J., Maniania, N., Fiaboe, K. and Ekesi, S. (2015). Interactions between *Phaedrotoma scabriventris* Nixon (Hymenoptera: Braconidae) and *Diglyphus isaea* Walker (Hymenoptera: Eulophidae), parasitoids of *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae). *Biological control, 80*, 8-13.

Amicarelli, V. and Camaggio, G. (2012). Amaranthus: a crop to rediscover. *Forum Ware International, 2*, 4-11.

Arivudainambi, S., Selvamuthukumaran, T. and Baskaran, P. (2010). Efficacy of herbal extracts in management of amaranth leaf caterpillar. *International journal of vegetable science, 16*(2), 167-173.

AVRDC. (2004). AVRDC Report 2003. *AVRDC Publication Number 04-599* (pp. 194). Shanhua, Taiwan: AVRDC— The World Vegetable Center.

Bailey, P. T. (2007). *Pests of field crops and pastures: identification and control*: CSIRO publishing.

Baltazar, A. (2009). Efficacy of *Spoladea recurvalis* as biocontrol agent against horse purslane (*Trianthema portulacastrum*) under small-plot conditions. AGRIS-*International Information System for the Agricultural Science and Technology*, 6-9.

Batra, H. and Bhattacherjee, N. (1960). Occurrence of *Hymenia recurvalis* (Fabricius) (Lepidoptera: Pyalidae) as a bad pest of some leaf vegetables. *Indian Journal of Entomology, 22*, 128-130.

Bellotti, A. C. and Arias, B. (2001). Host plant resistance to whiteflies with emphasis on cassava as a case study. *Crop protection, 20*(9), 813-823.

Bellows, T. S. and Fisher, T. W. (1999). *Handbook of biological control: principles and applications of biological control*. San Diego: Academic Press.

Benrey, B., Denno, R. F. and Kaiser, L. (1997). The influence of plant species on attraction and host acceptance in *Cotesia glomerata* (Hymenoptera: Braconidae). *Journal of Insect Behavior, 10*(5), 619-630.

Berryman, A. A. (1999). The theoretical foundations of biological control. In B. A. Hawkins & H. V. Cornell (Eds.), *Theoretical approaches to biological control* (pp. 3 - 21). Cambridge: Cambridge University Press.

Bhattacherjee, N. and Ramdas, M. (1964). Bionomics, biology and control of *Hymenia recurvalis* (Fabricius) (Pyralidae: Lepidoptera). *Indian Journal of Entomology, 26*(2), 176-183.

Blum, A. (1996). Crop responses to drought and the interpretation of adaptation. In E. Belhassen (Ed.), *Drought tolerance in higher plants: genetical, physiological and molecular biological analysis* (pp. 57-70). Dordrecht: Springer.

Brodeur, J., Geervliet, J. B. and Vet, L. E. (1996). The role of host species, age and defensive behaviour on ovipositional decisions in a solitary specialist and gregarious generalist parasitoid (*Cotesia* species). *Entomologia Experimentalis et Applicata, 81*(2), 125-132.

Bügler, M., Rempoulakis, P., Shacham, R., Keasar, T. and Thuijsman, F. (2013). Sex allocation in a polyembryonic parasitoid with female soldiers: an evolutionary simulation and an experimental test. *PLoS One, 8*(6), e64780.

Byeon, Y. W., Tuda, M., Takagi, M., Kim, J. H. and Kim, Y. H. (2009). Non-reproductive host killing caused by *Aphelinus asychis* (Hymenoptera: Aphelinidae), a parasitoid of cotton aphid, *Aphis gossypii* (Homoptera: Aphididae). *Journal of the Faculty of Agriculture, Kyushu University, 54*(2), 369-372.

Cardona, C. and Oatman, E. (1975). Biology and physical ecology of *Apanteles subandinus* Blanchard (Hymenoptera: Braconidae), with notes on temperature responses of *Apanteles scutellaris* Muesebeck and its host, the potato tuberworm. *Hilgardia, 43*(1), 1-51.

Chahal, K., Singh, B., Kang, B., Battu, R. and Joia, B. (1997). Insecticide residues in farmgate vegetable samples in Punjab. *Pesticide Research Journal, 9*(2), 256-260.

Chow, A. and Heinz, K. M. (2005). Using hosts of mixed sizes to reduce male-biased sex ratio in the parasitoid wasp, *Diglyphus isaea*. *Entomologia Experimentalis et Applicata, 117*(3), 193-199.

Clarke-Harris, D., Fleischer, S. and Fender, A. (1998). Major pests of callaloo. Identification Guide. *The Pennsylvania State University*, 1-16.

Clarke-Harris, D., Fleischer, S., Fuller, C. and Bolton, J. (2004). Evaluation of the efficacy of new chemistries for controlling major lepidoptera pests on

vegetable amaranth in Jamaica. *Caribbean Agricultural Research and Development Institute Review* (4), 12-19.

Clarke-Harris, D. and Fleischer, S. J. (2003). Sequential sampling and biorational chemistries for management of lepidopteran pests of vegetable amaranth in the Caribbean. *Journal of Economic Entomology, 96*(3), 798-804.

Colinet, H., Salin, C., Boivin, G. and Hance, T. (2005). Host age and fitness-related traits in a koinobiont aphid parasitoid. *Ecological Entomology, 30*(4), 473-479.

Cortesero, A., Stapel, J. and Lewis, W. (2000). Understanding and manipulating plant attributes to enhance biological control. *Biological control, 17*(1), 35-49. doi: https://doi.org/10.1006/bcon.1999.0777

Costea, M., Tardif, F. J. and David, M. B. (2003). The identity of a cultivated *Amaranthus* from Asia and a new nomenclatural combination. *Economic botany, 57*(4), 646-649.

Costea, M., Weaver, S. E. and Tardif, F. J. (2004). The biology of Canadian weeds. 130. *Amaranthus retroflexus* L., *A. powellii* S. Watson and *A. hybridus* L. *Canadian journal of plant science, 84*(2), 631-668.

Craufurd, P., Wheeler, T., Ellis, R., Summerfield, R. and Williams, J. (1999). Effect of temperature and water deficit on water-use efficiency, carbon isotope discrimination, and specific leaf area in peanut. *Crop Science, 39*(1), 136-142.

DAFF. (2010). Amaranthus-Production guideline. Pretoria: Department of Agriculture, Forestry and Fisheries. pp. 24.

Dannon, E. A., Tamo, M., van Huis, A. and Dicke, M. (2012). Assessing non-target effects and host feeding of the exotic parasitoid *Apanteles taragamae*, a potential biological control agent of the cowpea pod borer *Maruca vitrata*. *BioControl, 57*(3), 415-425.

Dannon, E. A., Tamò, M., van Huis, A. and Dicke, M. (2010). Functional response and life history parameters of *Apanteles taragamae*, a larval parasitoid of *Maruca vitrata*. *BioControl, 55*(3), 363-378.

de Pedro, L., Beitia, F., Ferrara, F., Asís, J. D., Sabater-Muñoz, B. and Tormos, J. (2017). Effect of host density and location on the percentage parasitism, fertility and induced mortality of *Aganaspis daci* (Hymenoptera: Figitidae), a parasitoid of *Ceratitis capitata* (Diptera: Tephritidae). *Crop protection, 92*, 160-167.

De Prins, J. and De Prins, W. (2014). Afromoths, online database of Afrotropical moth species (Lepidoptera). Retrieved 01 July 2014, from www.afromoths.net

Delplanque, A. and Gruner, L. (1975). The use of *Bacillus thuringiensis* Berliner against some Lepidoptera injurious to vegetable crops in the Antilles. *Nouvelles Agronomiques des Antilles et de la Guyane* (1), 71-82.

Diez-Rodríguez, G., Hübner, L., Antunes, L. and Nava, D. (2013). *Herpetogramma bipunctalis* (Lepidoptera: Crambidae) biology and

techniques for rearing on leaves of the blackberry (*Rubus* spp., Rosaceae). *Brazilian Journal of Biology, 73*(1), 179-184.

Dinssa, F., Hanson, P., Dubois, T., Tenkouano, A., Stoilova, T., Hughes, J. and Keating, J. (2016). AVRDC—The World Vegetable Center's women-oriented improvement and development strategy for traditional African vegetables in sub-Saharan Africa. *European Journal of Horticultural Science, 81*(2), 91-105.

Dombroskie, J. (2011). A matrix key to families, subfamilies and tribes of Lepidoptera of Canada. *Canadian Journal of Arthropod Identification, 17,* 1-129.

Dugdale, J. S. (1988). Lepidoptera-annotated catalogue, and keys to family-group taxa. *Fauna of New Zealand, 14,* 1-262.

Eben, A., Benrey, B., Sivinski, J. and Aluja, M. (2000). Host species and host plant effects on preference and performance of *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae). *Environmental entomology, 29*(1), 87-94.

Ebert, A. W., Wu, T.H. and Wang, S.T. (2011). Vegetable amaranth (*Amaranthus* L.). *AVRDC- The World Vegetable Center Publication* 11-754.

Eigenbrode, S. D. and Trumble, J. T. (1994). Host plant resistance to insects in integrated pest management in vegetable crops. *Journal of Agricultural Entomology, 11*(3), 201-224.

Ekesa, B., Walingo, M. K. and Onyango, M. (2009). Accesibility to and consumption of indigenous vegetables and fruits by rural households in Matungu division, western Kenya. *African Journal of Food, Agriculture, Nutrition and Development*, *9*(8), 1725-1738.

Erice, G., Louahlia, S., Irigoyen, J. J., Sanchez-Diaz, M. and Avice, J.-C. (2010). Biomass partitioning, morphology and water status of four alfalfa genotypes submitted to progressive drought and subsequent recovery. *Journal of Plant Physiology*, *167*(2), 114-120. doi: https://doi.org/10.1016/j.jplph.2009.07.016

Farahani, H. K., Bell, H. and Goldansaz, S. H. (2012). Biology of *Apanteles myeloenta* (Hymenoptera: Braconidae), a larval parasitoid of carob moth *Ectomyelais ceratoniae* (Lepidoptera: Pyralidae). *Journal of Asia-Pacific Entomology*, *15*(4), 607-610.

Farahani, H. K. and Goldansaz, S. H. (2013). Is host age an important factor in the bionomics of *Apanteles myeloenta* (Hymenoptera: Braconidae)? *European Journal of Entomology*, *110*(2), 277-283.

Fernández-arhex, V. and Corley, J. C. (2003). The functional response of parasitoids and its implications for biological control. *Biocontrol Science and Technology, 13*(4), 403-413.

Fernandez-Triana, J., Beaudin, M., van Achterberg, K., Agbodzavu, M. K., Othim, S. T., Nyamu, F. W. and Fiaboe, K. K. (2017). DNA barcodes, expanded distribution, and redescription of *Apanteles hemara* Nixon, 1965

(Hymenoptera, Braconidae, Microgastrinae), a potential biocontrol species against amaranth leaf-webbers in Africa. *Journal of Hymenoptera Research, 58*, 1-15.

Foba, C., Lagat, Z., Gitonga, L., Akutse, K. and Fiaboe, K. (2015). Interaction between *Phaedrotoma scabriventris* Nixon and *Opius dissitus* Muesebeck (Hymenoptera: Braconidae): endoparasitoids of *Liriomyza* leafminer. *African Entomology, 23*(1), 120-131.

Fox, L. R., Kester, K. M. and Eisenbach, J. (1996). Direct and indirect responses of parasitoids to plants: sex ratio, plant quality and herbivore diet breadth. *Entomologia Experimentalis et Applicata, 80*(1), 289-292.

Fox, L. R., Letourneau, D. K., Eisenbach, J. and Van Nouhuys, S. (1990). Parasitism rates and sex ratios of a parasitoid wasp: effects of herbivore and plant quality. *Oecologia, 83*(3), 414-419.

French, R. and Turner, N. (1991). Water deficits change dry matter partitioning and seed yield in narrow-leafed lupins (*Lupinus angustifolius* L.). *Australian Journal of Agricultural Research, 42*(3), 471-484.

García, A. A., Huato, M. Á. D., Lara, M. H., Sáenz-de-Cabezón, F. J., Pérez-Moreno, I., Marco-Mancebón, V. and López-Olguín, J. F. (2011). Insect occurrence and losses due to phytophagous species in the amaranth *Amaranthus hypocondriacus* L. crop in Puebla, Mexico. *African Journal of Agricultural Research, 6*(27), 5924-5929.

Garnier, E., Shipley, B., Roumet, C. and Laurent, G. (2001). A standardized protocol for the determination of specific leaf area and leaf dry matter content. *Functional ecology, 15*(5), 688-695.

Gatehouse, J. A. (2002). Plant resistance towards insect herbivores: a dynamic interaction. *New phytologist, 156*(2), 145-169.

Gikonyo, N., Mburu, M., Kenji, G. and Mwasaru, A. (2011). Properties of a complementary food based on amaranth grain (*Amaranthus cruentus*) grown in Kenya. *Journal of Agriculture Food and Technology, 1*, 153-178.

Gilbert, M. and Grégoire, J. C. (2003). Visual, semi-quantitative assessments allow accurate estimates of leafminer population densities: an example comparing image processing and visual evaluation of damage by the horse chestnut leafminer *Cameraria ohridella* (Lep., Gracillariidae). *Journal of Applied Entomology, 127*(6), 354-359.

Godfray, H. C. J. (1994). *Parasitoids: behavioral and evolutionary ecology*: Princeton University Press.

Gols, R., Bukovinszky, T., Van Dam, N. M., Dicke, M., Bullock, J. M. and Harvey, J. A. (2008a). Performance of generalist and specialist herbivores and their endoparasitoids differs on cultivated and wild Brassica populations. *Journal of Chemical Ecology, 34*(2), 132-143.

Gols, R., Wagenaar, R., Bukovinszky, T., Dam, N. M. v., Dicke, M., Bullock, J. M. and Harvey, J. A. (2008b). Genetic variation in defense chemistry in

wild cabbages affects herbivores and their endoparasitoids. *Ecology, 89*(6), 1616-1626.

Gols, R. and Harvey, J. A. (2009). Plant-mediated effects in the Brassicaceae on the performance and behaviour of parasitoids. *Phytochemistry Reviews, 8*(1), 187-206.

Gorai, M., Hachef, A. and Neffati, M. (2010). Differential responses in growth and water relationship of *Medicago sativa* (L.) cv. Gabès and *Astragalus gombiformis* (Pom.) under water-limited conditions. *Emirates Journal of Food and Agriculture*, 1-12.

Grovida, F. (2015). Vegetable pests. Retrieved on 26 May 2015 from http://www.grovida.us/vegetable-pests.html

Harbi, A., Beitia, F., Ferrara, F., Chermiti, B. and Sabater-Muñoz, B. (2018). Functional response of *Diachasmimorpha longicaudata* (Ashmead) over *Ceratitis capitata* (Wiedemann): Influence of temperature, fruit location and host density. *Crop protection, 109*, 115-122.

Harbison, J. L., Legaspi, J. C., Fabritius, S. L., Saldaña, R. R., Legaspi, B. C. and Enkegaard, A. (2001). Effects of age and host number on reproductive biology of *Allorhogas pyralophagus* (Hymenoptera: Braconidae) attacking the Mexican rice borer (Lepidoptera: Pyralidae). *Environmental entomology, 30*(1), 129-135.

Harvey, J. A. (2000). Dynamic effects of parasitism by an endoparasitoid wasp on the development of two host species: implications for host quality and parasitoid fitness. *Ecological Entomology, 25*(3), 267-278.

Harvey, J. A. (2005). Factors affecting the evolution of development strategies in parasitoid wasps: the importance of functional constraints and incorporating complexity. *Entomologia Experimentalis et Applicata, 117*(1), 1-13.

Harvey, J. A., Bezemer, T. M., Elzinga, J. A. and Strand, M. R. (2004). Development of the solitary endoparasitoid *Microplitis demolitor*: host quality does not increase with host age and size. *Ecological Entomology, 29*(1), 35-43.

Harvey, J. A. and Gols, R. (2011a). Development of *Mamestra brassicae* and its solitary endoparasitoid *Microplitis mediator* on two populations of the invasive weed *Bunias orientalis*. *Population Ecology, 53*(4), 587.

Harvey, J. A. and Gols, R. (2011b). Population-related variation in plant defense more strongly affects survival of an herbivore than its solitary parasitoid wasp. *Journal of Chemical Ecology, 37*(10), 1081.

Harvey, J. A., Gols, R., Wagenaar, R. and Bezemer, T. M. (2007a). Development of an insect herbivore and its pupal parasitoid reflect differences in direct plant defense. *Journal of Chemical Ecology, 33*(8), 1556-1569.

Harvey, J. A., Harvey, I. F. and Thompson, D. J. (1994). Flexible larval growth allows use of a range of host sizes by a parasitoid wasp. *Ecology, 75*(5), 1420-1428.

Harvey, J. A., Jervis, M. A., Gols, R., Jiang, N. and Vet, L. E. (1999). Development of the parasitoid, Cotesia rubecula (Hymenoptera: Braconidae) in *Pieris rapae* and *Pieris brassicae* (Lepidoptera: Pieridae): evidence for host regulation. *Journal of Insect Physiology, 45*(2), 173-182.

Harvey, J. A. and Strand, M. R. (2002). The developmental strategies of endoparasitoid wasps vary with host feeding ecology. *Ecology, 83*(9), 2439-2451.

Harvey, J. A. and Strand, M. R. (2003). Sexual size and development time dimorphism in a parasitoid wasp: an exception to the rule? *European Journal of Entomology, 100*(4), 485-492.

Harvey, J. A., Van Dam, N. M., Witjes, L., Soler, R. and Gols, R. (2007b). Effects of dietary nicotine on the development of an insect herbivore, its parasitoid and secondary hyperparasitoid over four trophic levels. *Ecological Entomology, 32*(1), 15-23.

Hassell, M. (2000). The spatial and temporal dynamics of host-parasitoid interactions. Oxford University Press, London.

HCD - Horticultural Crops Directorate. (2015-2016). Horticulture validated report 2015-2016. Nairobi, Kenya: Horticultural Crops Directorate, Agriculture and Food Authority. Retrieved on 10th January 2019 from

https://www.agricultureauthority.go.ke/wp-content/uploads/2016/09/Horticulture-2015_2016-Validated-Report3.pdf

Hillier, N., Dixon, P. and Larson, D. (2004). Trap captures of male *Grapholita libertina* (Lepidoptera: Tortricidae) moths: relationship to larval numbers and damage in wild lingonberry. *Environmental entomology*, *33*(2), 405-417.

Holling, C. S. (1959). Some characteristics of simple types of predation and parasitism. *The Canadian Entomologist, 91*(7), 385-398.

Horak, M. J. and Loughin, T. M. (2000). Growth analysis of four Amaranthus species. *Weed Science, 48*(3), 347-355.

Hsu, Y. C. and Srinivasan, R. (2012). Desert horse purslane weed as an alternative host for amaranth leaf webber, *Hymenia recurvalis* in Taiwan. *Formosan Entomologist, 32*, 297-302.

Ibeawuchi, I., Dialoke, S., Ogbede, K., Ihejirika, G., Nwokeji, E., Chigbundu, I., . . . Oyibo, P. O. (2007). Influence of yam/cassava based intercropping systems with legumes in weed suppression and disease/pest incidence reduction. *Journal of American Science, 3*(1), 49-59.

Idris, A. and Grafius, E. (1996). Effects of wild and cultivated host plants on oviposition, survival, and development of diamondback moth (Lepidoptera: Plutellidae) and its parasitoid *Diadegma insulare* (Hymenoptera: Ichneumonidae). *Environmental Entomology*, *25*(4), 825-833.

Infonet-biovision. (2018). Amaranth. Retrieved December 2018, from Infonet-biovision http://www.infonet-biovision.org/PlantHealth/Crops/Amaranth

Islam, N., Islam, W. and Mondal, K. (2006). Functional response of *Dinarmus basalis* (Rond.) (Hymenoptera: Pteromalidae) parasitizing *Callosobruchus maculatus* (F.). *Journal of Bio-Science, 14*, 11-16.

Jaenike, J. (1990). Host specialization in phytophagous insects. *Annual Review of Ecology and Systematics, 21*(1), 243-273.

James, B., Atcha-Ahowé, C., Godonou, I., Baimey, H., Goergen, H., Sikirou, R. and Toko, M. (2010). *Integrated pest management in vegetable production: A guide for extension workers in West Africa*. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. 120pp.

James, B., Godonou, I., Atcha-Ahowe, C., Glitho, I., Vodouhe, S., Ahanchede, A., . . . Goergen, G. (2007). Extending integrated pest management to indigenous vegetables. *Acta Horticulturae 752*, 89-94.

Jared, J. J., Murungi, L. K., Wesonga, J. and Torto, B. (2016). Steroidal glycoalkaloids: chemical defence of edible African nightshades against the tomato red spider mite, T*etranychus evansi* (Acari: Tetranychidae). *Pest Management Science, 72*(4), 828-836.

Jeyasankar, A. and Gokilamani, D. (2016). Biology and eco-friendly control of amaranths pests, *Hymenia recurvalis* Fabricius and *Psara basalis* Walker (Lepidoptera: Crambidae). *International Journal of Academic Studies, 2*, 218-230.

Jiang, C. X., Wright, R., Woo, S., DelMonte, T. and Paterson, A. (2000). QTL analysis of leaf morphology in tetraploid Gossypium (cotton). *Theoretical and Applied Genetics, 100*, 409-418.

Kagali, R. N., Kioko, E. N., Osiemo, Z., Muya, S. and Wachera, C. (2013). Insect abundance and diversity on cultivated *Amaranthus* spp.(Amaranthacea) in Meru County, Kenya. *American International Journal of Contemporary Research, 3*(7), 110-116.

Kahuthia-Gathu, R. (2013). Invasion of Amaranth by Spotted Beet Webworm *Spoladea recurlvalis* in semi-arid areas of Kenya. *In*, 13th Workshop on Sustainable Horticultural Production in the Tropics, Meru University of Science and Technology, Meru. Horticultural Association of Kenya.

Kahuthia-Gathu, R., Löhr, B. and Poehling, H. (2008). Effect of common wild crucifer species of Kenya on fitness of two exotic diamondback moth parasitoids, *Cotesia plutellae* and *Diadegma semiclausum*. *Crop protection, 27*(12), 1477-1484.

Karimzadeh, J. and Wright, D. J. (2008). Bottom-up cascading effects in a tritrophic system: interactions between plant quality and host-parasitoid immune responses. *Ecological Entomology, 33*(1), 45-52.

Kazmer, D. J. and Luck, R. F. (1995). Field tests of the size-fitness hypothesis in the egg parasitoid *Trichogramma pretiosum*. *Ecology, 76*(2), 412-425.

Kedar, S. and Kumaranag, K. (2013). Report on outbreak of *Spoladea recurvalis* (Fabricus) on *Trianthema portulacastrum* L. and its parasite from Haryana, India. *Journal of Entomological Research, 37*(2), 149-151.

Keinan, Y., Kishinevsky, M., Segoli, M. and Keasar, T. (2012). Repeated probing of hosts: an important component of superparasitism. *Behavioural Ecology, 23*, 1263-1268.

Ki-Yeol, L., KiSu, A., HyuJung, K., SungKyu, P. and TaeSu, K. (2002). Effects of temperature on reproduction and development of *Udea ferrugalis* Lepidoptera: Pyralidae. *Korean Journal of Applied Entomology, 41*, 205-209.

King, B. (2002). Sex ratio response to conspecifics in a parasitoid wasp: test of a prediction of local mate competition theory and alternative hypotheses. *Behavioral Ecology and Sociobiology, 52*(1), 17-24.

Kintomo, A. and Ojo, O. (2000). Non-destructive leaf area estimation in grain amaranth (*Amaranthus cruentus* L.). *Research on Crops, 1*(3), 267-270.

Kirby, J., Silver, A., Giri, J., Sebenik, M., Resstrepo, A. S., Velez, M. A. and Adeyinka, T. O. (2010). GEEBIZ Competition 2010 - Amaranth for Africa. Retrieved on 27th December 2018 from http://www.geebiz.org/downloads/Team%2018%20Report%20Winner.pdf

Kishore Kumar, V., Dharma Reddy, K. and Sharma, H. (2007). Expression of antixenosis and antibiosis components of resistance to spotted stem borer

Chilo partellus in sorghum under greenhouse conditions. *Journal of Semi-Arid Tropics Agricultural Research, 3*(1), 1-4.

Kitthawee, S., Sriplang, K., Brockelman, W. Y. and Baimai, V. (2004). Laboratory evaluation of density relationships of the parasitoid, *Spalangia endius* (Hymenoptera: Pteromalidae), with two species of tephritid fruit fly pupal hosts in Thailand. *ScienceAsia, 30,* 391-397.

Kogan, M. and Ortman, E. F. (1978). Antixenosis–a new term proposed to define Painter's "nonpreference" modality of resistance. *Bulletin of the Entomological Society of America, 24*(2), 175-176.

Kuruvilla, S. and Jacob, A. (1980). Pathogenicity of the entomogenous fungus *Paecilomyces farinosus* (Dickson ex Fries) to several insect pests. *Entomon, 5*(3), 175-176.

Landolt, P., Cha, D. and Davis, T. S. (2014). Attraction of the orange mint moth and false celery leaftier moth (Lepidoptera: Crambidae) to floral chemical lures. *Journal of Economic Entomology, 107*(2), 654-660.

Landolt, P., Jang, E., Carvalho, L. and Pogue, M. (2011a). Attraction of pest moths (Lepidoptera: Noctuidae, Crambidae) to floral lures on the island of Hawaii. *Proceedings of the Hawaiian Entomological Society, 43*, 49-58.

Landolt, P. J., Adams, T., Zack, R. S. and Crabo, L. (2011b). A diversity of moths (Lepidoptera) trapped with two feeding attractants. *Annals of the Entomological Society of America, 104*(3), 498-506.

Lauro, N., Kuhlmann, U., Mason, P. and Holliday, N. (2005). Interaction of a solitary larval endoparasitoid, *Microplitis mediator*, with its host, *Mamestra brassicae*: host acceptance and host suitability. *Journal of Applied Entomology, 129*, 567-573.

Lebreton, S., Chevrier, C. and Darrouzet, E. (2009). Sex allocation strategies in response to conspecifics' offspring sex ratio in solitary parasitoids. *Behavioral Ecology, 21*(1), 107-112.

Li, F., Bao, W., Wu, N. and You, C. (2008). Growth, biomass partitioning, and water-use efficiency of a leguminous shrub (*Bauhinia faberi* var. *microphylla*) in response to various water availabilities. *New Forests, 36*(1), 53-65.

Liu, F. and Stützel, H. (2002a). Leaf expansion, stomatal conductance, and transpiration of vegetable amaranth (*Amaranthus* sp.) in response to soil drying. *Journal of the American Society for Horticultural Science, 127*(5), 878-883.

Liu, F. and Stützel, H. (2002b). Leaf water relations of vegetable amaranth (*Amaranthus* spp.) in response to soil drying. *European Journal of Agronomy, 16*(2), 137-150.

Liu, F. and Stützel, H. (2004). Biomass partitioning, specific leaf area, and water use efficiency of vegetable amaranth (*Amaranthus* spp.) in response to drought stress. *Scientia Horticulturae, 102*(1), 15-27.

Liu, Z., Li, D., Gong, P. and Wu, K. (2004). Life table studies of the cotton bollworm, *Helicoverpa armigera* (Hübner)(Lepidoptera: Noctuidae), on different host plants. *Environmental Entomology, 33*(6), 1570-1576.

Losenge, T. (2005). Insect pest management for plant health: population dynamics of thrips in pure and mixed cropping, p. 35. *In*, 5th Horticultural Seminar on Sustainable Horticultural production in the Tropics, 23–26 November 2005, Njoro, Kenya. Horticultural Association of Kenya.

Lotter, D., Marshall, M., Weller, S. and Mugisha, A. (2014). African indigenous and traditional vegetables in Tanzania: Production, post-harvest management, and marketing. *African Crop Science Journal, 22*(3), 181-190.

Louw, S., Van Eeden, C. and Weekds, W. (1995). Curculionidae (Coleoptera) associated with wild and cultivated Amaranthus spp.(Amaranthaceae) in South Africa. *African Crop Science Journal, 3*, 93-98.

Luna, M. G., Sánchez, N. E. and Pereyra, P. C. (2007). Parasitism of *Tuta absoluta* (Lepidoptera, Gelechiidae) by *Pseudapanteles dignus* (Hymenoptera, Braconidae) under laboratory conditions. *Environmental entomology, 36*(4), 887-893.

Luoh, J. W., Begg, C. B., Symonds, R. C., Ledesma, D. and & Yang, R.-Y. (2014). Nutritional yield of African indigenous vegetables in water-deficient and water-sufficient conditions. *Food and Nutrition Sciences, 5*(09), 812.

Mackauer, M. and Sequeira, R. (1993). Patterns of development in insect parasites. In N. E. Beckage, S. N. Thompson and B. A. Federici (Eds.), *Parasites and Pathogens of Insects* (Vol. 1, pp. 1–12). San Diego, CA: Academic press.

Madl, M. and van Achterberg, C. (2014). A catalogue of the Braconidae (Hymenoptera: Ichneumonoidea) of the Malagasy subregion. *Linzer Biologische Beitraege, 46*(1), 5-220.

Mafi, S., and Ohbayashi, N. (2010). Some biological parameters of *Sympiesis striatipes* (Hymenoptera: Eulophidae), an ectoparasitoid of the citrus leafminer *Phyllocnistis citrella* (Lepidopetra: Gracillariidae). *Journal of Entomological Society of Iran, 30*, 29–40.

Magurran, A. E. (2004). *Measuring Biological Diversity*. Malden, MA: Blackwell Science Ltd.

Maini, S. and Burgio, G. (1990). Influence of trap design and phenylacetaldehyde upon field capture of male and female *Ostrinia nubilalis* (Hb.)(Lepidoptera: Pyralidae) and other moths. *Bollettino dell'Istituto di Entomologia della Università di Bologna, 45*, 157-165.

Malcicka, M. and Harvey, J. A. (2014). Trade-offs between developmental parameters of two endoparasitoids developing in different instars of the same host species. *Biological control, 74*, 52-58.

Malik, R., Dhankar, J. and Turner, N. (1979). Influence of soil water deficits on root growth of cotton seedlings. *Plant and soil, 53*, 109-115.

Mardani-Talaei, M., Nouri-Ganbalani, G., Naseri, B. and Hassanpour, M. (2012). Life history studies of the beet armyworm, *Spodoptera exigua* (Hübner)(Lepidoptera: Noctuidae) on 10 corn hybrids. *Journal of the Entomological Research Society, 14*(3), 9-18.

Martin, B., & Thorstenson, Y. R. (1988). Stable carbon isotope composition (δ13C), water use efficiency, and biomass productivity of *Lycopersicon esculentum, Lycopersicon pennellii*, and the F1 hybrid. *Plant Physiology, 88*(1), 213-217.

Martin, E., Baltazar, A., Ramo, J., Robles, R., De Data, S. and Kok, L. (2004). Efficacy of *Spoladea recurvalis* as biological control agent against the weed *Erianthema portusacastrum* L. *Philippine Entomologist, 18,* 174-176.

Martínez, G., Soler, R. and Dicke, M. (2013). Behavioral ecology of oviposition-site selection in herbivorous true bugs. *Advances in the Study of Behavior* (Vol. 45, pp. 175-207): Elsevier.

Masinde, P., Stützel, H., Agong, S. and Fricke, A. (2005). Plant growth, water relations, and transpiration of spiderplant [*Gynandropsis gynandra* (L.) Briq.] under water-limited conditions. *Journal of the American Society for Horticultural Science, 130*(3), 469-477.

Mbwambo, O., Abukutsa-Onyango, M., Dinssa, F. and Ojiewo, C. (2015). Performances of elite amaranth genotypes in grain and leaf yields in Northern Tanzania. *Journal of Horticulture and Forestry, 7*(2), 16-23.

Mlakar, S. G., Turinek, M., Jakop, M., Bavec, M. and Bavec, F. (2010). Grain amaranth as an alternative and perspective crop in temperate climate. *Journal for Geography, 5*(1), 135-145.

Mohan, M. C., Reddy, N. P., Devi, U. K., Kongara, R. and Sharma, H. C. (2007). Growth and insect assays of *Beauveria bassiana* with neem to test their compatibility and synergism. *Biocontrol Science and Technology, 17*(10), 1059-1069.

Monsreal-Ceballos, R. J., Ruiz-Sánchez, E., Ballina-Gómez, H. S., Reyes-Ramírez, A. and González-Moreno, A. (2018). Effects of botanical insecticides on Hymenopteran parasitoids: a meta-analysis approach. *Neotropical Entomology, 47*(5), 681-688.

Moran, P. J. and Showler, A. T. (2005). Plant responses to water deficit and shade stresses in pigweed and their influence on feeding and oviposition by the beet armyworm (Lepidoptera: Noctuidae). *Environmental Entomology, 34*(4), 929-937.

Moskova, C. (2013). Morphological and biological characteristics of species from the Amaranthus genus. *Scientific Papers Series A. Agronomy, 56*, 498-499.

Muchemi, S. K., Zebitz, C. P., Borgemeister, C., Akutse, K. S., Foba, C. N., Ekesi, S. and Fiaboe, K. K. (2018a). Acceptability and suitability of three Liriomyza Leafminer species as host for the endoparasitoid *Chrysocharis flacilla* (Hymenoptera: Eulophidae). *Journal of economic entomology, 111*(3), 1137-1143.

Muchemi, S. K., Zebitz, C. P., Borgemeister, C., Akutse, K. S., Foba, C. N., Ekesi, S. and Fiaboe, K. K. (2018b). Interaction between *Chrysocharis flacilla* and *Diglyphus isaea* (Hymenoptera: Eulophidae), two parasitoids of Liriomyza Leafminers. *Journal of economic entomology, 111*(2), 556-563.

Muchemi, S. K., Zebitz, C. P., Borgemeister, C., Akutse, K. S., Foba, C. N., Ekesi, S. and Fiaboe, K. K. (2018c). Acceptability and suitability of three Liriomyza species as host for the endoparasitoid *Halticoptera arduine* (Hymenoptera: Pteromalidae). *Environmental entomology, 47*(3), 684-691.

Mureithi, D. M., Komi, F. K., Ekesi, S. and Meyhöfer, R. (2017). Important arthropod pests on leafy Amaranth (*Amaranthus viridis, A. tricolor* and *A. blitum*) and broad-leafed African nightshade (*Solanum scabrum*) with a special focus on host-plant ranges. *African Journal of Horticultural Science, 11*, 1-17.

NAFIS - National Farmers Information Services (2011). Amaranth. Retrieved on 27th December 2018 from http://www.nafis.go.ke/vegetables/grain-amaranth/

Narayanan, E., Subba Rao, B. and Ramachandra Rao, M. (1957). *Hymenia recurvalis* F. and its parasite complex. *Proceedings: Plant Sciences, 46*(4), 241-246.

Nathan, S. S. and Kalaivani, K. (2006). Combined effects of azadirachtin and nucleopolyhedrovirus (SpltNPV) on *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) larvae. *Biological Control, 39*(1), 96-104.

Niveyro, S. L., Mortensen, A. G., Fomsgaard, I. S. and Salvo, A. (2013). Differences among five amaranth varieties (*Amaranthus* spp.) regarding secondary metabolites and foliar herbivory by chewing insects in the field. *Arthropod-Plant Interactions, 7*(2), 235-245.

Njau, G. M., Nyomora, A. M., Dinssa, F. F., Chang, J.-C., Malini, P., Subramanian, S. and Srinivasan, R. (2017). Evaluation of onion (*Allium cepa*) germplasm entries for resistance to onion thrips, *Thrips tabaci* (Lindeman) in Tanzania. *International Journal of Tropical Insect Science, 37*(2), 98-113.

NRC, (National Research Council) (1984). *Amaranth: Modern prospects for an ancient crop*. Washington, D.C: The National academy press.

Ode, P. J. and Hardy, I. C. (2008). Parasitoid sex ratios and biological control. In E. Wajnberg, C. Bernstein & J. van Alphen (Eds.), *Behavioural ecology of insect parasitoids: from theoretical approaches to field applications* (pp. 253-285). Malden, MA.: Blackwell Pub.

Ode, P. J. and Heinz, K. M. (2002). Host-size-dependent sex ratio theory and improving mass-reared parasitoid sex ratios. *Biological Control, 24*(1), 31-41.

Ode, P.J., Berenbaum, R. M., Zangerl, R. A. and Hardy, C. W. I. (2004). Host plant, host plant chemistry and the polyembryonic parasitoid *Copidosoma sosares*: indirect effects in a tritrophic interaction. *Oikos, 104*(2), 388-400.

Othim, S., Agbodzavu, K., Kahuthia-Gathu, R., Akutse, K., Muchemi, S., Ekesi, S. and Fiaboe, K. (2017). Performance of *Apanteles hemara* (Hymenoptera: Braconidae) on two Amaranth Leaf-webbers: *Spoladea recurvalis* and *Udea ferrugalis* (Lepidoptera: Crambidae). *Environmental Entomology, 46*(6), 1284-1291.

Othim, S. T., Kahuthia-Gathu, R., Akutse, K., Foba, C. and Fiaboe, K. (2018). Seasonal occurrence of amaranth Lepidopteran defoliators and effect of attractants and amaranth lines in their management *Journal of Applied Entomology*, *142*(7), 637-645.

Ouma, M. (2004). Indigenous Vegetable production and utilization in Suba district, Kenya: Improving health, unlocking the wealth in Suba district. *Bio Vision-TTU, ICIPE*.

Pande, Y. (1972). Some observations on the Bionomics of *Hymenia recurvalis* F.(Lepid., Pyralidae) feeding on *Trianthema monogyna* and *Amaranthus viridis* in India. *Journal of Applied Entomology, 72*, 362-366.

Pappas, M. L., Broekgaarden, C., Broufas, G. D., Kant, M. R., Messelink, G. J., Steppuhn, A., . . . van Dam, N. M. (2017). Induced plant defences in biological control of arthropod pests: a double-edged sword. *Pest Management Science, 73*(9), 1780-1788. doi: 10.1002/ps.4587

Peter, C. and Balasubramanian, R. (1984). New records of parasites of *Hymenia recurvalis* (Lepidoptera: Pyralidae) on Amaranthus. *Entomon (India), 9*, 71-72.

Phogat, B., Bhalla, S. and Bhagmal, B. (1994). Seasonal incidence of stem weevil (*Hypolyxus truncatulus*) and its effect on growth and grain yield of amaranth (*Amaranthus hypochondriacus*). *The Indian Journal of Agricultural Sciences, 64*(4), 261-262.

Radosevich, S., Holt, J. and Ghersa, C. (1997). *Weed ecology: implications for management*. New York: John Wiley and Sons.

Rakha, M., Bouba, N., Ramasamy, S., Regnard, J.-L. and Hanson, P. (2017a). Evaluation of wild tomato accessions (*Solanum* spp.) for resistance to two-spotted spider mite (*Tetranychus urticae* Koch) based on trichome type and acylsugar content. *Genetic Resources and Crop Evolution, 64*(5), 1011-1022.

Rakha, M., Hanson, P. and Ramasamy, S. (2017b). Identification of resistance to *Bemisia tabaci* Genn. in closely related wild relatives of cultivated tomato based on trichome type analysis and choice and no-choice assays. *Genetic Resources and Crop Evolution, 64*(2), 247-260.

Rakha, M., Zekeya, N., Sevgan, S., Musembi, M., Ramasamy, S. and Hanson, P. (2017c). Screening recently identified whitefly/spider mite-resistant wild tomato accessions for resistance to *Tuta absoluta*. *Plant Breeding, 136*(4), 562-568.

Sarfraz, M., Dosdall, L. and Keddie, B. (2009). Fitness of the parasitoid *Diadegma insulare* is affected by its host's food plants. *Basic and Applied Ecology, 10*(6), 563-572.

Sarfraz, R., Dosdall, L. and Keddie, B. (2012). Influence of the herbivore host's wild food plants on parasitism, survival and development of the parasitoid *Diadegma insulare*. *Biological Control, 62*(1), 38-44.

Seham, S., Mostafa, F., Aly, F. and Hussein, S. (2006). Certain biological aspects, threshold of development and thermal units for *Hymenia recurvalis* (Fab.),(Lepidoptera: Pyrallidae). *Arab Universities Journal of Agricultural Sciences, 14*(1), 447-456.

Sétamou, M., Jiang, N. and Schulthess, F. (2005). Effect of the host plant on the survivorship of parasitized *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) larvae and performance of its larval parasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae). *Biological Control, 32*(2), 183-190.

Shahrabian, E. and Soleymani, A. (2011). Effect of water deficit on morpho-physiologic behaviour of fodder cultivars of maize (*Zea mays* L.). *Research on Crops, 12*, 60-67.

Sharma, G. and Ramamurthy, V. V. (2009). A Checklist of lepidopterous pests of vegetables in India. Retrieved on 10th May 2015 from www.zsi.gov.in/zoological-survey-of-india/zsi-data/checklist/index.htm

Shi, Z. H., Liu, S. S. and Li, Y. X. (2002). *Cotesia plutellae* parasitizing *Plutella xylostella*: host-age dependent parasitism and its effect on host development and food consumption. *BioControl, 47*(5), 499-511.

Shirai, Y. (2006). Flight activity, reproduction, and adult nutrition of the beet webworm, *Spoladea recurvalis* (Lepidoptera: Pyralidae). *Applied Entomology and Zoology, 41*(3), 405-414.

Shroyer, J. P., Stegmeier, W. D., Mikesell, M. and China, S. A. (1990). Grain amaranth. *Kansas State University, Manhattan*.

Shuker, D. M., Pen, I., Duncan, A. B., Reece, S. E. and West, S. A. (2005). Sex ratios under asymmetrical local mate competition: theory and a test with parasitoid wasps. *The American Naturalist, 166*(3), 301-316.

Simmonds, M. S. (2003). Flavonoid–insect interactions: recent advances in our knowledge. *Phytochemistry, 64*(1), 21-30.

Slabbert, M. and Krüger, G. (2014). Antioxidant enzyme activity, proline accumulation, leaf area and cell membrane stability in water stressed *Amaranthus* leaves. *South African Journal of Botany, 95*, 123-128.

Spollen, W. G., Sharp, R. E., Saab, I. N. and Wu, Y. (1993). Regulation of cell expansion in roots and shoots at low water potentials. In J. A. C. Smith & H. Griffiths (Eds.), *Water Deficits: Plant Responses from Cell to Community* (Vol. 31, pp. 37–52). Oxford: Bios Scientific Publishers.

Srinivasan, R. (2012). Integrating biopesticides in pest management strategies for tropical vegetable production. *Journal of Biopesticides, 5*, 36-45.

Srinivasan, R. and Uthamasamy, S. (2005). Trichome density and antibiosis affect resistance of tomato to fruitborer and whitefly under laboratory conditions. *Journal of Vegetable Science, 11*(2), 3-17.

Stark, J. D. and Banks, J. E. (2003). Population-level effects of pesticides and other toxicants on arthropods. *Annual Review of Entomology, 48*(1), 505-519.

Summers, C. B. and Felton, G. W. (1994). Prooxidant effects of phenolic acids on the generalist herbivore *Helicoverpa zea* (Lepidoptera: Noctuidae): potential mode of action for phenolic compounds in plant anti-herbivore chemistry. *Insect Biochemistry and Molecular Biology, 24*(9), 943-953.

Tabashnik, B. E. (1994). Evolution of resistance to *Bacillus thuringiensis*. *Annual Review of Entomology, 39*(1), 47-79.

Taiz, L. and Zeiger, E. (2002). *Plant Physiology*. Sunderland, MA: Sinauer Associates Inc.

Tara, J., Azam, M., Ayri, S., Feroz, M. and Ramamurthy, V. (2009). Bionomics of *Hypolixus truncatulus* (F)(Coleoptera: Curculionidae. Lixinae: Lixini) a major pest of *Amaranthus caudatus* L. *Munis Entomology and Zoology, 4*(2), 510-518.

Tonnang, H. E., Nedorezov, L. V., Owino, J. O., Ochanda H. and Löhr, B. (2009). Evaluation of discrete host–parasitoid models for diamondback moth and *Diadegma semiclausum* field time population density series. *Ecological Modelling, 220*, 1735-1744.

Torres-Saldaña, G., Trinidad-Santos, A., Reyna-Trujillo, T., Castillo-Juárez, H., Bautista-Martínez, N. and León-González, D. (2004). Drilling of the stem of Amaranth by *Hypolixus truncatulus* (Coleoptera: Curculionidae) and

Amauromyza abnormalis (Diptera: Agromyzidae). *Acta Zoology Mexico, 20*, 131-140.

Tóth, M., Szarukán, I., Dorogi, B., Gulyás, A., Nagy, P. and Rozgonyi, Z. (2010). Male and female noctuid moths attracted to synthetic lures in Europe. *Journal of Chemical Ecology, 36*(6), 592-598.

Tran, D. H., and Takagi, M. (2006). Susceptibility of the stone leek leafminer *Liriomyza chinensis* (Diptera: Agromyzidae) to insecticides. *Journal of the Faculty of Agriculture Kyushu University, 50*, 383-390.

Trucco, F. and Tranel, P. J. (2011). Amaranthus. In C. Kole (Ed.), *Wild Crop Relatives: Genomic and Breeding Resources* (pp. 11-21): Springer-Verlag Berlin Heidelberg.

Tunca, H., Kilinçer, N. and Özkan, C. (2014a). Toxicity and repellent effects of some botanical insecticides on the egg-larval parasitoid *Chelonus oculator* Panzer (Hymenoptera: Braconidae). *Scientific Research and Essays, 9*(5), 106 - 113.

Tunca, H., Ozkan, C. and Beyarslan, A. (2014b). A rearing method of the larval parasitoid, *Apanteles carpatus* (Say)(Hymenoptera: Braconidae) on its new host *Monopis crocicapitella* (Clemens)(Lepidoptera: Tineidae). *African Journal of Agricultural Research, 9*(26), 2073-2076.

Turlings, T. C. and Benrey, B. (1998). Effects of plant metabolites on the behavior and development of parasitic wasps. *Ecoscience, 5*(3), 321-333.

Turner, N. C. (1996). Further progress in crop water relations. In D. L. Sparks (Ed.), *Advances in Agronomy* (Vol. 58, pp. 293-338): Elsevier.

van Alebeek, F. A., Koning, C., De Korte, E. and Van Huis, A. (1996). Egg-limited functional response of *Uscana lariophaga*, egg parasitoid of bruchid beetle pests in stored cowpea. *Entomologia Experimentalis et Applicata, 81*(2), 215-225.

van Emden, H. (1991). The role of host plant resistance in insect pest mis-management. *Bulletin of Entomological Research, 81*(2), 123-126.

Viqar, S. N. and Ali, S. V. (2012). Redescription of *Hymenia recurvalis* (Fabr.)(Lepidoptera: Pyraliidae: Pyraustini) with male genitalia from coastal belt of Sindh, Pakistan. *Federal Urdu University of Arts, Science and Technology Journal of Biology, 2*(1), 45-47.

Visser, M. E. (1994). The importance of being large: the relationship between size and fitness in females of the parasitoid *Aphaereta minuta* (Hymenoptera: Braconidae). *Journal of Animal Ecology*, *63*(4), 963-978.

Wajnberg, E., Bernstein, C. and Van Alphen, J. (2008). *Behavioral ecology of insect parasitoids: from theoretical approaches to field applications*: John Wiley & Sons.

War, A. R., Paulraj, M. G., Ahmad, T., Buhroo, A. A., Hussain, B., Ignacimuthu, S. and Sharma, H. C. (2012). Mechanisms of plant defense against insect herbivores. *Plant Signaling* and *Behavior, 7*(10), 1306-1320.

Weinberger, K. and Msuya, J. (2004). Indigenous vegetables in Tanzania: Significance and Prospects (Vol. Technical Bulletin No. 31). Arusha, Tanzania: AVRDC—The World Vegetable Center.

Wheeler, W. B. (2002). *Pesticides in Agriculture and the Environment*: CRC Press.

World Bank. (2011). Nutrition at a Glance: Tanzania. Retrieved on 27th December 2018 from http://www-wds.worldbank.org/external/default/WDSContentServer/WDSP/IB/2013/05/10/000442464_20130510154905/Rendered/PDF/772000BRI0Box000tanzania0April02011.pdf

Wright, G., Rao, R. and Farquhar, G. (1994). Water-use efficiency and carbon isotope discrimination in peanut under water deficit conditions. *Crop Science*, *34*(1), 92-97.

Yang, R. and Keding, G. B. (2012). Nutritional contributions of important African indigenous vegetables. In C. M. Shackleton, M. W. Pasquini & A. W. Drescher (Eds.), *African indigenous vegetables in urban agriculture* (pp. 105-144). London, UK: Earthscan.

Yu, D., Van Achterberg, C. and Horstmann, K. (2016). Taxapad 2016, Ichneumonoidea 2015. Database on flash-drive. Nepean, Ontario, Canada.

Zanuncio, J. C., Neto, M., da Costa, F., Tavares, W. d. S., Cruz, I., Leite, G. L. D. and Serrão, J. E. (2013). Functional and numerical responses and

reproduction of *Campoletis flavicincta* parasitizing *Spodoptera frugiperda* caterpillars. *Acta Scientiarum. Agronomy*, *35*(4), 419-426.

Zehnder, G., Gurr, G. M., Kühne, S., Wade, M. R., Wratten, S. D. and Wyss, E. (2007). Arthropod pest management in organic crops. *Annual Review of Entomology*, *52*, 57-80.

APPENDICES

Appendix 1: Publications emanating from the thesis

Othim, S.T.O., Kahuthia-Gathu, R., Ramasamy, S., Dubois, T., Ekesi, S. and Fiaboe, K.K.M. (2019). Effect of host age and density on the performance of *Apanteles hemara* (Hymenoptera: Braconidae), a larval endoparasitoid of *Spoladea recurvalis* (Lepidoptera: Crambidae). *Journal of economic entomology, 112* (5), 2131–2141.

Othim, S.T.O., Kahuthia-Gathu, R., Ramasamy, S., Dubois, T., Ekesi, S. and Fiaboe, K.K.M. (2019). The effects of pest-resistant amaranth accessions on the performance of the solitary endoparasitoid *Apanteles hemara* (Hymenoptera: Braconidae) against the amaranth leaf-webber *Spoladea recurvalis* (Lepidoptera: Crambidae). *Environmental entomology 48*(1), 163-172.

Othim, S.T.O., Kahuthia-Gathu, R., Ramasamy, S., Dubois, T., Dinssa, F.F., Ekesi, S. and Fiaboe, K.K.M. (2018). Screening for resistance against major lepidopteran and stem weevil pests of amaranth in Tanzania. *Euphytica, 214*(10), 182.

Othim, S.T.O., Kahuthia-Gathu, R., Ramasamy, S., Dubois, T., Ekesi, S. and Fiaboe, K.K.M. (2018). Expression of resistance in *Amaranthus* spp. (Caryophyllales: Amaranthaceae): effects of selected accessions on the behavior and biology of the amaranth leaf-webber, *Spoladea recurvalis* (Lepidoptera: Crambidae). *Insects, 9* (2), 62.