Characterisation of key pests of amaranth and nightshades in Kenya and development of integrated pest management (IPM) strategies

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Abstract

Scanty information is available concerning the identity of the major pests of amaranth and African nightshades in Kenya and associated damage. The natural enemies of these pests have also not been studied in detail. In this PhD study, field survey to identify the major pests of amaranth and nightshades, their abundance, distribution, and damage in six regions in Kenya was conducted. The natural enemies for these pests present in the amaranth and nightshade fields in these regions were also profiled. Based on the survey findings, field experiments to study the population dynamics and host range for the major pests of African nightshades was done. The performance of parasitoid *Aphidius colemani* Viereck for the control of *Aphis fabae* Scopoli and *Myzus persicae* Sulzer was also tested. Finally, the biology of the nightshade veinal mottle virus (NsVMV) was investigated. Survey findings showed that the damage by insect from various insect orders on amaranth was; Lepidoptera- 24.41±1.39%, Homoptera- 16.61±1.15%, Coleoptera- 14.99±0.89%, and Thysanoptera- 4.06±0.63%. However, the most destructive insect species on amaranth were *Spoladea recurvalis* Fabricius, and *Epicauta albovittata* Gestro in the rainy and dry season respectively. Four important amaranth pests that had not been reported as pests of amaranth in Kenya i.e. *Epicauta albovittata* Gestro, *Psara atritermina* Hampson, *Tuta absoluta* Meyrick and *Anyma octogueae* Guenèe were also observed. In the survey for the African nightshade pests, the greatest damage was caused by Homopterans (26.8 %), Coleoptera (16.5%), Lepidoptera (5.1%) and Thysanoptera (3.7%). We observed 47 Coleoptera species, 6 aphid species, 8 Lepidoptera species and 8 Thysanoptera species infesting the African nightshades. However, *A. fabae*, and *Epitrix silvicola* Bryant were the two most damaging pests on the crop. Majority of the natural enemies observed belonged to the Coleoptera and Hymenoptera insect orders among them the parasitoid *A. colemani* which was studied during this PhD project. In the population dynamics study, we showed that highest abundance of *A. fabae* was observed in the 2nd growing season at the mid altitude zone and in the 3rd growing season in the high altitude zone. For the *E. silvicola*, the highest abundance was observed in the 4th growing season at the mid altitude zone and in the 3rd growing season at the high altitude zone. For the Lepidopteran pests (*Spodoptera exigua*, *S. littoralis*, *Tuta absoluta* and *Plusia* sp.), the peak abundance was recorded in the 1st growing season at the mid altitude zone and 4th growing season at the high altitude zone. For most of the pests, colonization on African nightshades started early at the seedling stage. However, the population rose and fluctuated at different

phenological stages of crop growth. In the study of the performance of *A. colemani,* we showed for the first time that *A. colemani* has higher acceptance for *M. persicae* compared to *A. fabae* regardless whether the parasitoid was reared on *S. scabrum* or *S. villosum* as the host plants. However, higher parasitism was observed on *A. fabae*. Study on NsVMV revealed that *Solanum lycopersicum*, *Nicotiana occidentalis, Nicotiana.hesperis, Nicotiana debneyi, Nicotiana tabacum* cv. Samsun and *Nicandra sp* were the other hosts of the virus. There was no nightshade species/line resistant to the virus. In addition, 1000 seeds from NsVMV infected plants were germinated and found visually free from symptoms, indicating that the virus is if at all only to very low percentages seed-borne. Findings from the present study provide significant information necessary for designing and implementation of management interventions for the major pests of amaranth of African nightshades in Kenya.

Key words: *Amaranth pests, African nightshade pests, pest biodiversity, parasitoid, plant viruses*

Zusammenfassung

Es liegen nur wenige Informationen über die Identität der Hauptschädlinge von Amaranth und afrikanischem Nachtschatten und den damit verbundenen Schäden in Kenia vor. Auch die natürlichen Gegenspieler dieser Schädlinge wurden nicht im Detail untersucht. In der vorliegenden Doktorarbeit wurde deshalb eine Feldstudie durchgeführt, um die Hauptschädlinge von Amaranth und Nachtschatten zu identifizieren, sowie ihre Häufigkeit, Verbreitung und Schäden in sechs Regionen in Kenia zu analysieren. Auch natürliche Gegenspieler von Schädlingen, die in den Amaranth- und Nachtschattenfeldern in diesen Regionen vorhanden sind, wurden einbezogen. Basierend auf den Erhebungsergebnissen wurden Feldexperimente durchgeführt, um die Populationsdynamik und das Wirtsspektrum für die Hauptschädlinge afrikanischer Nachtschatten zu untersuchen. Die Leistung des Parasitoiden *Aphidius colemani* Viereck zur Bekämpfung von *Aphis fabae* Scopoli und *Myzus persicae* Sulzer wurde ebenfalls getestet. Schließlich wurde die Biologie des Nightshade Veinal Mottle Virus (NsVMV) untersucht. Die Erhebungsergebnisse zeigten, dass der Hauptschaden auf Amaranth durch Insekten von verschiedenen Insektenordnungen, d.h. Lepidoptera (24,41 ± 1,39%), Homoptera 16,61 ± 1,15%, Coleoptera 14,99 ± 0,89% und Thysanoptera 4,06 ± 0,63%, hervorgerufen wurde. Der größte Schaden an Amaranth wurde jedoch von dem Schmetterling *Spoladea recurvalis* Fabricius und dem Ölkäfer *Epicauta albovittata* Estra in der Regen- bzw. Trockenzeit hervorgerufen. Vier wichtige Amaranth-Schädlinge, die in Kenia bisher nicht als Amaranth-Schädlinge identifiziert worden waren, d. h. *Epicauta albovittata*, *Psara atritermina* Hampson, *Tuta absoluta* Meyrick und *Anyma octogueae* Guenèe, wurden ebenfalls beobachtet. Bei der Erhebung am afrikanischen Nachtschatten wurde der größte Schaden von Homoptera (26,8%), Coleoptera (16,5%), Lepidoptera (5,1%) und Thysanoptera (3,7%) verursacht. Insgesamt wurden 47 Käfer-Arten, 6 Blattlausarten, 8 Schmetterlings-Arten und 8 Thrips-Arten beobachteten, die deb afrikanischen Nachtschatten befallen. Die Blattlaus *A. fabae* und der Blattfloh *Epitrix silvicola* Bryant waren jedoch die beiden schädlichsten Insektenarten auf der Pflanze. Die Mehrheit der beobachteten natürlichen Feinde gehörte zu den Insektenordnungen der Coleoptera und Hymenoptera, darunter der Parasitoid *Aphidius colemani*, der im Rahmen dieser Doktorarbeit detaillierter untersucht wurde. In den Untersuchungen zur Populationsdynamik konnte gezeigt werden, dass die höchste Abundanz der Blattlaus *A. fabae* in der 2. Vegetationsperiode in der mittleren Höhenzone und in der 3. Vegetationsperiode in der oberen

Höhenzone beobachtet wurde. Für den Blattfloh *E. silvicola* wurde die höchste Abundanz in der 4. Vegetationsperiode in der mittleren Höhenzone und in der 3. Vegetationsperiode in der oberen Höhenzone beobachtet. Bei den Schadschmetterlingen (*Spodoptera exigua*, *S. littoralis*, *Tuta absoluta* und *Plusia* sp.) wurde die höchste Abundanz in der ersten Vegetationsperiode in der mittleren Höhenzone und in der vierten Wachstumsperiode in der oberen Höhenzone registriert. Bei den meisten Schädlingen begann die Kolonisierung des afrikanischen Nachtschatten früh im Keimlingsstadium. Die Population stieg jedoch an und schwankte bei verschiedenen phänologischen Wachstumsstadien. In der Untersuchung des Parasitierungsverhaltens von *A. colemani* zeigten wir zum ersten Mal, dass *A. colemani* eine höhere Akzeptanz für *M. persicae* im Vergleich zu *A. fabae* aufweist, unabhängig davon, ob der Parasitoid an *S. scabrum* oder *S. villosum* als Wirtspflanzen aufgezogen wurde. Bei *A. fabae* wurde jedoch eine höherer Parasitierungsleistung beobachtet. Die Untersuchungen zum erstmals beschriebenen Pflanzenvirus (NsVMV = Nightshade Veinal Mottle Virus) ergab, dass *Solanum lycopersicum, Nicotiana occidentalis, Nicotiana.hesperis, Nicotiana debneyi, Nicotiana tabacum* cv. Samsun und *Nicandra* sp zum Wirtspflanzenspektrum gehören. Es gab keine gegen das Virus resistente Nachtschattenart. Zusätzlich wurden 1000 Samen von NsVMV-infizierten Pflanzen zur Keimung gebracht. Visuell waren sie frei von Symptomen, was darauf hinweist, dass das Virus, wenn überhaupt, nur zu sehr geringen Prozentsätzen samenübertragbar ist. Die Ergebnisse der vorliegenden Untersuchungen liefern wichtige Informationen, die für die Planung und Durchführung von integrierten Pflanzenschutzmaßnahmen gegen Schadinsekten an Amaranth und afrikanischem Nachtschatten in Kenia notwendig sind.

Schlüsselbegriffe: *Schadinsekten, Amaranth, African nightshade, Biodiversität, Schlupfwespen, Pflanzenviren*

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Abbreviations

AIVs : African Indigenous vegetables

IPM : Integrated pest management

NsVMV : Nightshade veinal mottle virus

GDP : Gross Domestic Product

FAO : Food and Agriculture Organisation

HCDA : Horticultural Crop Development Authority

AEZ : Agro-ecological zone

asl : Above sea level

1. General Introduction

1.1 Role of AIVs in combating malnutrition and diseases

The highest incidences of malnutrition in the world are found in Sub-Saharan Africa (FAO, 2014). Moreover, cases of non-communicable lifestyle diseases such as cancers and heart ailments are also on the increase in the communities living in Africa (Tullao, 2002). The situation calls for urgent measures to arrest this challenge. African indigenous vegetables (AIVs), have numerous nutritional and health benefits and can be utilised to fight against malnutrition and lifestyle diseases in Africa (Nesamvuni et al., 2001; Yang and Keding, 2009). AIVs have higher content of protein, carotene, vitamin C, iron, calcium and magnesium than the exotic vegetables introduced to Africa from Europe (Maundu et al., 1999b). In particular leaves from amaranth (*Amaranthus viridis, A. cruentus* and *A. blitum*), African nightshade (*Solanum scabrum,, S. villosum, S. americanum, S. sarrachoides*), spider plant (*Cleome gynandra*), jute mallow (*Corchorus olitorius*, *C. tricularis*), sweet potato (*Ipomoea batatas*), cowpea (*Vigna unguiculata*), cassava (*Manihot esculenta*), and pumpkin (*Cucurbita* spp.) are a rich source of beta carotene and iron (Grubben and Denton, 2004; Weinberger and Swai, 2006). Additionally *Moringa stenopetala and M. esculenta* leaves have high content of vitamin c and e while *Pterocarpus mildbraedii* provides appreciable amounts of zinc. *Adansonia digitata and Rorippa madagascariensis* possesses antioxidant properties (Shackleton et al., 2009). AIVs also contain anti-oxidants that are useful in the fight against cancers and heart diseases (Uusiku et al., 2010).

1.2 Economic significance of AIVs

Agriculture is the backbone of the Kenyan economy with approximately 80 % of the population depending on it directly or indirectly. The agricultural sector accounts for 25% of Gross Domestic Product (GDP) (Muriuki et al., 2001). Horticulture is an important sub-sector in the agricultural sector. The sub-sector is dominated by small-scale holders who account for 70% of total horticultural production (McCulloch and Ota, 2002). The wide variety of horticultural crops enables small-scale farmers in Kenya who live in areas with varied climatic conditions to select crops suitable for their locality (Minot and Ngigi, 2004). Among the vegetable crops that are grown by Kenyan farmers are the African indigenous vegetables (AIVs). (Omiti et al., 2004). These are vegetable crops that have been grown and utilized as food in many African countries for generations. There are more than 210 species of AIVs in Kenya (Ngugi et al., 2006). Commonly grown AIVs include amaranth (*Amaranthus dubious, Amaranthus hybridus*), African nightshades (*Solanum scabrum, Solanum villosum, Solanum*

americanum, *Solanum macrocarpon*), spiderplant (*Cleome gynandra*), cowpea (*Vigna unguiculata*), and Ethiopian kale (*Brassica carinata*) among others (Maundu et al., 1999a).

Although previously referred as the poor man's vegetables, production and consumption of AIVs have increased greatly in the recent years due to consumer demand. The area put under the cultivation of AIVs also increased by 25 % between the years 2011 - 2013 (Cernansky, 2015). The market channels for AIVs have also expanded and currently they are sold both in open air markets and in the high-end markets such as the supermarkets (Irungu et al., 2007). The AIVs fetch better prices in the markets compared to the exotic vegetables making them play an important role in improving the income levels of the rural households who are the major producers (Lenne and Ward, 2010). For instance, a study conducted in Kiambu County, one of the regions where AIVs are cultivated in Kenya, revealed that production of AIVs earned farmers' up to three times the income they were obtaining from the production of exotic vegetables in the same unit of land (Muhanji et al., 2011).

1.3 Botanical characteristics and production of amaranth in Kenya

The word amaranth originates from a Greek work "amarantos" which means the one that does not wither or a never fading flower (Mosyakin and Robertson, 2003). Amaranth is an annual, dicotyledonous and herbaceous plant, 40 cm to over 3 m high, with a rigid upright stem, and flowers are a big inflorescence. Amaranth leaves are alternate, simple and petiolate, with some red or greenish colour and they are mostly edible. The flowers are very small purple or dark red or yellow-green. They are gathered in clusters grouped in spikes and panicles. Having a monocular ovary, fruits contain a tiny and lenticular shaped single seed (1.0 -1.5 mm diameter). Seeds have different colours and depending on plant species they may be white, gold, red and dark. Standard 1,000 amaranth seeds weight varies from 0.6 to 1.2 g (Bressani, 1993; Teutonico, 1985). There are more than 60 species in the genus amaranthaceae. However, only few of the species are used for human or animal consumption. *Amaranthus tricolor, A. lividus,* and *A. blitum* are grown for consumption of leaves whereas *A. cruentus/hybridus, A. caudatus,* and *A.hypocondriacus* are grown for grain production (Amicarelli and Camaggio, 2012).

A total of 17, 445 Mt of amaranth was produced in the year 2012, accounting for 2.6% of the total AIV produced in the Kenya. There is high concentration in production of amaranth (both grain and leaf) in the former Nyanza, Western, and Coast provinces of Kenya. Kisii, Nyamira, Kilifi Counties lead in production of amaranth in Kenya (HCDA, 2012).

1.4 Botanical characteristics and Production of African nightshades in Kenya

The African nightshade is an erect, many-branched herb growing 0.5 to 1.0 m high. The plant bears thin, oval, slightly purplish leaves up to 15 cm in length, has numerous white flowers and usually purple to black, round berries about 0.75 cm in diameter containing many small, flattened, yellow seeds. The species that are produced in Kenya include *S. macrocarpon*, *S. scabrum* and *S. villosum*. However *S. villosum* which bears orange berries is the most popular. Leaves from the plant are consumed after boiling them and discarding the water. They are a rich source of proteins, carbohydrates and vitamins. Fresh fruits are also consumed (www.infonet-biovision.org). With a total of 22,791 Mt produced in 3,440 hectares, African nightshade is the second most produced AIV after cowpeas in production (HCDA, 2012). Counties in the former Nyanza province are the leading producers of nightshade. Nyamira and Kisii counties account for 53% of the total national production. Other counties with high production of nightshade include; Bomet, Bungoma, Kakamega, Kisumu, Migori, and Narok counties (HCDA, 2012).

1.5 Pests of amaranth and African nightshades in Kenya

Production of AIVs in Kenya is constrained by arthropod pests and diseases that lower the quantity and quality of the produce (Gockowski and Ndumbe, 1997; Schippers, 2000). For instance, in Kenya, yield losses of between 20-100% by arthropod pests have been reported on amaranth and African nightshades (Sithanantham et al., 2003; Saunyama and Knapp, 2003). The major insect groups causing considerable losses to amaranth belong to the orders Lepidoptera, Coleoptera, Hemiptera, and Diptera (Clarke-Harris et al., 1998). More than 13 insect species belonging to orders Hemiptera, Lepidoptera, and Diptera as well as spider mites have been reported to attack African nightshades. In addition to direct losses, pests also lower the quality of the produce. For instance, aphid infestation significantly reduces product quality of produce through contamination with honeydew and subsequent growth of sooty mould, leading to frequent markets rejections (Varela and Seif, 2004). Moreover, leaves attacked by spider mites are generally twisted, webbed and therefore not marketable. Presence of pests and diseases in amaranth and African nightshades has lead to the overuse of chemical pesticides. As a result, the pesticide residues are more likely to be found on the produce thereby potentially causing health complications to the consumers and adverse effects to the environment. An integrated pest and disease management system for amaranth and African nightshades is necessary to enable production of these crops in a suitable manner.

1.6 Research aim

Until now, few studies on the identity and management of arthropod pests and diseases infecting amaranth and African nightshades in Kenya have been conducted. In particular, arthropod pests infesting amaranth and African nightshades in major production regions of Kenya have not been profiled in detail. Moreover, the seasonal abundances of the key pests of African nightshades in Kenya have not been studied extensively. The natural enemies occurring in amaranth and nightshade fields for control of arthropod pests infesting the two crops have not been identified and their potential for the management of amaranth and nightshade pests have not been explored. Moreover, the biological properties of the nightshade veinal mottle virus (NsVMV), a new plant virus infecting African nightshades (Schimmel et al., 2015), are unknown.

The overall objective in the current study was to develop knowledge on the key pests of amaranth and African nightshades and their natural enemies in Kenya as a pre-requisite for developing effective integrated pest management (IPM) measures for their control. Specifically, the study aimed to answer the following questions;

1. What are the biodiversity, abundance, distribution, and damage of arthropod pests infesting amaranth and African nightshades in key production counties in Kenya and their natural enemies?
2. How do agro-ecology, seasonality and crop phenological stages affect the abundance of major African nightshade pests and natural enemies?
3. Which are the alternative hosts for key pests infesting African nightshades among the crops/weeds growing around the African nightshade fields?
4. What is the potential of parasitiod *Aphidius colemani* in control of *Aphis gossypii* and *Myzus persicae* on African nightshades?
5. What are the host range, host resistance, aphid transmission and seed transmission of nightshade veinal mottle virus (NsVMV)?

Knowledge of the key pests of amaranth and nightshades and their distribution in Kenya will provide farmers and extension workers with critical information on which pests to prioritise on in their management practices in different seasons. The new information obtained on the pests and their natural enemies will be useful in development of IPM measures for these pests.

1.7 Outline of thesis

Amaranth and African nightshades are important AIVs in Kenya which are attacked by different types of pests. Neighbouring plants and wild plants occurring in and around the amaranth and nightshade crop may also play as alternative host to pests that infest the two crops. In **Chapter 2**, I take a critical review of the literature for major pest species of amaranth and African nightshades with a focus on their host plant ranges.

Occurrence and distribution of pests and their natural enemies of a particular crop may vary from one agro-ecological zone to another. In **Chapter 3** and **Chapter 4**, I examine the biodiversity, distribution, abundance, and damage of pest insects in major growing regions infesting amaranth and African nightshades on farmers fields. The natural enemies of these pests that are naturally occurring in amaranth and African nightshades fields are also discussed.

Agro-ecological zone, growing season and phenological stage of African nightshade influence the abundance of key pests. In **Chapter 5**, I study the influence of these factors on the abundance of *A. fabae*, *E. silvicola* and Lepidopteran pests (*Spodoptera exigua*, *S. littoralis*, *Tuta absoluta* and *Plusia* sp.) in four growing seasons in the year 2015 and 2016 under controlled conditions. In the same study, I examine the crops and wild plants growing in close proximity with the African nightshade fields for the presence of African nightshade pests.

Since aphids belong to the main pest species the promotion or release of aphid parasitoids seems to be an obvious strategy for IPM. But it's likely that the aphid host plant and the aphid species identity influence the performance of parasitoids. In **Chapter 6**, I evaluate the host acceptability and suitability of two aphid species, *A. fabae* and *M. persicae*, feeding on two different African nightshade species, *S. scabrum* and *S. villosum*, by the common aphid parasitoid *Aphidius colemani*.

Other than the pests, production of African nightshades in Kenya is faced with a threat of a new plant virus, the nightshade veinal mottle virus (NsVMV) which is transmissible by aphid *M. persicae*. However, little is known with regard to its biological properties. In **Chapter 7**, I test the host range, host resistance, transmission by aphid species A. fabae and by seed of NsVMV.

In **Chapter 8**, I review the most important finding from my PhD study and discuss their contribution in development of IPM measures for the key pests of amaranth and African nightshades in Kenya.

1.8 References

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

Bressani R. 1993. Amaranth, In: Macrae, R., Robinson, R.K., Sadler M.J., (Editors). Encyclopaedia of Food Science, Food Technology and Nutrition, vol. 1, Academic Press, London, pp. 135-140

Cernansky R. 2015. Super vegetables. Nature **522(7555)**: 146–148.

FAO, IFAD, WFP. 2014. The State of Food Insecurity in the World 2014. Strengthening the enabling environment for food security and nutrition. Rome, FAO.

Gockowski J, Ndumbe M. 1997. The transformation of leafy vegetable cropping systems along the humid forest margins of Cameroon in African Indigenous Vegetables. Proceedings of the NRI/IPGRI/CPRO workshop, 13-18 Jan. 1997, Limbe, Cameroon.. (R. Schippers & L. Budd, eds) ODA, UK.

Grubben GJH, Denton OA. 2004. Plant Resources of Tropical Africa 2: Vegetables, PROTA Foundation, Wageningen, Netherlands/Backhuys Publishers, Leiden, Netherlands/CTA, Wageningen, Netherlands

HCDA. 2012. Horticulture Validated report- Ministry of Agriculture & Horticultural Crops Development Authority

http://www.infonet-biovision.org/PlantHealth/Crops/African-Nightshade

Irungu C, Mburu J, Maundu P, Grum M, Hoescle-Zeledon I. 2007. Analysis of markets for African leafy vegetables within Nairobi and its environs and implications for on-farm conservation of biodiversity. A Consultancy Report for Global Facilitation Unit for Underutilised Species, Rome, Italy.

Lenne JM, Ward AF. 2010. Improving the efficiency of domestic vegetablemarketingsystems in East Africa constraints and opportunities. *Outlook on Agriculture* **39(1)**: 31–40.

Maundu PM, Ndungu GW, Kabuye CHS. 1999a. Traditional food plants of Kenya. National Museums of Kenya, Nairobi, Kenya.

Maundu PM, Njiro EI, Chweya JA, Imungi JK, Seme EN. 1999b. The Kenyan Case Study. In: Chweya, J.A. and P.B. Eyzaguirre (Eds.), The Biodiversity of Traditional Leafy Vegetables. IPGRI, Rome, pp: 51-84.

Mbugua G, Gitonga LN, Mureithi E, Karoga J, Manyeki L. 2006. Farmer-Participatory prioritization and development of agronomic practices for African Leafy Vegetables. Proceedings of 6th Horticultural Seminar on Sustainable Horticultural production in the Tropics held in Njoro, Kenya.

McCulloch N, Ota M. 2002. Export Horticulture and Poverty in Kenya. Working Paper No. 174, Institute of Development Studies, University of Sussex, Brighton.

Mosyakin SL, Robertson KR. 2003. Amaranthus. In: Flora of North America. North of Mexico. Oxford University Press, New York, USA

Muhanji G, Ralph LR, Webo C, Mwangi S. 2011. African indigenous vegetable enterprises and market access for small-scale farmers in East Africa. *International Journal of Agricultural Sustainability* **9: (1)**: 194-202.

Muriuki HG, Mwangi DM, Thorpe W. 2001. How smallholder dairy systems in Kenya contribute to food security and poverty alleviation: Results of recent collaborative studies. Proceeding at the 28th Tanzania Society of Animal Production Conference. Morogoro, August7- 9, 2001.

Nesamvuni C, Steyn N, Potgieter M. 2001. Nutritional value of wild, leafy plants consumed by the Vhavenda. *South African Journal of Science* **97**

Ngugi IK, Gitau R, Nyoro JK. 2006. Kenya Access to high value markets by smallholder farmers of African indigenous vegetables in Kenya. Tegemeo Institute, Egerton University.

Omiti JM, Omolo JO, Manyengo JU. 2004. Policy Constraints in Vegetable Marketing in Kenya. Discussion Paper No. 061/2004, Institute of Policy Analysis and Research (IPAR), Nairobi.

Uusiku NP, Oelofse A, Duodu KG, Bester MJ, Faber M. 2010. Nutritional value of leafy vegetables of sub-Saharan Africa and their potential contribution to human health: A review. *Journal of Food Composition and Analysis* **23(6)**: 499–509.

Yang R. -Y, Keding GB. 2009. Nutritional contributions of important African indigenous vegetables. London: African indigenous vegetables in urban agriculture. Earthscan, 105-144.

Schimmel J, Büttner C, Meyhöfer R, Maiß E. 2016. Nachweis eines neuen Potyvirus: Night shade veinal mottle virus (NSVMV) aus *Solanum nigrum* und *Nicotiana benthamiana*. *Phytomedizin Jahrgang* **46 (2)**: 6

Schippers RR. 2000. African Indigenous Vegetables. An Overview of the Cultivated Species. Natural Resources Institute/ACP-EU Technical Centre for Agricultural and Rural Cooperation, Chatham, UK. Pp 214.

Shackleton CM, Pasquini MW, Drescher AW. 2009. African Indigenous Vegetables in Urban Agriculture. Earthscan. 117-119

Teutonico RA, Knorr D. 1985. Amaranth: composition, properties and applications of a rediscovered food crop, In: Food Technology 39, pp. 49-61.

Tullao TS. 2002. The impact of economic globalization on noncommunicable diseases: Opportunities and threats. Diets and Noncommunicable Diseases: World Health Organization Globalization.

Weinberger K, Swai IS. 2006. 'Consumption of traditional vegetables in central and north-eastern Tanzania' *Ecology of Food and Nutrition* **45(2)**: 87–103

Saunyama IG, Knapp M. 2003. Effect of pruning and trellising of tomatoes on red spider mite incidence and crop yield in Zimbabwe. *African Crop Science Journal* **11:** 269-277

2 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 (based on Mureithi et al., 2017)

2.1 Abstract

Leafy amaranths and African nightshades are important African Indigenous vegetables (AIVs) with numerous nutritional and health benefits. However, their production is faced with several challenges - key among them integrated control of arthropod pests. The insect groups attacking these vegetables include a range of hemipterans, dipterans, lepidopterans, and coleopteran species. Moreover, other crop and weed species frequently serve as alternative hosts to amaranth and nightshade pests in absence of the crops or when pest management measures have been applied. This review will evaluate the major pests attacking leaf amaranth and African nightshades and their potential host ranges. Potential viral diseases transmitted by these insects on African nightshades will also be highlighted. The final aim of the project will be to characterize infection pathways in the production system and agricultural landscape to develop new options of pest control.

Key words**:** *African Indigenous vegetables, Infection pathways, Pest distribution.*

2.2 Introduction

Among the main African indigenous vegetables (AIVs) produced in Kenya are leafy amaranths and African nightshades (Mbugua et al., 2006; HCDA, 2012). *Amaranthus tricolor, A. lividus,* and *A. blitum* (Caryophyllales, Amaranthaceae) are grown for consumption of leaves (Amicarelli and Camaggio, 2012). The African nightshade species that are produced in Kenya include *Solanum macrocarpon*, *S. scabrum* and *S. villosum* (Solanales, Solanaceae). The insect groups attacking these two vegetables include; defoliators, sucking insects, stem borers, fruit/pod borers, leafminers and webbers (Schippers, 2000; Sithanantham et al., 2003). Development of sustainable integrated pest and disease management strategies are of high priority in the HORTINLEA project for production of healthy vegetables. In this context, World distribution of amaranth and nightshade pests in general and in East Africa in particular, host-ranges and their damage are discussed with the aim of characterizing their infection/infestation pathways in the production system and agricultural landscape.

2.3 Pests of amaranth and their host ranges

Amaranth is attacked by numerous herbivorous arthropod pests that feed on various plant parts such as roots, stems, leaves, flowers and seeds. The major insect groups causing considerable losses to amaranth belong to the orders Lepidoptera, Coleoptera, Hemiptera, and Diptera (Clarke-Harris et al., 1998). Beet webworm, *Spoladea recurvalis* Fabricius (Lepidoptera; Crambidae) is distributed in tropical and sub-tropical regions of Asia, Africa and Australia. Other than amaranth, Garden beet and swiss chards are other Chenopodeaceae crops commonly grown in amaranth production zones in East Africa and might serve as major hosts of *S. recurvalis*. The pest also attacks several weed species that are found in amaranth fields including *Chenopodium album* (Chenopodiaceae), *Portulaca oleracea* (Portulacaceae), and *Trianthema portulacastrum* (Aizoaceae) (Table 2-1) (Capinera, 2011; Kedar et al., 2013). Alternative hosts could serve to perpetuate the pest in absence of amaranth or further increase their population if present together with amaranth due to abundance in food sources. The larvae skeletonize the leaves before rolling them to provide shelter during pupation. Huge losses caused by *S. recurvalis* on amaranth have been reported in Nigeria (Aderolu et al., 2013).

Cotton leafworm *Spodoptera littoralis* Boisduval (Lepidoptera; Noctuidae) is a severe lepidopteran pest of amaranth and African nightshade. The pest is widely distributed throughout Africa including East African countries of Kenya, Uganda and Tanzania. It is also

present in other tropical and sub tropical regions of Asia and Europe (Miller, 1976; Sidibe and Lauge, 1977). *Spodoptera littoralis* is a highly polyphagous species which is able to feed on more than 87 plant species covering 40 different families such as Amaranthaceae, Brassicaceae, Liliaceae, Malvaceae, Chenopodiaceae, Fabaceae, Solanaceae, Curcubitaceae, and Poaceae. Besides amaranth, onion, cabbage, capsicum, beans, maize, potato, tomato and eggplants are potential major hosts of the pest grown in amaranth production areas. Minor hosts in the familyApiaceae such as carrots are also grown in amaranth production areas and could serve as an alternative host to *S. littoralis*. Wild hosts such as lantana (Verbenaceae), jatropha (Euphorbiaceae) and wild strawberries (Rosaceae) could also provide food resources (nectar and foliage) to the pest (Table 1) (Salama et al., 1970; Brown and Dewhurst, 1975; Badr, 1982; Rizk et al., 1988; Holloway, 1989; Mohamed, 2003). The ability of the pest to fly long distances could enable *S. littoralis* to reach many other hosts which may be far away in absence of amaranth crop and later return to infest newly established amaranth. The pest is a voracious feeder shredding leaves of the host plant and leaving large irregular holes. Considerable yield losses on amaranth have been reported in Nigeria and Mexico (Aragón et al., 1997; Aderolu et al., 2013).

Amaranth stem weevils, *Hypolixus* sp. (Coleoptera; Curculionidae) are among the most serious coleopteran pests of amaranth. Species known to be destructive to the crop include *H. truncatulus*, *H. haerens*, and *H. nubilosus* (Gupta and Rawat, 1954; Louw et al., 1995; Torres-Saldaña et al., 2004; Kagali et al., 2013). Besides Amaranth, no other host plant has been documented for *Hypolixus* sp suggesting that the pest could be managed by cultural practices such as closed season or crop rotation (Table 2-1). Weevil larvae damage the stem by burrowing and feeding on the stem tissues and leaving their excreta therein while the adults are leaf-feeders. Feeding by the pest causes stunting, reduction in leaf yield, development of tumours on the stem and eventual drying up of the plant (Tara et al., 2009; Imam et al., 2010). Plant infestation of up to 81 % has been reported in India.

The pea leafminer, *Liriomyza huidobrensis* Blanchard (Diptera; Agromyzidae) is among the leafminer flies challenging the production of amaranth. *L. huidobrensis* is widespread in the Mediterranean region. However, it has colonized other regions of the world (America, Asia, Africa and the Oceania). In East Africa, it has been reported in Kenya and Tanzania (Chabi-Olaye et al., 2008; EPPO 2014; Foba et al., 2015). *Liriomyza huidobrensis* is highly polyphagous and is known to attack host plants from 14 different families, both cultivated and wild including amaranth. Other popular crops grown alongside amaranth which the pest

uses as host include faba beans, onions, garlic and snowpeas. Oxalis, datura and tagetes are wild hosts of *L. huidobrensis* that invade amaranth farms leading to higher epidemics of the pest (Table 2-1) (Mujica and Kroschel, 2011; Foba et al., 2015). The pest manifests itself by burrowing irregular white mines with dampened black and dried brown areas on the leaves. Yield losses of between 20-100 % on different crops have been reported in Kenya (Spencer 1973, 1990; OEPP/EPPO, 2005).

The green peach aphid, *Myzus persicae* Sulzer (Hemiptera; Aphididae) is distributed throughout the world except in areas with extreme temperatures or moisture. The pest is present in East African countries including Kenya (Millar, 1994; CIE, 1979; Remaudiere and Autrique, 1985). *M. persicae* is a serious pest of Amaranth. Groundnuts, capsicums, carrots, maize, beans, potato, tomato and eggplants which are cultivated in amaranth growing regions of East Africa also serve as alternative hosts of *M. persicae* leading to high population build-up of the pest (Table 2-1) (Heathcote, 1962; Tamaki 1975). Significant yield losses have been reported in potato, sugarbeets and peach (Barbagallo et al., 2007). The pest vectors important plant viruses such as Potato leaf roll virus (PLRV), Potato virus Y (PVY), Cucumber mosaic virus (CMV), and Pepper veinal mottle virus (PVMV). Among the listed viruses, PVY is the only one that has been shown to infect amaranth experimentally. However, the other virus could also infect amaranth as they are hosted by other plant species that also grow in the same neighbourhood as amaranth such as potato, tomato, capsicums, and pumpkin. Common weeds in amaranth fields such as datura and *Physalis ungulata* are also hosts of the viruses listed (http://www.cabi.org; Kennedy et al., 1962).

Other important pests infesting leaf amaranth that have been reported in Africa include; *Sylepta derogota* (Lepidoptera; Pyralidae), *Herpetogramma bipunctalis* (Lepidoptera, Crambidae), *Liriomyza sativae* (Diptera; Agromyzidae), and *Empoasca* sp. (Hemiptera; Cicadellidae) (Table 2-1) (Aragón et al.,1997; Garcia et al., 2011; Sæthre et al., 2011; Aderolu et al., 2013; Kagali et.al., 2013). Although scanty information is available on some of these pests with regard to their geographical distribution in Africa, host range, virus transmission and economic importance, they pose a serious challenge in production of Amaranth due to their long distance flight capability particularly the Lepidopterans and the Dipterans.

Table 2-1: Pests of Amaranth and host range on crops and weeds (--- = no information available).

Order	Family	Species	Distribution	Major hosts	Other hosts	Weed hosts	Damage	Importance	Reference
Coleoptera	Curculionidae	*Hypolixus sp* Amaranth stem weevil	India, Mexico, Nigeria, South Africa, Kenya	*Amaranthus* sp	---	---	Stem burrowing by larva Adults feed on leaves	A serious pest of amaranth in Mexico, India, South Africa and Kenya	Gupta and Rawat 1954 ; Louw *et al.*,, 1995; Torres-Saldaña *et al.*,, 2004; Kagali *et al.*,, 2013; Tara *et al.*,, 2009; Imam *et al.*,, 2010
Diptera	Agromyzidae	*Liriomyza huidobrensis* Sepentine leafminer/ Pea leafminer	Mediterraneam region, present in several countries in America, Asia, Oceania, Africa inchuding Kenya	*Amaranth* sp, *Gypsophila* sp, *Vicia faba*, *Allium cepa*, *Allium sativum*, *Dianthus caryophyllus*, *Cucumis sativus*, *Lactuca sativa*, *Solanum tuberosum*, *Spinacia oleracea*, *Pisum sativum*, *Raphanus sativus*	---	*Oxalis sp*, *Datura stramonium*, *Sonchus* sp., *Tagetes* sp.	Irregular white mines with dampened black and dried brown areas on the leaves.	Lowering of aesthetic value of ornamentals,yield reduction in vegetables	CABI/EPPO 2002; EPPO 2014; Spencer 1973 1990; OEPP/EPPO 2005; Mujica and Kroschel 2011
Diptera	Agromyzidae	*Liriomyza sativae* Vegetable leafminer	Worldwide, in Africa, reported from Kenya, Sudan, Nigeria, Cameroon, and Zimbabwe	*Medicago sativa*, *Solanum melongena*, *Capsicum* sp. *Solanum lycopersicum*, *Solanum tuberosum*, *Pisum sativum*	*Amaranthus* sp., *Aster* sp., *Cucumis sativus*, *Apium graveolens*, *Lathyrus* sp, *Citrullus lanatus*, *Tropaeolum* sp	*Erechtites hieraciifolia*, *Synedrella nodiflora*, *Deeringia amarantoides*, *Ipomoea aquatic*, *Basella alba*	Mines on the leaves	Losses of up to 80 % have been recorded in celery and *Medicago sativa*, severe yield loss in tomato and other field crops Transmission of Celery mosaic potyvirus	Smith *et al.*,, 1962; Musgrave et al.,, 1975; Zitter *et al.*,, 1980 ; Spencer, 1982; CIE 1986
Hemiptera	Aphididae	*Myzus persicae* Green peach aphid	Worldwide except in areas with extreme temperatures and moisture	*Apium graveolens*, *Arachis hypogaea*, *Capsicum* sp, *Carika papaya*, *Citrullus lanatus*, *Daucus carota*, *Nicotiana tabacum*, *Phaseolus vulgaris*, *Zea mays*, *Solanum esculentum*, *Solanum tuberosum*, *Solanum melongena*	*Pisum sativum*, *Vigna unguiculata*, *Solanum nigrum*	Dicotyledonous weeds	Direct damage through sucking of plant sap Transmission of plant viruses	Heavy losses have been reported on potato, sugarbeets, and peach	Millar 1994; CIE 1979; Remaudiere & Autrique 1985; Heathcote 1962; Tamaki 1975; Barbagallo *et al.*, 2007
Hemiptera	Cicadellidae	*Empoasca sp* Leafhopper	Nigeria	*Amaranthus* sp. *Nicotiana tabacum*	---	---	Sucking plant sap from the leaves causing "hopper burn"	Vectoring viruses, bacteria, and fungi	Aragón *et al.*,, 1997; Kallenbach *et al.*, 2012

Table 2-1: Pests of Amaranth and host range on crops and weeds (continued) (--- = no information available).

Order	Family	Species	Distribution	Major hosts	Other hosts	Weed hosts	Damage	Importance	Reference
Hemiptera	Miridae	Lygus lineolaris Tarnished plant bug	Canada, Mexico, USA, Nigeria	*Amaranthus* sp, *Daucus carota*, *Gossypium hirsutum*, *Phaseolus lunatus*, *Medicago sativum*, *Phaseolus vulgaris*, *Glycine max*, *Solanum esculentum*, *Malus domestica*, *Prunus avium*, *Prunus persica* , *Pyrus communis*, *Fragaria Ananassa*	Most vegetable crops	---	Yellowing and distortion of terminal growth, ragged and discoloured leaves Flower abortion	Losses of up to 50 % have been reported on nursery stock	Haseman 1918 ; Tingey and Pillemer 1977; Young 1986; Aragón *et al.*,, 1997; Capinera 2001
Lepidoptera	Crambidae	*Herpetogramma bipunctalis* Southern beet webworm moth	Many tropical and sub-tropical regions of the world	*Beta vulgaris subsp. vulgaris*, *spinacia oleracea*, *Amaranthus* sp	*Capsicum* sp, *Zea mays*, *Gossypium hirsutum*, *Brassica* sp, *Medicago sativum*, *Arachis hypogaea*, *Solanum tuberosum*, *Solanum esculentum*	Purslane, *Portulaca oleracea* *Solanum nigram*, *Chenopodium album*, *S. indicum*	Larva burrows and feed on the stem tissues causing lodging and death of the plants.	---	Allyson 1984; Solis 2006 ; Capinera 2011; www.africanmoths.com
Lepidoptera	Crambidae	*Spoladea recurvalis*, Hawaiian beet webworm	**Many African countries**	*Beta vulgaris*, *Amaranthus* sp	---	*Chenopodium album*, *Portulaca oleracea*, *Trianthema portulacastrum*	Sclerotization and rolling of the leaves	Most abundant pest of amaranth in Nigeria	Capinera 2011; Aderolu *et al.*,, 2013; Kedar *et al.*,, 2013
Lepidoptera	Noctuidae	*Spodoptera littoralis* Cotton leafworm	Subtropical and tropical range, **Africa**, Asia, Turkey, Spain, Greece	*Amaranthus* sp, *Allium cepa*, *Brassica* sp, *Capsicum* sp, Curcubitaceae, *Gossypium hirsutum*, *Phaseolus vulgaris*, *Zea mays*, *Spinacea oleracea*, *Solanum tuberosum*, *Solanum esculentum*, *Solanum melongena*	Apium graveolens, *Trigonella Foenum*, *Musa domestica*, *Asparagus officinalis*	*Lantana camara*, *Jatropha curcas* , *Hibiscus rosa-sinensis*	Shredding of leaves Premature fruit drop Holes on fruits	Considerable leaf yield losses on amaranth Severe damage to flowering and fruiting points on cotton and cowpea	Miller 1976; Sidibe and Lauge 1977; Salama *et al.*,, 1970 ; Brown and Dewhurst 1975; Badr 1982; Rizk *et al.*,, 1988; Holloway 1989; Mohamed 2003; Aragón *et al.*, 1997 Aderolu *et al.*,, 2013
Lepidoptera	Pyralidae	*Sylepta derogota* Cotton leaf roller	**Africa**, Asia, Oceanic	*Abelmoschus esculentus*, *Gossypium hirsutum*, *Manihot esculenta*, *Corchorus* olitorius	*Solanum esculentum*, *Solanum melongena*, *Amaranthus* sp, *Durio zibethinus*, *Coleus* sp.	---	Feeding on leaf margins Leaf rolling	Losses of between 10-14 % have been reported on cotton	Odebiyi 1982; Zang 1994; CABI 2007; The Natural History Museum 2007

2.4. Pests of African nightshades and their host ranges

African nightshade is attacked mainly by herbivorous arthropod pests that feed on leaves. More than 13 insect species belonging to orders Hemiptera, Lepidoptera, and Diptera as well as spider mites have been reported to attack African nightshades. The most serious pests on African nightshades are discussed. Aphids (Hemiptera; Aphididae) are among the most important sucking insects attacking African nightshades. The leaves infested by aphids curl and fold causing distorted and retarded growth of young apical shoots. Moreover, aphid infestation significantly reduces crop quality through contamination with honeydew and subsequent sooty mould, leading to frequent markets rejections. (AVRDC, 2003; Varela and Seif, 2004). The major aphid species attacking African nightshades include *Aphis gossypii, A. craccivora,* and *A. fabae* (Ashilenje et al., 2011; Suganthy and Sakthivel, 2012; Singh et al., 2014).

The cotton aphid, *A. gossypii* Glover is present worldwide including the East African region. It can survive in both hot and cold regions of the world (UK CAB International, 1968). The pest has a wide host range in over 92 plant families. Among the primary hosts are crops in the Malvaceae, Cucurbitaceae, and Solanaceae families such as cotton, pumpkins, cucumber tomato, and nightshades. Other hosts of the pest include; maize, beans, cabbages, kales, and *Bidens pilosa* (Table 2-2) (Ebert and Cartwright., 1997). Presence of the mentioned host plants in East Africaenables the perpetual survival of *A. gossypii* in farmlands throughout the year and recolonisation of the new nightshade crop upon establishment. Transmission of viruses is the most devastating impact of the pest with a potential of transmitting over 30 plant viruses such as Cucumber mosaic virus (CMV), Pepper veinal mottle virus (PVMV) and Potato leafroll virus (PLRV) (http://www.cabi.org ; Kennedy et al., 1962; Ebert and Cartwright, 1997). These viruses are present in East Africa and have been reported to infect nightshades alongside other Solanaceae crops such as tomato, potato, capsicums, and weed species such as datura and *Physalis ungulata*. Melon, Pumpkin, common beans, faba beans, maize and Oxalis are also hosts of CMV and could serve as a reservoir of the virus in absence of nightshades. Yield losses of up to 80 % have been reported on cotton in Zambia. However, losses on African nightshades are yet to be quantified.

The cowpea aphid, *A. craccivora* Koch, has a wide distribution in the tropics where it is among the most common aphid species. Among other East African countries, the pest is also present in Kenya (CIE, 1983; Blackman and Eastop, 2000). Although *A. craccivora* has higher preference for plants in the Fabaceae family, it is a polyphagous pest that uses 18 other

plant families such as Amaranthaceae, Solanaceae, and Malvaceae. Host crops for *A. craccivora* that are found areas where nightshades are grown in East Africa include; beans, cowpea, mung beans, pigeon peas, groundnuts, pepper, amaranth and citrus fruits. Wild hosts to the pest include; *Commelina benghalensis, Palisota hirsute, Boerhavia diffusa,* and *Portulaca oleracea* (Table 2-2)(Sæthre et al., 2011). *A. craccivora* transmits about 30 different plant viruses including Cucumber mosaic virus (CMV), and Alfalfa mosaic virus (AMV) that are known to infect nightshades and other common vegetables present in nightshade growing areas of East Africa such as tomato, potato, pepper, common beans, faba beans, eggplant, and beetroot. (http://www.cabi.org; Jones, 1967; Bock 1973).

The black bean aphid, *A. fabae* Scopoli, is highly polyphagous and plants in the families Solanaceae, Amaranthaceae, Chenopodiaceae, Brassicaceae, Cucurbitaceae, and Fabaceae serve as suitable hosts. Among the potential alternative cultivated hosts of *A. fabae* in nightshade production areas are; common beans, runner beans, and broad beans. Common weeds found in nightshade farms that could serve as alternative hosts to *A. fabae* are *Chenopodium album, Physalis wrightii, Sonchus oleraceus Amaranthus retroflexus,* and *Amysynchia intermedia* (Table 2-2). The major damage by this pest is through direct feeding (Cammell and Way, 1983). Although *A. fabae* transmits over 30 viruses, the damage is low on other plants except *Beta vulgaris*. Important virus transmitted by *A. fabae* and is present in East Africa is Potato virus Y (PVY). The virus not only infects nightshades but other crops and weed species discussed earlier in this review that are present in nightshade growing areas.

Spidermites, *Tetranychus* spp. (Trombidiformes; Tetranychidae), are a menace in production of African nightshades particularly in dry weather conditions. The underside of African nightshade leaves attacked by spidermites turn bronze, rusty or yellowish. Severe infestation results to cobwebbing on the plant and may lead to the death of the plant. *Tetranychus evansi* Baker & Pritchard and *Tetranychus urticae* Koch cause most serious damage to African nightshades (Jepson et al., 1975; Moraes et al., 1987; Park and Lee 2002; Fiaboe et al., 2006; Murungi et al., 2011). Tomato red spider mite, *T. evansi* originated from South America. However, it is currently distributedin many African countries including Kenya (Migeon & Dorkeld, 2006-2012). *T. evansi* is a specialist spidermite species mainly foraging on plants in the Solanaceae family. Tomato, potato and eggplant commonly grown in the same agroecological zone or in the same field with African nightshades are the other preferred Solanaceae hosts (Moraes et al., 1987). Minor hosts are in Asteraceae, Fabaceae,

Cucurbitaceae, Malvaceae, Poaceae, Chenopodiaceae, Euphorbiaceae, Amaranthaceae and Brassicaceae families among others (Migeon and Dorkeld 2006–2012). *Chenopodium* sp., *Conyza* sp., and *Sonchus* sp. are common weeds in nightshade fields that also serve as alternative refuge to *T. Evansi* (Table 2). Losses of 90 % have been reported in field trials in Namibia (Jeppson et al., 1975; Gutierrez and Etienne, 1986).

The two spotted spider mite, *Tetranychus urticae* is widely spread in many parts of the world. It was reported in Kenya in 1996 (IIE, 1996; Bolland et al., 1998). *T. urticae* has a wide host range from wild plants, ornamentals, vegetable plants, and fruits. Other than African nightshades, it forages on many other crops such as tomato, common beans, cucumber, eggplant, pepper, sorghum onion, garlic and cotton, many of which are grown in similar areas as nightshades thereby serving to perpetuate the pest further (Table 2-2). (Jepson *et al.,* 1975; Bolland et al., 1998). Economic damage of 13 % has been recorded on Soybean.

Flea beetles (*Phyllotreta* sp. and *Epitrix* sp.), *Herpetogramma bipunctalis*, *Agrotis* sp., Sp*odoptera* sp., *Tuta absoluta*, whiteflies, thrips, *Liriomyza* sp. and nematodes (*Meloidogyne* sp.) are other important pests of African nightshades and many other crops and weed species in nightshade growing zones. Flea beetles have particularly been observed to cause immense damage in African nightshades farms in Kenya although they have not been properly documented.

Table 2-2: Pests of African nightshades and host range on crops and weeds.

Order	Family	Species	Distribution	Major hosts	Other hosts	Weed hosts	Damage	Importance	References
Coleoptera	Chrysomelidae	*Epitrix* sp Potato flea beetle	North, Central and South America, Portugal, Kenya (during recent surveys)	*Solanum tuberosum*, *Solanum scabrum*, *Solanum melongena*, *Nicotiana tabacum*, *Capsicum sp*, *Solanum esculentum*	Chenopodiaceae, Cucurbitaceae, Fabaceae, Brassicaceae, Poacea,	*Datura stramonium*, *Solanum nigrum*, *S. trifolium*	Shot-holes on the leaves	Yield losses up to 20 % have been reported	Neilson & Finlayson 1953; Wallis 1957; CABI 2007; Boavida & Germain 2009
Coleoptera	Chrysomelidae	*Phyllotreta* sp Striped flea beetle	No information, but observed in Kenya during 2014 survey	Brassicaceae	*Amaranthus* sp *Beta vulgaris*	Weed plants in the families Euphorbiaceae, Asteraceae, Solanaceae	Shot-holes on the leaves	Leaf damage of up to 71 % has been observed on *Brassica oleraceae* var. *capitata*	Mayori & Mikunthan 2009
Hemiptera	Aleyrodidae	*Bemisia tabaci* Sweet potato whitefly	Africa, Asia, North America, South America, Oceania	Many plant families	Many plant families	Many	Leaf chlorosis Viruses transmission	Yield losses to crops of between 20 and 100 % have been reported from Geminiviruses	Mound & Halsey 1978; IAPSC 1985; CIE 1986 ; Brown & Bird 1992; Perring *et al.*,, 1993
Hemiptera	Aleyrodidae	*Trialeurodes vaporariorum* Greenhouse whitefly	Widespread in many parts of the world including Africa	Many vegetable and agricultural crops	Many vegetable crops	*Stellaria media*	Necrotic spots on leaves, tissue distortion, dwarfing	Direct feeding, virus transmission(Beet pseudo-yellows virus, Strawberry pallidosis virus)	Byrne *et al.*,, 1990; EPPO 2014
Hemiptera	Aphididae	*Aphis craccivora* Cowpea Aphid/ Groundnut Aphid	Abundant in subtropical and tropical regions and the Mediterranean	*Phaseolus vulgaris*, *Vigna unguiculata*, *Vigna radiata*, *Arachis hypogaea*, *Cajanus cajan*	*Solanum scabrum*, *Lactuca sativa*, *Gossypium hirsutum*, *Capsicum* sp, *Citrus* sp, *Amaranthus* sp	*Commelina benghalensis*, *Palisota hirsute*, *Boerhavia diffusa*, *Portulaca oleracea*	Direct feeding, virus transmission.	Transmits about 30 plant viruses on Groundnuts, Beans, Peas, Brassicaceae, Cucurbits, and Beets.	Jones 1967; Bock 1973; CIE 1983 ; Blackman & Eastop 2000
Hemiptera	Aphididae	*Aphis fabae* Black bean aphid	Worldwide	*Beta vulgaris*, *Phaseolus vulgaris*, *Phaseolus coccineus*, *Vicia faba*	Many vegetable and agricultural crops	*Chenopodium album*, *Physalis wrightii*, *Sonchus oleraceus* *Amaranthus retroflexus*, *Amysynchia intermedia*	stunting of the plants or death in severe infestation.	Yield and quality reduction particularly on crops in Fabaceae family, serious injury due to transmission of viruses has only been witnessed on *Beta vulgaris*	Cammell and Way 1983; Fernandez-Quintanilla *et al.*,,2002
Hemiptera	Aphididae	*Aphis gossypii* Cotton Aphid/ Melon aphid	Widespread worldwide	*Carica papaya*, *Cucurbita pepo*, *Cucumis sativus*, *Gossypium hirsutum*. *Solanum esculentum*	Many crops in the families; Brassicaceae, Fabaceae, Solanaceae, Poaceae	*Bidens pilosa*, *Commelina benghalensis*, *Brachiaria lata*	Yellowing and curling of leaves, sooty moulds on leaves	Over 30 plant viruses transmitted including Potato leafroll virus, Pepper veinal mottle and virus	Kennedy *et al.*, 1962; UK CAB International 1968; Ebert & Cartwright 1997

Table 2-2: Pests of African nightshades and host range on crops and weeds (Continued).

Order	Family	Species	Distribution	Major hosts	Other hosts	Weed hosts	Damage	Importance	References
Lepidoptera	Gelechiidae	*Tuta absoluta* Tomato leafminer	South America, Israel, **Several African countries including; Kenya, Tanzania, Ethiopia,** Senegal, Nigeria, Niger, Egypt, Algeria	*Solanum esculentum*	*Solanum scabrum Solanum tuberosum*	*Solanum elaeagnifolium, Solanum puberulum, Datura stramonium, Datura ferox, Nicotiana glauca*	Burrows into the leaves lowering the photosynthetic rate of the plants	100 % economic loss has been reported on tomato Ban on trade on commodities infested by the pest	Garcia & Espul 1982; Zappalà *et al.*, 2012; Zlof & Suffert 2012; CABI/EPPO 2013; IPPC 2014
Lepidoptera	Noctuidae	*Agrotis* sp. Cutworm	Widely distributed in **Africa**	Allium cepa, *Abelmoschus esculentus, Arachis hypogaea*, Brassicaceae, *Cicer arietinum, Solanum esculentum, Solanum tuberosum, Zea mays*	*Agrostis palustris, Poa pratensis Prunus persica, Prunus domestica*	*Mentha* sp. *Solanum nigrum, Convolvulus* sp	Cutting the seedling stems at the ground level	May cause economic injury to seedlings of maize, many vegetables, cotton, tobacco, turf grasses	CIE 1969
Nematoda	Tylenchormopha	*Meloidogyne javanica Meloidogyne enterelobii Meloidogyne incognita*	Tropical and sub tropical regions of the world including Kenya	*Solanum scabrum, Solanum villosum, Solanum esculentum*	Many agricultural crops such as. *Curcubita pepo, Citrullus lanatus, Amaranthus* sp. *Coffea* sp	*Bidens pilosa, Ageratum conyzoides, Emex australis, Galinsoga parviflora*	Development of root-knots Yellowing accompanied by stunted growth	10-100 % yield loss	CABI/EPPO, 2002a; CABI/EPPO, 2002b; Chitambo et al., 2016
Trombidiformes	Tetranychidae	*Tetranychus evansi* Tomato red spider mite	Many **African** countries, South America	*Solanum esculentum, Solanum melongena, Nicotiana tabacum, Solanum tuberosum*	Plants in the following families; Asteraceae, Fabaceae, Cucurbitaceae, Malvaceae, Poaceae, Chenopodiaceae, Euphorbiaceae, Amaranthaceae Brassicaceae	*Chenopodium* sp., *Convolvus* sp., *Conyza* sp., *Diplotaxis* sp., *Hordeum murinum, Lavatera* sp., *Sonchus* sp.	Leaves turn bleached yellow-orange followed by rapid death	Most important dry season pest of tomato in South Africa and in Reunion; losses of 90 % have been reported in field trials in Namibia	Jepson *et al.*, 1975; Gutierrez & Etienne 1986; Moraes *et al.*,1987; Migeon & Dorkeld 2006-2012
Trombidiformes	Tetranychidae	*Tetranychus urticae* Red spider mite/ two spotted red spider mite	Widely distributed in the world including **Africa**	Solanum esculentum, Phaseolus vulgaris, *Gossypium hirsutum, Zea mays, Cucumis sativus, Sorghum bicolor, Solanum melongena, Capsicum* sp	*Allium cepa, Allium sativum*	Many weeds in the families; Solanaceae, Fabaceae, Malvaceae, Poaceae, Cucurbitaceae, Liliaceae, Chenopodiaceae	Reduction to the photosynthetic rate of leaves	Yield reduction on cotton, tomato, apple, peach and strawberry. Fruit damage-reduction in fruit quality on tomato	Jepson *et al.*, 1975;Sances *et al.*,1982;Mobley & Marini 1990; Nihoul *et al.*,1992;Bondada *et al.*,1995;IIE 1996 ;Bolland *et al.*, 1998;Meck *et al.*,2012

2.5 Conclusion

Although the major pests of leafy amaranth are chewing insects mainly Lepidopterans and Coleopterans, production of African nightshades is chiefly constrained by sucking insects particularly the aphids and spider mites. The importance of the mentioned key pests is due their abundance in amaranth and nightshade farms, and the direct and indirect damage they cause on the crop. This has been supported by own survey done in Kenya (unpublished data). The plant host range for many pests of the two crops are broad, cutting across many vegetable, agricultural crops as well as and weed species commonly found in or around amaranth or nightshade growing fields. However, some of the pests are specialist herbivores mainly feeding on Amaranthaceae or Solanaceae families. Sucking pests know to transmit plant viruses are more important in Nightshades, therefore it is likely that viral diseases play a larger role in constraining production of African nightshades compared to Amaranth. There are also a higher number of other hosts and weed species for pests of African nightshades as compared to Amaranth possibly due to a lower number of sucking insects infesting amaranth or due to missing information on host range of some of the pests of Amaranth. In considering integrated pest management measures for both crops, whole farm evaluation should be done taking in to account not only the crop of interest but also the other crops and weed species present in the farm. Larger areas should be considered for management of amaranth pests due to their ability to fly longer distances particularly the Lepidopterans.

2.6 References

ADAS. 1991. *Protected crops technical notes*. Agricultural Development and Advisory Service, Ministry of Agriculture, Fisheries and Food, UK. No. 144

Aderolu IA, Omooloye AA, Okelana FA. 2013. Occurrence, Abundance and Control of the Major Insect Pests Associated with Amaranths in Ibadan, Nigeria. *Entomol Ornithol Herpetol* **2**: 112.

Allyson S. 1984. Description of last instar larvae of 22 species of North American Spilomelini (Lepidoptera: Pyralidae: Pyraustinae) with a key to species. *Canadian Entomologist* **116**: 1301-1334

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

Aragón GA, Tapia-Rojas AM, Huerta-Sánchez SIMT. 1997. Insects associated with the Amaranth crop *Amaranthus hypocondriacus* L. (Amaranthaceae) in the Tehuacán Valley, Puebla, Mexico. *Folia Entomol. Mex.*, **100**: 33-43.

Ashilenje DS, Omunyin ME, and Okalebo JR. 2011. Influence of potassium on aphid incidence and yield of vegetable African nightshades (*Solanum* L. section *Solanum*). *Annals of Biological Research* **2 (3)**:317-326

Aunu RA, Merle S, Marshall WJ. 2010. Leafminers in vegetables, ornamental plants and weeds in Indonesia: Surveys of host crops, species composition and parasitoids. *International Journal of Pest Management* **46(4)**:257-266

AVRDC. 2003. Broad-leaved Nightshade, International Cooperators Fact Sheet 8, Arusha, Tanzania.

Badr, NA. 1982. Biological and ecological studies on the cotton leaf worm, Spodoptera littoralis (Boisd.) Ph.D. Thesis, Fac. Agric., Cairo Univ

Barbagallo S, Cocuzza G, Cravedi P, Komazaki S. 2007. IPM case studies: deciduous fruit trees. In: Aphids as crop pests [ed. by Emden, H. F. van\Harrington, R.]. Wallingford, UK: CABI, 651-661.

Blackman RI, Eastop VF. 1985. Aphids on the World's Crop: An Identification Guide. John Wiley and Sons. London.

Boavida C. 2009. Presence in Portugal of Epitrix similaris Gentner (Coleoptera: Chrysomelidae: Alticinae), an exotic pest of potato tubers. *Boletı´n de Sanidad Vegetal Plagas* **35(1)**:73–74

Bock KR, 1973. Peanut mottle virus in East Africa. *Annals of Applied Biology*. 74(2):171-179

Bolland HR, Gutierrez J, Flechtmann CHW. 1998. World Catalogue of the Spider Mite Family (Acari: Tetranychidae). Leiden, Netherlands: K. Brill, pp 392.

Bondada BR, Oosterhuis DM, Tugwell NP, Kim KS. 1995. Physiological and cytological studies of two spotted spider mite, Tetranychus urticae K., injury in cotton. *Southwestern Entomologist*. **20(2):**171-180

Brown ES, Dewhurst CF. 1975. The genus Spodoptera (Lepidoptera, Noctuidae) in Africa and the Near East. *Bulletin of Entomological Research*. **65(2):**221-262.

Brown, JK, Bird J. 1992. Whitefly-transmitted geminiviruses and associated disorders in the Americas and the Caribbean Basin. *Plant Disease* **76**:220-225.

Brunt AA, Kenten RH, Phillips S. 1978. Symptomatologically distinct strains of Pepper veinal mottle virus from four (4) West African Solanaceous crops. *Ann App Biology* **88**:115-119

Byrne DNJr, Bellows TS, Parrella MP. 1990. Whiteflies in agricultural systems, In: D. Gerling (ed.). Whiteflies: their bionomics, pest status, and management. Intercept, Ltd., Andover, Hants., UK. pp 227–261.

CABI/EPPO. 2002a. Meloidogyne incognita. Distribution Maps of Plant Diseases. No. 854. Wallingford, UK. CAB International.

CABI/EPPO, 2002b. Meloidogyne javanica. Distribution Maps of Plant Diseases, No. 855. Wallingford, UK. CAB International.

CABI/EPPO. 2013. Tuta absoluta. [Distribution map]. Distribution Maps of Plant Pests, No.December. Wallingford, UK CABI Map 723 (2nd revision).

CABI, 2007. Datasheet on *Haritalodes derogata* (cotton leaf roller). CABI Crop protection compendium.

Cammell ME, Way MJ. 1983. Aphid Pests. *In* P. D. Hebblethwaite [ed.], The Faba Bean (*Vicia faba* L.), A Basis for Improvement. Butterworths, London. pp. 315-346.

Capinera JL. 2001. Handbook of Vegetable Pests. Academic Press, New York. Pp 729.

Chabi-Olaye A, Mujica N, Lo¨hr B, Kroschel J. 2008. Role of agroecosystems in the abundance and diversity of Liriomyza leafmining flies and their natural enemies: In Proceedings, XXIII International Congress of Entomology, 6-12th July 2008,in Durban, South Africa.

Chitambo O, Haukeland S, Fiaboe KKM, Kariuki GM, and Grundle FMW. 2016. First Report of the Root-Knot Nematode Meloidogyne enterolobii Parasitizing African Nightshades in Kenya. *Plant Disease PDIS PDN*. 11-15-1300

CIE. 1979. Distribution Maps of Plant Pests. Wallingford, UK: CAB International. No. 45.

CIE. 1986. Distribution Maps of Pests, Series A. CAB International, Wallingford, UK. No. 477

CIE. 1986. Distribution Maps of Pests, Series A. CAB International, Wallingford, UK. No. 284

Clarke-Harris D, Fleischer SJ, Fender A. 1998. Identification Guide: Major Pests of Callaloo. Pennsylvania State University. University Park, PA, USA.

Degri MM, Onu I, Dike MC, Ayuba MM. 2007. Host plants of the serpentine leafminer, *Liriomyza sativae* Blanchard (Diptera: Agromyzidae) in Bauchi state, Nigeria. *Niger. J. Entomol.,* 24:122-130.

Degri MM, Ayuba MM, Yoriyo KP. 2012. Bio-efficacy of some aqueous plant extracts and cyromazine (Trigard 169) in the management of leafminer (*Liriomyza* sp.) on egg plant in the Northern Guinea Savanna of Nigeria. *Niger. J. Exp. Appl. Biol.*, 13(2):125-130.

Duarte VS, Silva RA, Wekesa VW, Rizzato FB, Dias CTS, Delalibera IJr. 2009. Impact of natural epizootics of the fungal pathogen Neozygites floridana (Zygomycetes: Entomophthorales) on population dynamics of Tetranychus evansi (Acari: Tetranychidae) in tomato and nightshade. *Biological Control*, **51(1)**:81–90

Ebert AW, Wu T, Wang S. 2011. Vegetable amaranth (*Amaranthus* L.) International Cooperator's guide. AVRDC – The World Vegetable Center: pp 11-754.

Ebert T A, Cartwright B. 1997. Biology and ecology of Aphis gossypii Glover (Homoptera: Aphididae). *Southwestern Entomologist*. **22(1)**:116-153

EPPO. 2013. PQR database. Paris, France: European and Mediterranean Plant Protection Organization. http://www.eppo.int/Databases/pqr/pqr.htm

EPPO. 2014. PQR database. Paris, France: European and Mediterranean Plant Protection Organization. http://www.eppo.int/DATABASES/pqr/pqr.htm

Fernandez-Quintanilla C, Fereres A, Godfrey L, Norris RF. 2002. Development and reproduction of *Myzus persicae* and *Aphis fabae* (Hom., Aphididae) on selected weed species surrounding sugar beet fields. *J. Appl. Ent.* **126**:198–202

FOBA CN, Salifu D, Lagat ZO, Gitonga LM, Akutse KS, Fiaboe KKM. 2015. Species Composition, Distribution, and Seasonal Abundance of Liriomyza Leafminers (Diptera: Agromyzidae) Under Different Vegetable Production Systems and Agroecological Zones in Kenya. Environmental Entomology **44(2)**:223-232. 2015

Fiaboe KKM, Fonseca RL, de Moraes GJ, Ogol CKPO, Knapp M. 2006. Identification of priority areas in South America for exploration of natural enemies for classical biological control of *Tetranychus evansi* (Acari: Tetranychidae) in Africa. *Biological Control* **38**:373–379.

Garcia MF, Espul JC, 1982. Bioecology of the tomato moth (Scrobipalpula absoluta) in Mendoza, Argentine Republic. *Revista de Investigaciones Agropecuarias*. **17(2):**135-146

Gupta RL, Rawat RR. 1954. Life-history of *Hypolixus truncatulus* (Boh.) (Lixus branchyrhinus Boh.) The Rajgira Weevil. *Indian Journal of Entomology* **16 (2):**142-144.

Gutierrez J, Etienne J. 1986. Tetranychidae of Reunion Island Indian Ocean and some of their predators. *Agronomie Tropicale* **41(1):** 84-91.

Haseman L. 1918. The tarnished plant-bug and its injury to nursery stock. University of Missouri, College of Agriculture. *Agricultural Experiment Station Research Bulletin* **29**:26

Heathcote GD. 1962. The suitability of some plant hosts for the development of the peach-potato aphid, *Myzus persicae* (Sulzer). *Entomologica Experimentalis et Appliciata* **5**:114-118.

Holloway JD, 1989. The moths of Borneo: family Noctuidae, trifine subfamilies: Noctuinae, Heliothinae, Hadeninae, Acronictinae, Amphipyrinae, Agaristinae. *Malayan Nature Journal*. **42(2-3):**57-228

HCDA. 2012. Horticulture Validated report- Ministry of Agriculture & Horticultural Crops Development Authority

IAPSC.1985. Distribution Maps of Major Crop Pests and Diseases in Africa. Inter-African Phytosanitary Council, Yaoundé, Cameroon. No. 188

Igwegbe ECK, Waterworth HE. 1982. Properties and serology of the strain of Pepper veinal mottle virus isolated from eggplant (Solanum melongena L.) in Nigeria. *Phytopathologische Zeitschrift* **103(1)**:9-12

IIE. 1996. Distribution maps of pests. Wallingford, UK: CAB International. No. 562

Imam TS, Yusuf AU, Mukhtar MD. 2010. A survey of some insect pests of cultivated vegetables in three selected irrigation areas along Jakara river, Kano, Nigeria

Int. J. Biol. Chem. Sci. 4(2): 400-406

IPPC. 2014. New pest in Kenya: Preliminary surveillance report on *Tuta absoluta* - preliminary report by Kenya. IPPC Official Pest Report, No. KEN-01/2. Rome, Italy: FAO. https://www.ippc.int/

Jeppson LR, Keifer HH, Baker EW. 1975. Mites injurious to economic plants. University of California Press, Berkley

Jones MG. 1967. Observations on two races of the groundnut aphid, *Aphis craccivora*. *Entomologia Experimentalis Applicata* 10: 31-38

Kedar SC, Kumaranag KM. 2013. Report on outbreak of Sp*oladea recurvalis* (Fabricus) on Trianthema portulacastrum L. and its parasite from Haryana, India. *Journal of Entomological Research*. **37(2):**149- 151

Kagali RN, Kioko EN, Osiemo Z, Muya S, Wachera C. 2013. Insect abundance and diversity on cultivated *Amaranthus* Sp. (Amaranthaceae) in Meru County, Kenya. *American International Journal of Contemporary Research* **3**: 7

Kallenbach M1, Bonaventure G, Gilardoni PA, Wissgott A, Baldwin IT. 2012. Empoasca leafhoppers attack wild tobacco plants in a jasmonate-dependent manner and identify jasmonate mutants in natural populations. *Proc Natl Acad Sci U S A* **12(109)(24):**E1548-57

Kennedy JS, Day MF, Eastop VF. 1962. A Conspectus of Aphids as Vectors of Plant Viruses. Wallingford, UK: CAB International.

Ladipo JL, Roberts IM. 1977. Pepper veinal mottle viru associated with a streak disease of tomato in Nigeria. *Ann Appl Biol* **87**:133-138

Marullo R. 1998. Impact of an introduced pest thrips on the indigenous natural history and agricultural systems of southern Italy. Thrips and tospoviruses: proceedings of the 7th international symposium on thysanoptera held from 2-7th of July 2001 in Calabria, Italy.

McDonald JG and Singh RP. 1996. Host range, symptomology and serology of isolates of Potato virus Y (PVY) that share properties with both the PVY^N and PVY^O strain groups. *Amer. Pot. J.* **73:** 309- 314

Mayoori K, Mikunthan G. 2009. Damage pattern of cabbage flea beetle, *Phyllotreta cruciferae* (Goeze) (Coleoptera : Chrysomelidae) and its associated host of crops and weeds. *Amer. – Eurasian. J. Agric & Environ. Sci*., **6 (3):**303-307.

Mbugua G, Gitonga LN, Mureithi E, Karoga J, Manyeki L. 2006. Farmer-Participatory prioritization and development of agronomic practices for African Leafy Vegetables. Proceedings of 6th Horticultural Seminar on Sustainable Horticultural production in the Tropics held in Njoro, Kenya.

Meck ED, Walgenbach JF, Kennedy GG. 2012. Association of *Tetranychus urticae* (Acari: Tetranychidae) feeding and gold fleck damage on tomato fruit. *Crop Protection* **42**:24-29.

Miller GW. 1976. Cold storage as a quarantine treatment to prevent the introduction of Sp*odoptera littoralis* (Boisd.) into glasshouses in the UK. *Plant Pathology* **25(4):**193-196.

Migeon A, Dorkeld F. 2006–2012. Spider Mites Web: a comprehensive database for the Tetranychidae. INRA, http://www.montpellier.inra.fr/CBGP/spmweb/

Millar IM. 1994. A catalogue of the aphids (Homoptera: Aphidoidea) of sub-Saharan Africa. Plant Protection Research Institute Handbook No. 4. Pretoria, South Africa: Agricutural Research Council.

Mobley KN, Marini RP. 1990. Gas exchange characteristics of apple and peach leaves infested by European red mite and twospotted spider mite. *Journal of the American Society for Horticultural Science* **115(5):**757-761

Moraes GJD, McMurtry JA, Baker EW. 1987. Redescription and distribution of the spider mites *Tetranychus evansi* and *T. marianae*. *Acarologia* **28**:333–334.

Mohamed, HM. 2003. Comparative study of host plants on growth, development and fecundity of the cotton leaf worm Sp*odoptera littoralis* (Boisduval), Noctuide: Lepidoptera. *J. Egypt. Ger. Soc. Zool.* **42E**:167-183.

Mound LA, Halsey SH. 1978. Whiteflies of the world, a systematic catalogue of the Aleyrodidae (Homoptera) with host plant and natural enemy data. British Museum (Natural History), London, UK.

Mujica N, Kroschel J. 2011. Leafminer Fly (Diptera: Agromyzidae) Occurrence, Distribution, and Parasitoid Associations in Field and Vegetable Crops along the Peruvian Coast. *Environ. Entomol.* **40(2)**: 217-230

Murungi LK, Knapp M, Salifu D, Wesonga J, Nyende A, Masinde P, Torto B. 2011. Differential effects of various African nightshade species on the fecundity and movement of *Tetranychus evansi* (Acari: Tetranychidae). *International Journal of Tropical Insect Science* **31(4):**269–276

Musgrave CA, Poe SL, Weems HV. 1975. The vegetable leafminer *Liriomyza sativae* Blanchard. *Entomology Circular, Florida Department of Agriculture and Consumer Services, Division of Plant Industry* **162**:1-4.

Neilson CL, Finlayson DG. 1953. Notes on the biology of the tuber flea beetle, *Epitrix tuberis* Gentner (Coleoptera: Chrysomelidae) in the interior of British Colombia. *The Canadian Entomologist* **85:**31-32.

Nihoul P, Hance T, Impe G, Marechal B. 1992. Physiological aspects of damage caused by spider mites on tomato leaflets. *Journal of Applied Entomology* **113(5):**487-492

Okunlola AI, Ofuya TI, Aladesanwa RD. 2008. Efficacy of plant extracts on major insect pests of selected leaf vegetables of Southwestern Nigeria. *Agricultural Journal* **3**: 181-184.

Okunlola A I, Akinrinnola O. 2014. Effectiveness of botanical formulations in vegetable production and bio-diversity preservation in Ondo State, Nigeria. *Journal of Horticulture and Forestry* **6(1):**6-13

Oliveira CM, Walter QR, Amabilio JAC, Marina RF. 2012. First record of a damage by an insect pest in a commercial amaranth crop in Brazil. *Sci. Agric*. **69(4)**:271-274.

Perring TM, Cooper AD, Rodriguez RJ, Farrar CA, Bellows TS. 1993. Identification of a whitefly species by genomic and behavioural studies. *Science* **259**:74-77.

Remaudiere G, Remaudiere M. 1997. Catalogue of the world's Aphididae: Homoptera Aphidoidea. Catalogue des Aphididae du monde: Homoptera Aphidoidea., pp 473.

Richard, RW. 1989. Studies of insects feeding on grain Amaranth in the mid west. *J. Kansas Entomol. Soc* **62(4):**440-448.

Rizk GA, Hussein SM, Hafez HF. 1988. Studies on biotic potential of the cotton leaf worm Sp*odoptera littoralis* (Boisd.); with special reference to the effect of host plants on larval susceptibility to synthetic pyrethroids. *Bull. Ent. Soc. Egypt, Econ. Ser* **17**:47-55.

Rodriquez CL 1997. The leafminer (*Liriomyza* sp.) (Diptera Agromyzidae) a serious insect pest of vegetables and horticultural crops in the tropics. *CAB Abstracts* **15(1):** 39-42.

Sæthre M.-G, Godonou I, Hofsvang T , Tepa-Yotto GT, James B. 2011. Aphids and their natural enemies in vegetable agroecosystems in Benin. *International Journal of Tropical Insect Science* **31(1–2):**103–117.

Salama HS, Dimetry NZ, Salem SA. 1970. On the host preference and biology of the cotton leaf worm Sp*odoptera littoralis*. *Zeitung für Angewandte Entomologie* **67**:261-266.

Sances FV, Toscano NC, Oatman ER, LaPr´e, LF, Johnson MV, Voth V. 1982. Reductions in plant processes by *Tetranychus urticae* (Acari: Tetranychidae) feeding on strawberry. *Environ. Entomol.* **11**:733–737.

Schippers RR. 2000. African Indigenous Vegetables. An Overview of the Cultivated Species. Natural Resources Institute/ACP-EU Technical Centre for Agricultural and Rural Cooperation, Chatham, UK. Pp 214.

Sidibe B, Lauge G, 1977. Effect of warm periods and of constant temperatures on some biological criteria in Sp*odoptera littoralis* Boisduval (Lepidoptera Noctuidae). *Annales de la Societe Entomologique de France* **13(2):**369-379

Singh G, Singh NP, Singh R. 2014. Food plants of a major agricultural pest *Aphis gossypii* glover (homoptera: aphididae) from india: an updated checklist. *Int. J. LifeSc. Bt & Pharm*. **3**: 2

Sithanantham S, Matoka CM, Maundu M, Jakari M, Agong SG. 2003. Integrated crop protection research for sustainable production of Indigenous vegetable crops in Eastern Africa. Proceedings of 4th Horticultural Seminar on Sustainable Horticultural production in the Tropics held in Njoro, Kenya.

Smith FF, Boswell AL, Wave HE. 1962. New chrysanthemum leafminer species. *Florists' Review* **130**:29-30.

Solis MA. 2006. Key to Selected Pyraloidea (Lepidoptera) Larvae Intercepted at U.S. Ports of Entry: Revision of Pyraloidea in "Keys to Some frequently intercepted Lepidopterous larvae" by Weisman 1986. University of Nebraska, Lincoln, NE, USA.

Sorensen KA. 1995. Insect pest of vegetables. North Carolina Agricultural Extension service AG-404.

Sparle AN, Liu TX. 2001. A key to common caterpillar pests of vegetables. Texas A and M University system. B-6110.

Spencer K*A* 1973. Agromyzidae (Diptera) of economic importance. *Dr. W. Junk B.V., The Hague.* P 418.

Spencer KA. 1981. A revisionary study of the leaf-mining flies (Agromyzidae) of California. University of California, Division of Agricultural Sciences Publication 3273.

Spencer K*A***.** 1992. Flycatcher: Memoirs of an amateur entomologist. SP*B Academic Publishing, The Hague.* P 414.

Suganthy M, Sakthivel P. 2012. Efficacy of botanical pesticides against major pests of black nightshade, solanum nigrum. *Int j pharm bio sci* 3(3):220 – 228

Tamaki G. 1975. Weeds in orchards as important alternate sources of green peach aphids in late spring. *Environmental Entomology* **4**:958-960

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

The Natural History Museum. 2007. Lepidopteran Hostplant Database:
http://internt.nhm.ac.uk/jdsml/research-curation/projects/hostplants/index.dsml

http://www.cabi.org/isc/datasheet/16970

http://www.cabi.org/isc/datasheet/42783

http://www.cabi.org/isc/datasheet/4346

Tingey WM, Pillemer EA. 1977. Lygus bugs: crop resistance and physiological nature of feeding injury. *Bulletin of the Entomological Society of America* **23:** 277-287.

Torres-Saldaña G, Trinidad SA, Reyna TT, Castillo JH, Bautista MN, De León GF. 2004. Drilling of the stem of Amaranth by *Hypolixus truncatulus* (Coleoptera: Curculionidae) and *Amauromyza abnormalis* (Diptera: Agromyzidae). *Acta Zool. Mex.* **20**:131-140.

UK CABI. 1968. *Aphis gossypii*. [Distribution map]. Distribution Maps of Plant Pests. Wallingford, UK: CAB International, Map 18 (Revised).

Varela AN, Seif A. 2004. A guide to IPM and Hygiene Standards in Okra Production in Kenya. ICIPE Science Press, Nairobi ICIPE

Wallis RL, 1957. Seasonal abundance and host plants of the tuber flea beetle in the Rocky Mountain region. *Journal of Economic Entomology* **50(4):**435-437.

Wool D, Hales D and Sunnucks P. 1995. Host Plant Relationships of *Aphis gossypii* Glover (Hemiptera: Aphididae) in Australia. *J. Gust. ent. SOC.* **34**:265-271

www.africanmoths.com

Young OP. 1986. Host plants of the tarnished plant bug, *Lygus lineolaris* (Heteroptera: Miridae). *Annals of the Entomological Society of America* **79**:747-762.

Zappalà L, Bernardo U, Biondi A, Cocco A, Deliperi S, Delrio G, Giorgini M, Pedata P, Rapisarda C, Garzia GT, Siscaro G, 2012. Recruitment of native parasitoids by the exotic pest *Tuta absoluta* in Southern Italy. *Bulletin of Insectology* **65(1)**:51-61.

Zhang BC, 1994. Index of economically important Lepidoptera. Wallingford, UK CAB International.

Zitter TA, Tsai JH, Harris KF. 1980. Flies. In: Vectors of plant pathogens Ed. by Harris, K.F.; Maramorosch, K, Academic Press, New York, USA: pp 165-176

Zlof V, Suffert M. 2012. Report of the EPPO/FAO/IOBC/NEPPO Joint International Symposium on management of *Tuta absoluta* (tomato borer) held from 16th to 18th November 2011in Aghadir, Morocco.

3 Altitudinal zone and seasonality affects the biodiversity and abundance of amaranth (*Amaranthus* spp.) pests in Kenya.

3.1 Abstract

Vegetable amaranth, an important indigenous vegetable for nutritional security and income generation in Africa is attacked by different types of arthropod pests. To understand the effects of altitude and seasonality on biodiversity and abundance of amaranth pests, field studies were conducted in 188 amaranth farms in low, mid and high altitudinal zones of Kenya. The damage caused by these pests and natural enemies associated with them were also investigated. We show for the first time that the highest diversity of amaranth pests is found in mid altitude zone followed by low and high altitudes respectively. Moreover, the highest diversity of amaranth pest occurs in the rainy season. A total of 975 Coleopterans, 426 Lepidopterans, 2875 aphids, and 363 Thysanopterans were collected among them four important amaranth pests that have not been reported as pests of amaranth in Kenya i.e. *Epicauta albovittata* Gestro, *Psara atritermina* Hampson , *Tuta absoluta* Meyrick and *Anyma octogueae* Guenèe. The pest damage were; Lepidoptera-24.41±1.39%, Homoptera-16.61±1.15%, Coleoptera- 14.99±0.89%, and Thysanoptera- 4.06±0.63%. The most destructive insect species were *Spoladea recurvalis* Fabricius, in the rainy season and *Epicauta albovittata* Gestro during the dry season. Twenty two (22) natural enemies of amaranth pests were also observed. Findings from this study will assist amaranth farmers in formulating crop protection measures at various altitudinal zones and seasons of the year.

Key words**:** *Indigenous vegetables; pest diversity; pest abundance; pest distribution*

3.2 Introduction

There is a re-awakening on the importance of African indigenous vegetables (AIVs) for food and nutritional security as well as health benefits across the African continent. Amaranth is among the leafy AIVs that has received great interest in production and consumption. Currently, amaranth is the 4th most produced AIVs in Kenya (HCDA, 2013). Amaranth leaves are a rich source of protein, fat, iron, calcium and vitamin C (Uusikua, 2010; Amicarelli and Camaggio, 2012). Some amaranth species have anti oxidant properties and could play a role in management of ailments such as cancer (Sreelatha et al., 2012). Arthropod pests are among the major constraints affecting production of AIV (Gockowski and Ndumbe, 1997; Schippers, 2000). Twenty percent (20%) amaranth yield losses due to insect pests has been reported in Kenya from the year 1996 to 1998 (Sithanantham et al., 2003). The insect groups attacking AIV include defoliators (beetles and caterpillars), sucking arthropods (aphids, mites, thrips, and bugs), stem borers, fruit/pod borers, leafminers and webbers (Schippers, 2000; Sithanantham et al., 2003; Sithanantham et al., 1997; HortCRSP, 2012; Kagali et al., 2013).

The diversity and abundance of insects in the tropical regions vary in different seasons of the year (Pinheiro et al. 2002). The variation is brought about by changes in climatic factors such as temperature, photoperiod, rainfall, humidity and landscape composition (Wolda, 1988). The abundance of insects declines rapidly in case of severe dry season when there is low availability of food resources or in the mid of the wet season (Wolda, 1988; Pinheiro et al. 2002). Moreover, altitude affects climatic factors such as temperature, rainfall, CO_2, UV, and soil fertility. These factors have an impact on the on the physical and physiological properties of the host plants growing at various altitudinal levels with implications on the diversity and abundance insect species (Kronfuss and Havranek, 1999). Presence of natural enemies and interspecific competition among insect species among other factors also affect pest diversity and abundance (Goeden and Teerink, 1996; Hodkinson and Bird, 1998).

Altitude affected the abundance of various Agromyzidae leafminers species in Kenya. While there was higher abundance of *Liriomyza huidobrensis* in the high altitude compared to the mid and low altitudes, the highest abundance of another two species, *L. sativae* and *L. trifolli* was found on the mid altitude zone (Foba et al., 2015). In other studies, the abundance of Heteroptera, Homoptera and Coleoptera species affecting birch, *Betula pubescens* decreased with an increase of altitude levels in Sogndal, Norway while the species richness of Psocopterans infesting mango, *Mangifera indica* increased with an increase in the altitudinal gradient in Jamaica (Turner and Broadhead, 1974; Hägvar, 1976). The insect diversity could

also remain unaltered despite the changes in altitudinal gradient as observed for insects infesting branken, *Pteridium aquilinum* in United Kingdom (Lawton et al., 1987).

To our knowledge, no studies on the effects of altitude and seasonality on amaranth pests in Kenya have been conducted. In this present study, we present comprehensive national-wide data showing the diversity, distribution, seasonality, and damage of arthropod pests infesting amaranth in the main production regions in Kenya. We also characterize natural enemies occurring naturally in these regions which could be exploited in an integrated pest management program.

3.3 Methodology

3.3.1 Field survey methodology

The survey was done in two different seasons of the year 2014. The first season was conducted from February to May, and was relatively dry with scanty rainfall while the second season covering October to November was cool and witnessed heavy rains. The survey involved visiting leading Counties in production of amaranth in Kenya. A total of 15 counties were surveyed and further categorized into low altitude zone (< 1000 m asl), mid altitude zone (1000-1800 m asl), and high altitude zone (> 1800 m asl) (Hassan, 1998). Mombasa, Kilifi, Lamu, and some parts of Embu County were located in the low altitude zone; Machakos, Embu, Kajiado, Kisii, Narok, Kisumu, Kakamega, Busia, and parts of Nyamira, Kirinyaga, and Tranzoia Counties were in the mid altitude zone; Kiambu, and some parts of Kirinyaga (Gichugu sub-county), Nyamira, and Transzoia Counties were in the high altitude zone.

In each of the selected Counties, 5 farms each in 2 Sub-counties were selected making a total of 10 farms per County. Each farm was divided into 4 equal quadrants and ten plants were randomly selected and insects sampled following a diagonal transect across the quadrant. Each of the selected plants was examined for the presence of pests and natural enemies using the beating and picking methods. The beating method was used for small cryptic insects particularly the Coleopterans, Thysanopterans, some Homopterans, and the young instar larva of Lepidopterans. For this purpose,, a tray (32 x 23.5 cm) smeared with 70% alcohol at its base was held below the plant with one hand and the other hand used to tap the plant vigorously for 10 seconds to dislodge the insects that are on the plants. The insects that fell on the tray were collected with fine camel brush (No. 1) into vials containing 70% alcohol. The vials were labeled appropriately with details of farm, host crop, insect development stage, and insect damage.

The picking method was used for sampling insects that burrow within the leaves/stems such as leafminers, amaranth stem weevils, and insects that are not very mobile such as mites, aphids, big sized caterpillars and parasitoid mummies. The picked adult insects were directly put in vials containing 70% alcohol while the caterpillars/mummies/mined plant tissues were put in lunch boxes (19 cm ×13 cm × 8 cm). The lunchboxes were lined with paper towels to absorb excess leaf moisture and fitted with fine wire screen mesh on top. The insects in lunchboxes were transferred to the laboratory at *icipe* to allow further insect development to adulthood for identification and potential parasitism recording. The larval stages of the insects were fed with fresh plant material on a daily basis to enable them to develop to maturity. The pupae were provided with suitable pupation sites. Where insects had burrowed into the plant tissues such as leaves or stems, the infested plant part was collected into the lunchboxes and kept in similar conditions as the immature live insects until adult emergence. The insect samples collected in 70% alcohol and the mature adults emerging from insects reared in lunchboxes were sorted into the various insect orders, mounted using insect pins or insect cards and later identified using morphological keys at the Entomology laboratory at the Nairobi National Museum, 1.2740° S, 36.8145° E.

3.3.2 Insect damage scoring guidelines

Significant of pest is due to the damage they cause to the plants. Moreover, insect abundance does not necessarily translate into insect damage. Therefore the damage of the pests was scored alongside the insect abundance following the guide on table 3-1.

Table 3-1: damage guide used to score for various insect orders during the survey to identify the major pests of amaranth in Kenya.

Insect	**Damage score**	**Description of damage**	**Reference**
Aphids	0	Plant appear healthy, may have small chrotic spots	Webster et al. 1987
	1	Chlorosis and leaf folding 26-50 % of total leaf area	
	2	Chlorosis and leaf folding 26-50 % of total leaf area	
	3	Chlorosis and leaf folding 51-75 % of total leaf area	
	4	Chlorosis and leaf folding > 75% of total leaf area	
Coleoptera	0	No visible damage on the crop	Smith, 2000 modified
	1	1-20% leaf consumed	
	2	21-40% leaf consumed	
	3	41-60% leaf consumed	
	4	61-80% leaf consumed	
	5	81-100% leaf consumed	
Lepidoptera	0	No leaf damage	Said and Itulya, 2003 modified
	1	1-25 % of leaf consumed	
	2	26-50 % of leaf consumed	
	3	51-75 % of leaf consumed	
	4	76-100 % of leaf consumed	
Thysanoptera	1	No leaf damage	Nyasani et al., 2011
	2	Few silvery streaking (≤25%)	

Table 3-1: damage guide used to score for various insect orders during the survey to identify the major pests of amaranth in Kenya (Continued).

Insect	Damage score	Description of damage	Reference
Thysanoptera	3	Moderate streaking (26-50%)	Nyasani et al., 2011
	4	Heavy streaking (51-75%)	
	5	Severe streaking and drying of attacked leaves (≥75%)	
Mites	0	No leaf damage	Hussey & Parr 1963 modified
	1	1-20 % of leaf damaged	
	2	21-30 % of leaf damaged	
	3	31-50 % of leaf damaged	
	4	51-70 % of leaf damaged	
	5	71-0 % of leaf damaged	

3.3.3 Data analysis

ANOVA for the average pest abundance per farm for each of the 4 most important orders and for the three most abundant species per order at different altitudinal zones and seasons was done using R. program (R version 3.3.1, 2016). The insect count data was log transformed before the analysis was done. The pest damage data which was recorded in percentage was Arcsin-squareroot transformed before statistical analysis. Where significant differences were observed, the means were separated using the Tukeys test. All tests were carried out at 5% level of significance. Insect species diversity on amaranth crop across different altitudinal zones and in different seasons were analysed using the Renyi diversity profiles. Biodiversity analyses were done using R (version 3.3.1, 2016). V*egan* (Oksanen et al., 2005) and *BiodiversityR* (Kindt & Coe, 2005) packages were used to calculate the diversity and plot the graphics.

The Renyi diversity profiles order species in an ecosystem from species richness to species evenness. Other common diversity indices used by ecologist such as the Shannon index, or Simpson index are specific cases of Rényi entropy formula. In Renyi diversity profile, the diversity values on the y-axis (H-alpha) are related to the scalar parameter "alpha" on the x-axis. H_0 = species richness, H_1 = Shannon Diversity, H_2 = Simpson Diversity and H_∞ = Berger-Parker Index (Legendre & Legendre, 1998, Kindt *et al.* 2006). H-alpha is based on the frequency of each component species (proportional abundances "pi" = abundance of species i/ total abundance) and a scale parameter (α) ranging from zero to infinity (Tóthmérész, 1995).

$$H_\alpha = \frac{\ln\left(\sum p_i^\alpha\right)}{1-\alpha}$$

A given insect community X is said to have higher species richness than community Y if it has a higher value at alpha=0 compared to community Y. Similarly, insect community X is said to have higher evenness than community Y if it has a higher value at alpha=∞ than community Y. Species diversity is a combination of species richness and species evenness. A given insect community X is regarded as more diverse than a community Y if its diversity profile line is everywhere above that of community Y in Renyi diversity graph (Kindt R, Coe R. 2005). This means that community X has higher species richness and higher species evenness than community Y. If the profile lines of different insect communities cross each other, it is not possible to order the diversity of these communities from the most diverse to the least diverse. This is because one community for instance community X could have higher species richness than another community Y but at the same time community Y could be having higher species evenness than community X. In that case, we can only discuss species richness and species evenness separately (Legendre and Legendre). The values of the series for the three altitudinal zones and in the two seasons were calculated for the scales of α = {0, 0.25, 0.5, 1, 2, 4, 8, ∞} and plotted as diversity profiles for each altitudinal zone (high, mid, and low) in a single graph or diversity profiles for each of the seasons (Season 1 and season 2) in a single graph.

3.4 Results

3.4.1 Diversity of Amaranth pests in Kenya at different altitudes and seasons

When different altitudinal zones were considered, the mid altitude zone had the highest pest species richness of amaranth pests followed by low altitude zone and high altitude zone respectively. The H-alpha value at alpha=0 (representing species richness) was 3.87 for the mid altitude zone, 3.67 for the low altitude zone and 3.25 for the high altitude zone. Moreover the mid altitude zone had the highest pest species evenness of the amaranth pests. The H-alpha value at alpha=∞ (representing species evenness) was 0.82 for the mid altitude zone. However, the high altitude zone had higher pest species evenness compared to the low altitude zone. The H=∞ values were 0.68 for high altitude zone and 0.50 for the low altitude zone. When pest species diversity, which combines both species richness and species evenness was considered, the mid altitude zone had the highest amaranth pest species diversity since its diversity profile was everywhere above the diversity profiles for the high and low altitudinal zones at all alpha values i.e. it had the highest h-alpha value at alpha=0, 2, and∞. However, the amaranth pest species diversity between the high and low altitude zones could not be ordered since their diversity profiles were criss-crossing each other. This is

because the low altitude zone had higher pest species richness compared to the high altitude zone while the high altitude zone had greater pest species evenness than the low altitude zone (Fig 3-1a).

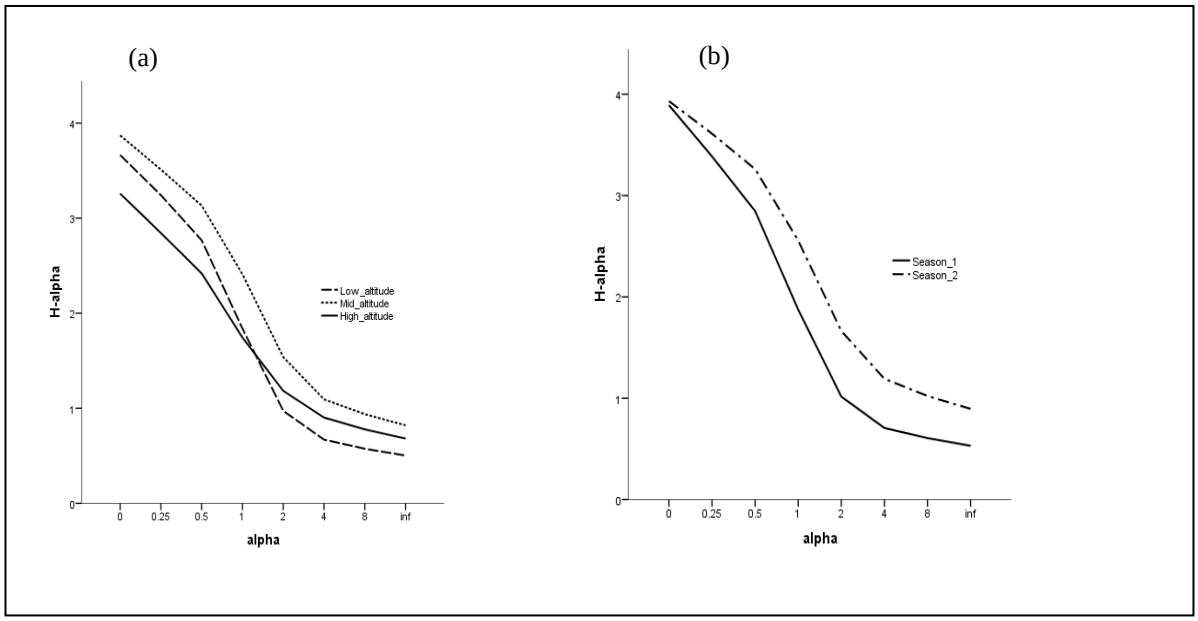

Fig 3-1: Renyi diversity indices for amaranth pests in a study conducted in Kenya across three agro-ecological zones. These are: high altitude, mid altitude and low altitude zones (a). Renyi diversity indices for amaranth pests in a study conducted in Kenya across the six regions producing amaranth for the two seasons of 2014 (b).

When different seasons were considered, the species richness was more or less the same for the two seasons. The H-alpha values at alpha=0 were rather tight for both seasons, 3.89 for season 1 and 3.93 for season 2. However, at alpha=∞, which represents species evenness, the H-alpha values were 0.89 for season 2 and 0.53 for season 1 indicating that the insect communities in season 2 were more evenly distributed compared to those in season 1. However, when the overall pest species diversity was considered, the diversity profile for amaranth pests in season 2 was everywhere above that of season 1 indicating that there was a higher pest species diversity in season 2 as compared to season 1. (Fig 3-1b).

3.4.2 Abundance and damage of major insect orders attacking amaranth

A total of 5047 individual insects were collected during the survey. The five most abundant insect orders infesting amaranth in were Homoptera- 2876 (56.61%), Coleoptera- 959 (18.88 %), Lepidoptera- 448 (8.82 %), Diptera 434 (8.54%), and Thysanoptera- 363 (7.15%). There was significantly higher number of the Homopterans and Coleopterans compared to the Lepidopterans, Dipterans and Thysanopterans. However, there was no significant difference in the abundance of the Coleopterans and Homopterans. Moreover, there were no significant

differences in the number of Lepidopterans, Dipterans, and Thysanopterans ($F_{4,\ 941}$=18.52; P=<0.001**)** (Fig 3-2).

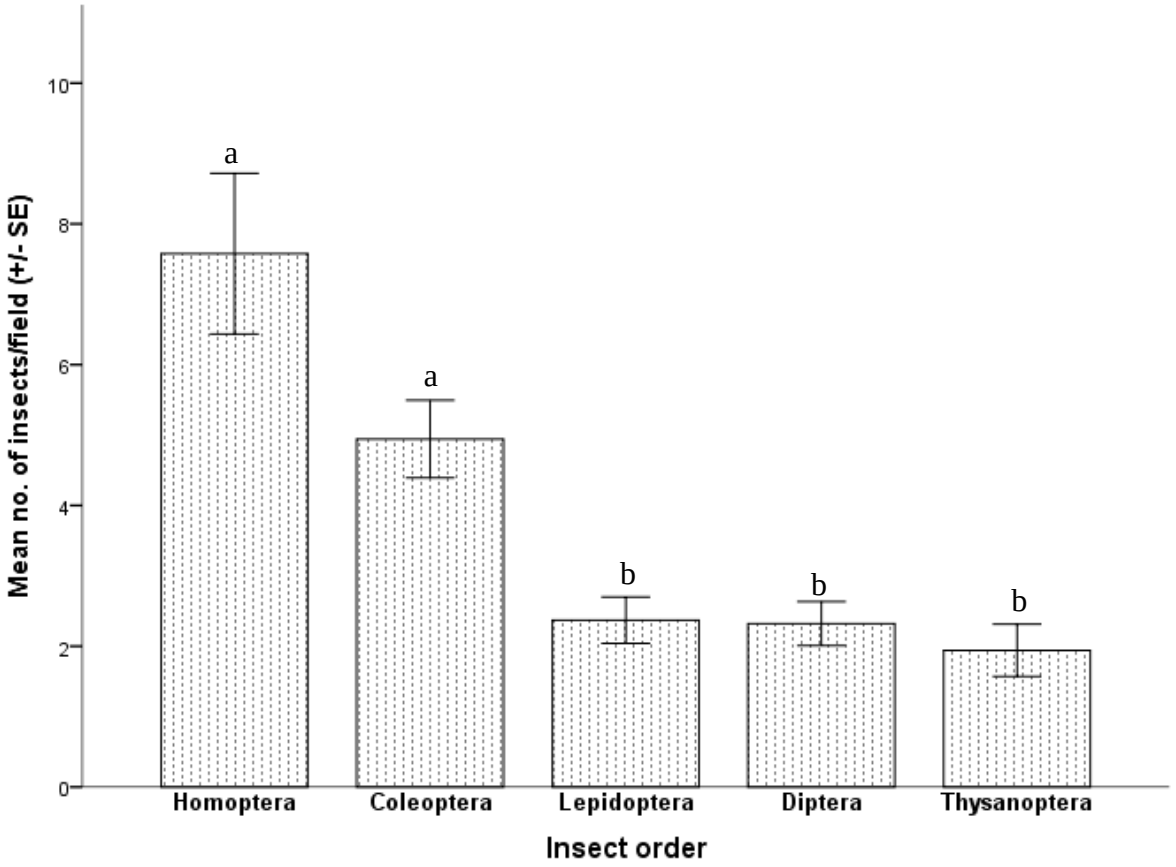

Fig 3-2: Mean number (+/- SE) of insects/ field infesting amaranth from the major orders from a study conducted in two seasons of year 2014 across the three altitudinal zones (low, mid and high) growing amaranth in Kenya. The insect count data was log transformed before the analysis. Anova was carried out at P≤0.05. Where significance difference was observed, Tukey test was used to separate the means.

Although the highest abundance was observed on insect from the Homopteran order, insects from the Lepidopteran order recorded the highest damage. There was significantly higher damage from the Lepidopterans compared to pests from the other orders. Moreover, there was significantly higher damage from insects in the Homopteran and Coleopteran orders compared to the Dipterans and the Thysanopterans. However, there was no significant difference in the damage arising from the Coleopterans and the Homopterans. Furthermore, there was no significant difference in the damage caused by Dipterans and the Thysanopterans ($F_{4,943}$ = 132.87; P<0.001) (Fig 3-3).

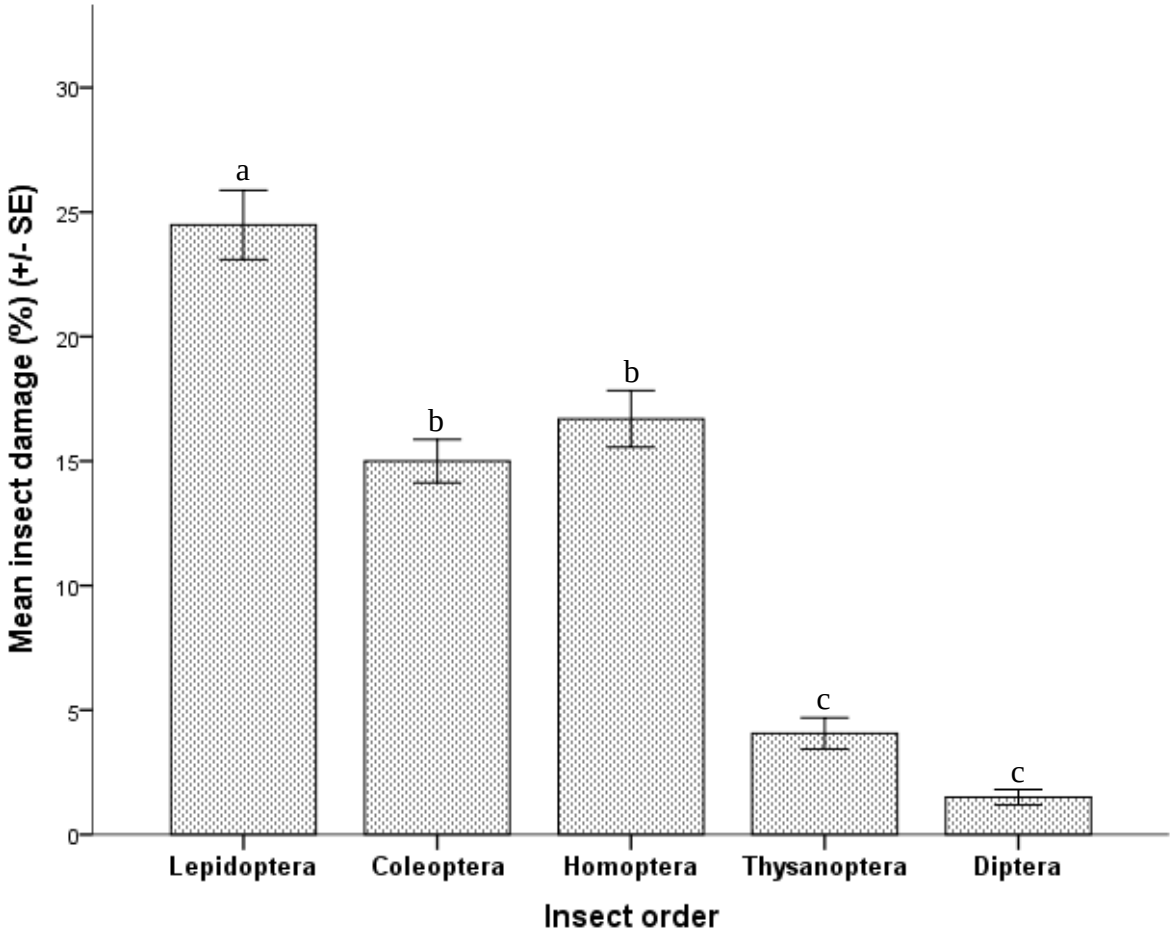

Fig 3-3: Mean percentage damage (+/- SE) by insects infesting amaranth from the major orders in a study conducted in two seasons of year 2014 across the three altitudinal zones (low, mid and high) growing amaranth in Kenya. The percentage data was arcsine square root transformed before the analysis. Anova was carried out at P≤0.05. Where significance difference was observed, Tukey test was used to separate the means.

3.4.3 Abundance of Coleopteran species on amaranth

Thirty eight Coleopteran species infesting amaranth belonging to 15 families and 35 genera were observed during the survey. Nine species were from Chrysomelidae family. Similarly, nine species were from Curculionidae family. Four of the species were from Tenebrionidae family while three species were from Bruchidae family. Two species were from Alleculidae family while one came from each of the following families; Aderidae, Anthicidae, Apionidae, Brenthidae, Cerambycidae, Elateridae, Histeridae, Melyridae, and Nitidulidae families. The most abundant species across the two seasons were *Lixus* sp. (Coleoptera: Curculionidae), *Apion* sp. (Coleoptera: Apionidae) and *Epicauta albovittata* Gestro (Coleoptera: Meloidae) (Table 3-2).

Table 3-2: Mean number (+/- SE) of Coleopteran insects infesting amaranth fields during a study conducted in two seasons in the year 2014 across the three altitudinal zones (low, mid and high) growing amaranth in Kenya.

Family	**Species**	**Abundance (%)**	**Count/ field**	**Range of abundance**
Curculionidae	*Lixus* sp.	13.9	0.516±0.35	0 - 17
	Baris sp.	8.0	0.298±0.20	0 - 14
	Hypolixus pulvisculosus (Boheman)	4.3	0.160±0.12	0 - 6
	Systates crenatipennis (Fairmaire)	3.9	0.144±0.17	0- 12
	Balanogastris sp.	3.3	0.122±0.13	0 - 7
	Anaplesius sp.	1.9	0.069±0.11	0 - 9
	Babauitia sp.	0.9	0.032±0.06	0 - 5
	Cylas sp.	0.4	0.016±0.03	0 - 3
	Nematocerus sp.	0.1	0.005±0.01	0 - 1
Apionidae	*Apion* sp.	11.7	0.436±0.35	0- 24
Meloidae	*Epicauta albovittata*	10.6	0.394±0.31	0- 19
	Mylabris amplectens (Gerstaecker)	1.3	0.048±0.10	0 - 9
Chrysomelidae	*Aulacophora foveilcollis* (Lucas)	8.6	0.319±0.35	0 - 23
	Epitrix silvicola (Bryant)	8.5	0.314±0.25	0- 13
	Phyllotreta sp.	4.4	0.165±0.21	0- 17
	Monolepta cruciata (Guerin De Meneville)	1.1	0.043±0.05	0 - 3
	Hapsidolema nigroparallela (Crow)	1.1	0.043±0.09	0- 8
	Hyperacantha semipalliata (Fair)	0.9	0.032±0.07	0 - 6
	Ceralces natalensis (Baly)	0.6	0.021±0.05	0 - 4
	Colasposoma tokeri (Bryant)	0.3	0.011±0.02	0- 2
	Monolepta leuce (Weise)	0.1	0.005±0.01	0- 1
Anthicidae	*Formicomus* sp.	5.2	0.191±0.14	0 - 8
Aderidae	*Hylophilus* sp.	0.1	0.005±0.01	0 - 1
Alleculidae	*Synallecula* sp.	2.3	0.085±0.11	0 - 7
	Allecula lesnei (Pic)	0.1	0.005±0.01	0- 1
Brenthidae	*Cerobates* sp.	0.1	0.005±0.01	0- 1
Bruchidae	*Bruchus* sp.	1.1	0.043±0.06	0 - 4
	Acanthoscelides obtectus (Say)	0.4	0.016±0.03	0- 3
	Callosobruchus chinensis (Linnaeus)	0.1	0.005±0.01	0 - 1
Cerambycidae	*Xystrocera dispar* (Fahraeus)	0.6	0.021±0.05	0 - 4
Elateridae	*Cardiophorus sp.*	1.3	0.048±0.10	0 - 5
Histeridae	*Atholus* sp.	0.1	0.005±0.01	0 - 1
Melyridae	*Hapalochrus elgonensis* (Champ)	0.4	0.016±0.03	0 - 2
Nitidulidae	*Pria* sp.	0.3	0.011±0.02	0 - 2
Tenebrionidae	*Himatismus tyrivialis* (Gerstaecker)	0.6	0.021±0.05	0 - 4
	Lagria cyanicollis (Borchmann)	0.4	0.016±0.03	0 - 2
	Derolagria erythrocephala (Borchmann)	0.4	0.016±0.03	0 - 2
	Lagria sp.	0.3	0.011±0.02	0 - 2

When these three most abundant species were considered separately, there was significantly higher abundance of *Apion* sp. in season 1 compared to season 2 in the low altitude zone ($t = 58.24$; $df = 49$; $P<0.001$). Similarly, there was significantly higher abundance of *Apion* sp. at

the mid altitude zone in season 1 compared to season 2 (T=18.64; df= 104; P<0.001) (Table 3-3).

Table 3-3: Mean number of insects/field from the three major Coleoptera species infesting amaranth from a study conducted in two seasons in the year 2014 across the three altitudinal zones (low, mid and high) growing amaranth in Kenya.

Species	**Season**	**Altitudinal zone**		
		Low	**Mid**	**High**
Apion sp	Season 1	3.43±1.28aA	0.15±0.08aB	0.00B
	Season 2	0.07±0.07bA	0.00bA	0.00A
E. albovittata	Season 1	0.07±0.07bA	0.00B	0.00B
	Season 2	0.23±0.18aA	0.00A	0.00A
Lixus sp	Season 1	1.48±0.89A	0.00aA	0.43±0.25aA
	Season 2	1.60±0.69aA	0.00aB	0.00aB

Same lower case letter for the same zone in a particular insect species denotes no significant difference for the two seasons. Same upper case letter for the same season in a particular insect species denotes no significant difference in various altitudinal zones. P≤0.05

When season 1 was considered separately, there was significantly higher abundance of *Apion* sp. at the low altitude zone compared to the mid and high altitude ($F_{2,85}$=19.96; P<0.001) (Table 3-3).. When season 2 was considered separately, there was no significant difference in the abundance of *Apion* sp. across all the altitudinal zones ($F_{2,97}$=1.17; P=0.314) (Table 3-3).
When *E.albovittata* was considered, there was significantly higher abundance of the pest in season 2 compared to season 1 (t=34.68; df= 49; p<0.001) (Table 3-3). When each season was considered separately, there was significantly higher abundance of *E. albovittata* in low altitude zone compared to the mid and high altitude zone ($F_{2,85}$=23.94; P<0.001) (Table 3-3).
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When *Lixus* sp. was considered, there was no significance difference in the abundance of the pest at the low altitude zone between the two seasons (t =0.59; df= 49; p=0.78). Similarly, there was no significance difference in the abundance of *Lixus* sp. in the mid altitude zone between the two seasons (t= 11.85; df= 104; p=0.103). Moreover, no significance difference was observed in the abundance of *Lixus* sp. in the high altitude between the two seasons (T = 25.61; df = 29; P = 0.056) (Table 3-3). When each season was considered separately, there was no significant difference across the three altitudinal zones in season 1 ($F_{2,85}$=2.83; P=0.065). However, in the second season, there was significantly higher abundance of *Lixus* sp. at the low altitude zone compared to the mid and high altitude zones ($F_{2,97}$=10.65; P<0.001) (Table 3-3).

3.4.4 Abundance of Homopteran species on amaranth

Four species of aphids belonging to family Aphididae were observed during the survey: *Aphis fabae* Scopoli (Homoptera: Aphididae), *Myzus persicae* Sulzer (Homoptera: Aphididae), *Aphis craccivora* Koch (Homoptera: Aphididae) and *Toxoptera sp* (Homoptera: Aphididae). However, the most abundant species were *A. fabae* (79.9 %), *M. persicae* (13.0 %) and *A. craccivora* (6.1%) (Table 3-4).

Table 3-4: Mean number (+/- SE) of aphid species infesting amaranth fields during a study conducted in two seasons in the year 2014 across the six regions (Central, Coast, Eastern, Nyanza, Rift, and Western) growing amaranth in Kenya.

Family	**Species**	**Abundance (%)**	**Count/ field**	**Range of abundance**
Aphididae	*Aphis fabae* (Scopoli)	79.9	22.03±10.60	0 - 462
	Myzus persicae (Sulzer)	13.0	3.60±1.59	0 - 64
	Aphis craccivora (Koch)	6.1	1.69±0.80	0 - 34
	Toxoptera sp.	1.0	0.26±0.42	0 - 27

Seasonality had no significant effect on abundance of *A. fabae* at the low altitude zone between the two seasons (t = 5.39; df=49; p=0.102). Moreover, there was no significant difference in the abundance of *A. fabae* in the mid altitude zone between the two seasons (t = 2.99; df = 102; p = 0.111). Similarly, there was no significance difference in the population of *A. fabae* at the high altitude between season 1 and season 2 (t= 0.00; df= 29; p=0.549) (Table 3-5).

Table 3-5: Mean number of insects/ field from the three major aphid species infesting amaranth from a study conducted in two seasons in the year 2014 across the three altitudinal zones (low, mid and high) growing amaranth in Kenya.

Order	**Season**	**Altitudinal zone**		
		Low	**Mid**	**High**
A. fabae	Season 1	49.90±28.62aA	9.00±4.23aA	11.33±6.22aA
	Season 2	9.47±6.47aA	3.48±1.89aA	9.94±5.19aA
M. persicae	Season 1	7.38±3.87aA	1.21±0.50aA	4.33±2.84aA
	Season 2	0.00bA	1.15±0.61aA	1.50±1.03aA
A. craccivora	Season 1	0.00B	0.77±0.37aAB	3.00±1.81aA
	Season 2	0.00B	0.44±0.23aB	4.00±2.31aA

Same lower case letter for the same zone in a particular insect species denotes no significant difference for the two seasons. Same upper case letter for the same season in a particular insect species denotes no significant difference in various altitudinal zones. P≤0.05

There was also no significant difference in the abundance of *A. fabae* across the three altitudinal zones in season 1 ($F_{2,85}$= 1.77; P=0.177). Similarly, there was also no significant difference in the abundance of *A. fabae* across the low, mid, and high altitudinal zones in the second season ($F_{2,95}$= 1.77; P=0.177) (Table 3-5). When the abundance of *M. persicae* was considered, there was significantly higher population of the pest in season 1 compared to

season 2 in the low altitude zone (t= 45.20; df = 49; P = 0.005). However, there was no significant difference in the abundance of *M. persicae* between the two seasons at the mid altitude zone (t = 1.91; df =102; P=0.317). Moreover, there was no significant difference in the abundance of *M. persicae* in the high altitude between the two seasons (t = 2.49; t= 29; p=0.317) (Table 3-5). . When different seasons were considered separately, there was no significant difference in the abundance of *M. persicae* at the low, mid and high altitude zones in the first season ($F_{2,85}$= 1.28; P=0.286). Moreover, there was no significant difference in the abundance of *M. persicae* at the low, mid and high altitude zones in the second season ($F_{2,95}$= 2.10; P=0.128) (Table 3-5).

There was no significant difference in the abundance of *A. craccivora* in the mid altitude zone between the two seasons (t= 1.56; df= 102; p=0.529). Moreover, there was no significant difference in the abundance of *A. craccivora* at the high altitude zone between the two seasons (t = 0.19; df = 29; P =0.798). *A. craccivora* was absent at the low altitude zone in both seasons (Table 3-5). When the seasons were considered separately, there was significantly higher abundance of *A. craccivora* in the high altitude zone compared to the low altitude zone in the first season. However, there was no significant difference in the abundance of *A. craccivora* between the low and mid altitude or between the mid and high altitude zones in the first season ($F_{2,85}$= 2.77; P = 0.068). Moreover, there was significantly higher abundance of *A. craccivora* in second season at the high altitude zone compared to the mid and low altitude zones ($F_{2,95}$= 5.97; P = 0.004). (Table 3-5).

Nineteen species of Coccinelids beetles associated with predation of aphids were observed in amaranth fields during the survey. The beetles were from 11 different genera, however, majority of the species were from the Scymnus genus (6 species). Moreover, hoverflies (Syrphidae) larvae were also seen predating aphids on amaranth fields. *Aphidius colemani* Verick (Hymenoptera: Brachonidae) was also observed parasitizing aphids in amaranth fields. The most abundant aphid predators were *Hippodamia variagata* Goeze (Coleoptera: Coccinellidae) (10±7.0 insects/farm), *Platynapsis vittigera* (Coleoptera: Coccinellidae) (3.3±1.00 insects/farm), *Cheilomenes sulphurea* Olivier (Coleoptera: Coccinellidae) (3.1±0.69 insects/farm) and *Scymnus luteus* Sicard (Coleoptera: Coccinellidae) (3 insects/farm) (Table 3-6).

Table 3-6: Natural enemies of aphids observed in amaranth fields during a study conducted in two seasons in the year 2014 across the three altitudinal zones (low, mid and high) growing amaranth in Kenya.

Order	**Family**	**Species**	**Insects/field**
Coleoptera	Coccinelidae	*Hippodamia variagata* (Goeze)	10±7.0
		Platynapsis vittigera (Muls)	3.3±1.00
		Cheilomenes sulphurea (Olivier)	3.1±0.69
		Scymnus luteus (Sicard)	3
		Scymnus trepidulus (Weise)	2.6±0.63
		Scymnus sp.	2.6±0.39
		Brumoides fulviventris (Fairmare)	2.4±0.75
		Exochomus nigrimaculatus (Goeze)	2.2±0.42
		Scymnus kibonotensis (Weise)	2.0±1
		Stethorus sp.	2
		Scymnus morelleti (Muls)	1.3±0.25
		Cheilomenes aurora (Gerstäcker)	1
		Chnotriba similes (Thunberg)	1
		Henesepilacha hirta (Thunberg)	1
		Hyperaspis sp.	1
		Scymnus pruinosus (Weise)	1
Hymenoptera	Braconidae	*Aphidius colemani* (Viereck)	5.5±0.71
Diptera	Syrphidae	Hoverfly	1.7±0.24

3.4.5 Abundance and damage caused by Lepidopteran species on amaranth

Seventeen Lepidopteran species belonging to 4 families and 15 genera were found to attack amaranth. The two families having the highest number of species were Noctuidae with eight species and Crambidae with five species. Two species belonged to Geometriidae family while Acraeidae and Gelechiidae families had one species each. The three most abundant species were *S. recurvalis* Fabricius (Lepidoptera: Crambidae) (28.9 %), *P. atritermina* Hampson (Lepidoptera: Crambidae) (24.2 %) and *A. octogueae* Guenèe (Lepidoptera: Noctuidae) (11.0%) (Table 3-7).

Table 3-7: Mean number (+/- SE) of Lepidopteran species infesting amaranth fields during a study conducted in two seasons in the year 2014 across the six regions (Central, Coast, Eastern, Nyanza, Rift, and Western) growing amaranth in Kenya.

Family	**Species**	**Abundance (%)**	**Count/field**	**Range of abundance**
Crambidae	*Spoladea recurvalis* (Fabricius)	28.9	0.690±0.44	0 - 20
	Psara atritermina (Hampson)	24.2	0.578±0.38	0 - 16
	Sameodes cancellalis (Zeller)	2.7	0.064±0.07	0 - 4
	Udea ferrugalis (Hübner)	1.6	0.037±0.05	0 - 4
	Orphanostigama absuptalis	0.4	0.011±0.02	0 - 2
Noctuidae	*Anyma octogueae* (Guenèe)	11.0	0.262±0.21	0 - 10
	Helicoverpa armigera (Hübner)	8.5	0.203±0.56	0 - 11

Table 3-7: Mean number (+/- SE) of Lepidopteran species infesting amaranth fields during a study conducted in two seasons in the year 2014 across the six regions (Central, Coast, Eastern, Nyanza, Rift, and Western) growing amaranth in Kenya (Continued).

Family	**Species**	**Abundance (%)**	**Count/field**	**Range of abundance**
Noctuidae	*Spodoptera litorralis* (Boisduval)	7.0	0.166±0.10	0 - 5
	Spodoptera exigua (Hübner)	5.6	0.134±0.15	0 - 12
	Plusia sp.	5.4	0.128±0.08	0 - 4
	Anomis sabulifera (Guenée)	0.9	0.021±0.05	0 - 4
	Spodoptera exempta (Walker)	0.7	0.016±0.03	0 - 2
Acraea	*Acraea eponina* (Cramer)	1.6	0.037±0.08	0 - 7
Gelechiidae	*Tuta absoluta* (Meyrick)	0.7	0.016±0.03	0 - 2
	Eupithecia sp.	0.2	0.005±0.01	0 - 1
	Traminda pallid (Warren)	0.2	0.005±0.01	0 - 1
Scythrididae	*Eretmocera sp.*	0.4	0.011±0.02	0 - 2

When the abundance of the three most dominant species was considered, there was significantly higher abundance of. *P. atritermina* in the second season compared to first season at the low altitude zone (t= 123.36; df=49; p<0.001). However the pest was absent at the mid and high altitude zones during the two seasons when the study was conducted (Table 3-8).

Table 3-8: Mean number of insectst/field from the three major Lepidopteran species infesting amaranth from a study conducted in two seasons in the year 2014 across the three altitudinal zones (low, mid and high) growing amaranth in Kenya.

Order	**Season**	**Altitudinal zone**		
		Low	**Mid**	**High**
P. atritermina	Season 1	0.00b	0.00	0.00
	Season 2	3.60±0.94aA	0.00B	0.00B
S. recurvalis	Season 1	0.00bA	0.17±0.12aA	0.27±0.27aA
	Season 2	1.57±0.72aA	1.31±0.58aA	0.06±0.06aA
A. octogueae	Season 1	0.19±0.11aA	0.06±0.03aA	0.00A
	Season 2	1.30±0.56aA	0.06±0.04aB	0.00B

Same lower case letter for the same zone in a particular insect species denotes no significant difference for the two seasons. Same upper case letter for the same season in a particular insect species denotes no significant difference in various altitudinal zones. P≤0.05

When each season was considered separately, there was significant difference in abundance of *P. atritermina* in the second season at the low altitude zone compared to the mid and high altitude zones ($F_{2,95}$= 22.82; P<0.001). Moreover, the pest was not observed during the first season in all of the altitudinal zones (Table 3-8).

Significantly higher abundance of *S. recurvalis* was observed in season 2 compared to season 1 at the low altitude zone (t = 27.62; df = 49; P = 0.024). However, there was no significant

difference in the abundance of *S. recurvalis* between the two season at the mid altitude zone (t = 14.09; df = 103; P = 0.089). Moreover, there was no significant difference on the abundance of *S. recurvalis* between the two seasons at the high altitude zone (t = 1.47; df = 29; P = 0.587). (Table 3-8). When each season was compared separately, there was no significant difference in the abundance of *S. recurvalis* among the three altitudinal zones in the first season ($F_{2,86}$= 0.71; P = 0.493). Moreover, there was no significant difference in the abundance of *S. recurvalis* at the low, mid, and high altitudinal zones during the second season ($F_{2,95}$= 1.38; P = 0.256) (Table 3-8).

No significant difference was observed in the abundance of *A. octogueae* between the two seasons at the low altitude zone (T = 12.18; df = 49; P = 0.156). Moreover, there was no significant difference in the abundance of *A. octogueae* between the two seasons at the mid altitude zone (T = 0.066; df = 103; P = 0.886). (Table 3-8). When both seasons were considered separately, there was significantly higher abundance of *A. octogueae* at the low altitude zone compared to the mid and high altitude zones during the second season ($F_{2,95}$= 6.24; P = 0.003). However, there was no significant difference in the abundance of *A. octogueae* among the three altitudinal zones during the first season ($F_{2,86}$= 1.88; P = 0.158) (Table 3-8). One parasitoid species, *Apanteles* sp. attacking *S. recurvalis* was observed during the survey. The parasitoid was mainly observed in the coastal and Eastern regions of Kenya.

3.4.6 Abundance and damage caused by Thysanopteran species on amaranth

Eight thrips species belonging to 3 families and 8 different genera were observed during the survey. Six of the species belonged to the Thripidae family while there was one species each in the families Aeolothripidae and Phlaeothripidae. The most dominant species were *F. Schultzei* Trybom (Thysanoptera: Thripidae) (37.2 %), *M. Sjostedti* Trybom (Thysanoptera: Thripidae) (24.5 %) and *H. gowdeyi* Franklin (Thysanoptera: Phlaeothripidae (16.6 %) (Table 3-9).

Table 3-9: Mean number (+/- SE) of Thysanopteran species infesting amaranth fields during a study conducted in two seasons in the year 2014 across the six regions (Central, Coast, Eastern, Nyanza, Rift, and Western) growing amaranth in Kenya.

Family	Species	Abundance %	Count/ field	Range of abundance
Thripidae	*Frankliniella schultzei* (Trybom)	37.19	0.722±0.56	0 - 39
	Megalurothrips sjostedti (Trybom)	24.52	0.476±0.35	0 - 24
	Dendrothrips sp.	9.92	0.193±0.14	0 - 5
	Franklinothrips megalops (Trybom)	8.54	0.166±0.24	0 - 19
	Sericothrips adolfifriderici (Karny)	1.65	0.032±0.04	0 - 2
	Chirothrips frontalis (Williams)	0.55	0.011±0.02	0 - 1
Aeolothripidae	*Microcephalothrips abdominalis* (Crawford)	0.83	0.016±0.03	0 - 2
Phlaeothripidae	*Haplothrips gowdeyi* (Franklin)	16.80	0.326±0.18	0 - 8

There was significantly higher abundance of *F. schultzei* at the low altitude zone in the first season compared to the second season (t = 27.91; df = 49; P = 0.015). Moreover, there was significantly higher abundance of *F. schultzei* in season 1 than in season 2 at the mid altitude zone (t = 22.95; df = 103; P = 0.027). However, there was no significant difference in the abundance of *F. schultzei* between the two seasons at the high altitude zone (t = 0.062; df = 29; P = 0.905) (Table 3-10).

Table 3-10: Mean number of insects/ field from the three major Thysanoptera species infesting amaranth from a study conducted in two seasons in the year 2014 across the three altitudinal zones (low, mid and high) growing amaranth in Kenya.

Order	Season	Altitudinal zone		
		Low	Mid	High
F. schultzei	Season 1	1.43±0.76aA	1.62±0.84aA	0.47±0.40aA
	Season 2	0.10±0.10bA	0.08±0.05bA	0.31±0.22aA
H. gowdeyi	Season 1	0.52±0.31aA	0.70±0.23aA	0.20±0.20aA
	Season 2	0.13±0.13aA	0.12±0.09bA	0.00aA
M. sjostedti	Season 1	1.38±0.59aA	0.79±0.47aA	0.07±0.07aA
	Season 2	0.33±0.33bA	0.13±0.12aA	0.00aA

Same lower case letter for the same zone in a particular insect species denotes no significant difference for the two seasons. Same upper case letter for the same season in a particular insect species denotes no significant difference in various altitudinal zones. P≤0.05

When each season was considered separately, there was no significant difference in the abundance of *F. schultzei* among the three altitudinal zones in the first season ($F_{2,86}$= 0.52; P = 0.595). Moreover, there was no significance difference in the abundance of *F. schultzei* among the three altitudinal zones in the second season ($F_{2,95}$= 1.15; P = 0.322) (Table 3-10). There was significantly higher population of *M. sjostedti* in season 1 compared to season 2 at the low altitude zone (t = 19.29; df = 49; P = 0.031). The mean number of *M. sjostedti* in season 1 at the low altitude zone was 1.38±0.59 as compared to 0.33±0.33 in season 2.

However, there was no significant difference in the abundance of *M. sjostedti* between the two seasons at the mid altitude zone (t = 12.76; df = 103; P = 0.076). The mean number of *M. sjostedti* in season 1 at the mid altitude zone was 0.79±0.47 compared to 0.13±0.12 in the second season. Moreover, there was no significant difference in the abundance of *M. sjostedti* between the two seasons at the high altitude zone (t= 4.96; df= 29; p=0.310) (Table 3-10). When each season was considered separately, there was no significant difference in the abundance of *M. sjostedti* across the altitudinal zones during the first season ($F_{2,86}$= 2.25; P=0.111). Moreover, there was no significant difference in the abundance of *M. sjostedti* across the three altitudinal zones during the second season ($F_{2,95}$= 0.33; P=0.722) (Table 3-10).

Significantly higher population of *H. gowdeyi* was observed in season 1 compared to season 2 in the mid altitude zone (t = 30.00; df = 103; P = 0.012). However, there was no significant difference in the abundance of *H. gowdeyi* between the seasons at the low altitude zone (t = 8.17; df = 49; P = 0.146). Moreover, there was no significant difference in the abundance of *H. gowdeyi* between the two seasons at the high altitude zone (t= 4.96; df = 29; P = 0.310). (Table 3-10). When each season was considered separately, there was no significance difference in the abundance of *H. gowdeyi* across the three altitudinal zones during the first season ($F_{2,86}$= 0.71; P = 0.493). Moreover, there was no significant difference in the abundance of *H. gowdeyi* across the three altitudinal zones ($F_{2,95}$= 0.29; P = 0.752) (Table 3-10).

3.5 Discussion

Biodiversity includes a collection of all species of animals, plants and microorganisms preset and interacting in a given ecosystem (Vandermeer and Perfecto, 1995). Agricultural lands that are characterized by monocultures have less pest diversity as compared with the ones that are practicing polycultures or ones with a variety of vegetation types (Altieri and Letourneau, 1984; Altieri, 1994). Changes in climatic factors due to changes in altitude may also affect the biodiversity of insect species in an ecosystem (Pickett, 1989). Therefore, climatic factors and agricultural practices impact heavily on the biodiversity of insect communities in a given ecosystem. In our study, the environment in the mid altitude zone could have provided more niches for different insect species than in the other zones leading to higher species richness and evenness. The mid altitude zone in Kenya is characterised by temperature range of between 17-32˚C and annual rainfall of between 500-1000 mm/year (Hassan, 1998). These climatic conditions particularly the warm weather and adequate rainfall in the mid altitude

zone could have supported growth of a variety of host plant species that could not only provide food for the insects but also offer suitable habitats for their growth and reproduction. A stable environment, i.e. an environment that provides more realisable niches for different species has higher species richness (Legendre, 1998).

Higher altitudes are characterized by relatively lower temperatures (Kronfuss and Havranek, 1999). In general, an increase of 1000 m in altitude results to a 5.5 – 6.5 ˚C decrease in temperature (Anslow & Shawn, 2002). It might have been that many insects have low thermal tolerance range and few could have survived in the high altitude zone which is characterized by low temperatures. The low altitude had the lowest species evenness indicating some species were dominant as compared to the other species. Species evenness can be used to evaluate the biological activity in an ecosystem. Low evenness corresponds to high biological activity in a given site leading to dominance of some species as compared to the others on the ecosystem (Legendre, 1998). In the case of low altitude zone in Kenya, the Lepidopterans, being voracious feeders could have out-competed the other species leading to their dominance in that region.

The abundance of insects at different altitudinal zones is not only affected directly by prevailing climatic factors, but interaction with other living organisms in the ecosystem such as host plant and natural enemies (Hodkinson, 2005). For instance, population of phloem feeding psyllid, *Strophingia ericae,* decreased at higher altitudes when the UV-B levels increased by 15% compared to the low altitude (Salt et al., 1998). In our study, the abundance of the major Coleopterans decreased at higher attitudes. Higher UV-B exposure changes the host-plant morphology and biochemistry by increasing leaf thickness and trichome leaf density, leading to a higher UV-B protection on the plants. Moreover, more exposure to UV-B increases the concentration of carotenes and fouranocoumerins. Higher exposure of UV-B to *Citrus jambhiri,* a host plant of moth *Trichoplusia ni*. lead to a slower development of its larvae (McCloud and Berenbaum, 1994). The biochemistry of the amaranth crop could have been altered by increased exposure to UV-B at high altitude with negative effects on the growth of the Coleopterans feeding on this crop.

We observed the stripped blister beetle, *E. albovittata* only at the low altitude zone of Kenya along the coast. This pest has not been reported as a pest of amaranth in Africa. The high temperatures and humidity prevailing at the Kenyan coast could have favoured the survival and development of the pest in that region. The pest has also been reported in Mexico, a country with tropical climate that is characterized by high temperatures and humidity (Aragon et al, 1997). Our study also reported two other major Lepidopteran pests that had

previously not been documented as pests of amaranth in Kenya. These are *P. atritermina* and *A. octogueae*. *P. atritermina* which is a leaf webber was particularly a menace in the coastal area causing similar damage as that caused by *S. recurvalis*. Moreover, *A. octogueae, a* leaf worm, was observed in our study particularly in the low altitude zone.

Seasonality had an effect on the Lepidopterans, Coleopterans, and Thysanopterans. Lepidopteran species particularly *S. recurvalis* and *P. atritermina* were more abundant in season 2 which was immediately after the onset of short rains in Kenya. The outbreak of these pests could have been triggered by the lush vegetation of host plants that grows after the onset of rains. A similar observation was made in India where S. *recurvalis* population increased during the rainy seasons (National Research Council, 1984). Apart from the cultivated amaranth, other host plants for *S. recurvalis* are wild amaranth, *Amaranthus* spp., and the devils horsewhip A*chyranthes aspera* (Kahuthia-Gathu., 2011). Moreover, during the rainy season, there is higher cultivation of spinach, *Beta vulgaris* by many small scale farmers in the same regions growing amaranth. Spinach is an alternative host plant of *S. recurvalis*. Proliferation of these weed plants and high cultivation of spinach after the onset of the rainy season could have provided *S. recurvalis* with abundant food resources for their growth and reproduction leading to their high abundance. However, in another study conducted in Nigeria, the abundance of *S. recurvalis* did not differ between rainy and dry season (Aderolu et al., 2013). Perhaps there were adequate food resources in both seasons in that region.

Significantly higher abundance of major Coleopteran and Thysanopteran species were observed in the dry season compared to the wet season. Dry and hot weather conditions like those witnessed during the first season survey could have shortened the development time for the Coleopterans and Thysanopterans during the dry season. MacDonald et al., 1998, observed that high temperatures reduce the development time of thrips making them have many and overlapping generations within one season of the crop. Moreover, majority of thrips pupate in the soil (Berndt et al., 2004). Therefore wet soils in rainy season could have suffocated the pupal stages of thrips in the soil leading to low population.

Altitude and seasonality affects the diversity and abundance of amaranth pests in Kenya. The highest diversity of amaranth pests is found in the mid altitude zone. There is also higher abundance of Coleopterans and Thysanopterans in the dry season than in the rainy season. Conversely, abundance of Lepidopterans is highly favoured by the rainy season. Coleoptera, Homoptera and Lepidoptera are the primary insect groups affecting amaranth and management measures for their control need to be prioritised. Farmers in the mid altitude

zone are likely to face greater challenge in management of amaranth pests owing to their high diversity in that region. Moreover, farmers should be alert of threat of Coleopterans during the dry season and Lepidopterans during the rainy season and prepare in advance the management interventions. The wide range of indigenous predators (coccinelid ladybeetles and hoverfly) and parasitoids (*Aphidius colemani*, *Apanteles sp*) observed during the survey could be exploited for the management of these pests in a biological plant protection program.

3.6 References

Amicarelli V, Camaggio G. 2012. Forum Ware International **2**: 4-11.

Anslow FS, Shawn, MJ. 2002. An investigation of local alpine terrestrial lapse rates in the Canadian Rockies. pp. 1. Proceedings of the 32nd Annual Arctic Worshop, INSTAAR, University of Colorado, Boulder.

Altieri MA, Letourneau DK. 1984. Vegetation diversity and insect pest outbreaks. *CRC Critical Reviews in Plant Sciences* **2**: 131–169.

Altieri, M.A., 1994. Biodiversity and Pest Management in Agroecosystems. Haworth Press, New York, 185 pp.

Aragón GA, Tapia-Rojas AM, Huerta-Sánchez SIMT. 1997. Insects associated with the Amaranth crop Amaranthus hypocondriacus L.. (Amaranthaceae) in the Tehuacán Valley, Puebla, Mexico. *Folia Entomológica Mexicana* **100**: 33-43

Berndt O, Meyhöfer R, Poehling H.-M. 2004. The edaphic phase in the ontogenesis of *Frankliniella occidentalis* and comparison of *Hypoaspis miles* and *H. aculeifer* as predators of soil-dwelling thrips stages. *Biological Control* **30**: 17-24.

Degri MM, Randy S. 2014. Effect of variety and spacing on insect pest infestations and growth of *Amaranthus spp* in Alau Dam, Maiduguri, Nigeria. *World Journal of Agricultural Sciences* **2(3)**: 26-30.

Ebert AW, Wu TH, Wang ST. 2011. Vegetable amaranth (Amaranthus L.). *AVRDC Publication* **11**: 754.

Foba CN, Salifu D, Lagat ZO, Gitonga LM, Akutse KS, Fiaboe KKM. 2015. Species Composition, Distribution, and Seasonal Abundance of Liriomyza Leafminers (Diptera: Agromyzidae) Under Different Vegetable Production Systems and Agroecological Zones in Kenya. *Environtal Entomology* **44(2)**: 223–232

Goeden RD, Teerink JA. 1996. Life history and descriptions of adults and immature stages of Aciurina idahoensis Steyskal (Diptera : Tephritidae) on Chrysothamnus viscidiflorus (Hooker) Nuttall in southern California. *Proceedings of the Entomological Society of Washington* **98**: 681–694.

Ha¨Gvar S. 1976. Altitudinal zonation of the invertebrate fauna on branches of birch (Betula pubescens Ehrh.). *Norwegian Journal of Entomology* **23**: 61–74.

Hassan RM. 1998. Maize technology development and transfer. A GIS application for research planning in Kenya. CAB International,Wallingford, United Kingdom.

Hodkinson DI. 2005. Terrestrial insects along elevation gradients: species and community responses to altitude. *Biological Reviews* **80**: 489–513.

Hodkinson ID, Bird J. 1998. Host-specific insect herbivores as sensors of climate change in arctic and Alpine environments. *Arctic and Alpine Research* **30**: 78–83.

Hussey NW, Parr WJ. 1963. The Effect of Glasshouse Red Spider Mite (Tetranychus Urticae Koch) on the Yield of Cucumbers. *Journal of Horticultural Science* **38(4)**: 255–263.

Kagali RN, Kioko EN, Osiemo Z, Muya S, Wachera C. 2013. Insect abundance and diversity on cultivated *Amaranthus* Sp. (Amaranthaceae) in Meru County, Kenya. *American International Journal of Contemporary Research* **3**: 7

Kamga RT, Kouamé C, Atangana AR, Chagomoka T, Ndango R. 2013. Nutritional evaluation of five African indigenous vegetables. *Journal of Horticultural Research* **21(1)**: 99-106

Kavallieratos N, Tomanović Z, Sarlis G, Vayias B, Žikić V, Emmanouel N. 2007. Aphids (Hemiptera: Aphidoidea) on cultivated and self-sown plants in Greece. *Biologia **62*(3)**: 335-344.

Kindt R, Coe R. 2005. Tree diversity analysis. A manual and software for common statistical methods for ecological and biodiversity studies. In. World Agroforestry Centre (ICRAF), Nairobi (Kenya).

Kindt R, van Damme P, Simons AJ. 2006. Tree diversity in western Kenya: Using profiles to characterise richness and evenness. *Biodiversity and Conservation* **15**: 1253-1270.

McDonald JR, Bale JS, Walters KFA. 1998. Effect of temperature on development of the western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae). *European Journal of Entomology* **95**: 301–306.

Kronfuss H, Havranek WM. 1999. Effects of elevation and wind on the growth of Pinus cembra L. in a subalpine afforestation. *Phyton-Annales Rei Botanicae* **39**: 99–106.

Lawton JH, Macgarvin M, Heads PA. 1987. Effects of altitude on the abundance and species richness of insect herbivores on bracken. *Journal of Animal Ecology* **56**: 147–160.

Legendre L, Legendre P. 1998. Numerical ecology, 2 edn. Elsevier.

McCloud ES, Berenbaum MR. 1994. Stratospheric ozone depletion and plant-insect interactions – effects of UVb radiation on foliage quality of Citrus jambhiri for Trichoplusia ni. *Journal of Chemical Ecology* **20**: 525–539.

Mureithi, DM, Fiaboe KKM, Ekesi S, Meyhöfer R. 2017. Important arthropod pests on leafy Amaranth (*Amaranthus viridis*, *A. tricolor* and *A.* 1 *blitum*) and broad-leafed African nightshade (*Solanum scabrum*) with a special focus on 2 host-plant ranges. *African Journal of Horticultural science* **11**: 1-17

National Research Council. 1984. Amaranth: Modern prospects for an ancient crop. *National Academies* **47**

Nyasani JO, Meyhöfer R, Subramanian S, Poehling H.-M. 2012. Effect of intercrops on thrips species composition and population abundance on French beans in Kenya. *Entomologia Experimentalis et Applicata* **142**: 236–246.

Ogedegbe AB, Ezeh AE. 2015. Effect of variety and nutrient on insect pest infestation of *Amaranthus* spp. *J. Appl. Sci. Environ. Manage*. **19 (2)**: 251-256

Oke OA, Odiyi CA, Ofuya TI. 2015. Insects associated with underutilized crop: grain, leafy and ornamental amaranth in Ibadan, Nigeria. *J Agric Ecol Res Int*, ***2*(2)**: 145-155.

Oksanen M, Pietarinen J. 2004. Philosophy and biodiversity. Cambridge University Press, Edinburgh, UK.

Pickett STA. 1989. Space-for-time substitution as an alternative to long-term studies. In Long-term studies in Ecology (ed. G. Likens), pp. 110–135. Springer-Verlag, Heidelberg.

Pinheiro F, Diniz FIR, Coelho D, Bandeira MPS. 2002. Seasonal pattern of insect abundance in the Brazilian cerrado. *Austral Ecology* **27**: 132–136

Said M, Itulya FM. 2003. Intercropping and nitrogen management effects on diamondback moth damage and yield of collards in the highlands of Kenya. *African Crop Science Journal* **11(1)**: 35-42.

Salt DT, Moody SA, Whittaker JB, Paul ND. 1998. Effects of enhanced UVB on populations of the phloem feeding insect Strophingia ericae (Homoptera: Psylloidea) on heather (Calluna vulgaris). *Global Change Biology* **4**: 91–96.

Schippers, R R. 2000. African Indigenous Vegetables. An Overview of the Cultivated Species. Natural Resources Institute/ACP-EU Technical Centre for Agricultural and Rural Cooperation, Chatham, UK. Pp 214.

Sithanantham S, Matoka CM, Maundu M, Jakari M, Agong SG. 2003. Integrated crop protection research for sustainable production of Indigenous vegetable crops in Eastern Africa. Proceedings of 4th Horticultural Seminar on Sustainable Horticultural production in the Tropics held in Njoro, Kenya.

Sreelatha DE, Uma C. 2012. Antioxidant Properties of Rajgira (Amaranthus paniculatus) Leaves and Potential Synergy in Chemoprevention. *Asian Pacific J Cancer Prev* **13**: 2775-2780.

Tóthmérész B. 1995. Comparison of different methods for diversity ordering. *Journal of Vegetation Science* **6**: 283-290.

Turner BD, Broadhead E. 1974. The diversity and distribution of psocid populations on Mangifer indica L. in Jamaica and their relationship to altitude and micro-epiphyte diversity. *Journal of Animal Ecology* **43**: 173–190.

Uusiku NP, Oelofsea A, Duodub KG, Besterc MJ, Faberd M. 2010. Nutritional value of leafy vegetables of sub-Saharan Africa and their potential contribution to human health: A review. *Journal of Food Composition and Analysis* **23**: 499–509.

Vandermeer, J., Perfecto, I. 1995. Breakfast of biodiversity: the truth about rainforest destruction. Food First Books, Oakland, 185 pp.

Wolda H. 1988. Insect seasonality: Why? *Annual Review of Ecology, Evolution, and Systematics* **19**: 1–18.

4 Effect of elevation and seasonality on the abundance and diversity of major pests of African nightshades (*Solanum* sp) in Kenya and their associated natural enemies.

4.1 Abstract

There has been a renewed interest in production and consumption of African nightshades due to their superior nutritional content and health benefits. However, pests continue to pose great challenge to their production. Scanty information is available on the abundance, distribution and damage of pests that infest this crop in Kenya and the naturally occurring natural enemies that could be exploited for their management. To understand the diversity, distribution, seasonality and damage caused by the major insect pests infesting African nightshades, studies were conducted in all the major African nightshade growing areas in Kenya during the wet and dry season. A total of 132 African nightshade farms were examined for the insect pests and associated natural enemies using the beating and the picking methods. 29 farms were located in the high altitude zone, 91 in the mid altitude zone and 12 in the low altitude zone. A total of 8190 individual insects were collected of which 6220 Homopterans, 1466 Coleopterans, 444 Thysanopterans and 60 Lepidopterans. For the first time, we report 47 Coleoptera species, 6 aphid species, 8 Lepidoptera species and 8 Thysanoptera species infesting African nightshades in Kenya. The highest diversity of African nightshade pests was in the mid altitude zone and during the dry season. The greatest damage was caused by Homopterans (26.8 %), Coleoptera (16.5%), Lepidoptera (5.1%) and Thysanoptera (3.7%). The most destructive species of African nightshades were *Aphis gossypii*, *Epitrix silvicola* and *Phyllotreta* sp. The findings of this study will be useful in prioritization of key pests in development of management measures for African nightshade pests in various regions of Kenya and in different seasons.

Key words: *Pest distribution, species richness, species composition*

4.2 Introduction

African nightshade is the 2nd most produced African indigenous vegetable (AIV) in Kenya (HCDA, 2012). African nightshades are a valuable source of vitamins A and C, calcium, potassium, iron and proteins (IPGRI, 2003; Kamga et al., 2013). The vegetable is also recommended for the people living with human immune deficiency virus (HIV/AIDS) due to its therapeutic properties (Abukutsa-Onyango, 2007). Pests are among the major constraint in production of African nightshades. Aphids and red spider mites have been reported as the main pests infesting nightshade in Kenya with yield losses as high as 36-100% particularly during the dry spell (Sithanantham et al., 2003; Mureithi et al., 2017). In addition to direct losses, insects such as aphids significantly reduce product quality through contamination with honeydew and subsequent sooty mould, leading to frequent markets rejections (Varela and Seif, 2004). Especially leaves attacked by spider mites are generally twisted, webbed and unmarketable.

Few studies have been conducted on pests of African nightshades in Kenya. For instance, a study conducted in Western and Rift valley regions of Kenya showed that whiteflies, aphids, leafminers, leaf hoppers and grass hoppers were reported as the main pest infesting the African nightshade in western and rift valley regions of Kenya. A related study in Central region reported that cutworm (*Agrotis* spp), white grubs, crickets, aphids (*Myzus persicae*), African bollworm (*Helicoverpa armigera*) and leafminer (*Liriomyza* sp) were reported as the main pests attacking nightshade in central region of Kenya (Mbugua et al., 2006).

Several factors influence the diversity, distribution and abundance of insects in a given area. Factors that may affect the insect population include; altitudinal gradient, climatic factors, availability of host plants, natural enemy complex, and interspecific competition (Lawton et al. 1987; Torres and Madi-Ravazzi 2006). In particular, altitudinal gradient could influence the diversity of insect communities in a given ecosystem. Previous studies have given contrasting conclusion on the effect of altitudinal gradient on the abundance and diversity of insect communities in an ecosystem. In their study, Romero-Alcaraz and Avila (2000) reported higher species richness and diversity of scarabaeoid dung beetles in high altitude zones than in the low altitudes zones in a study conducted at altitude zones between 900-2271 m above sea level in Sierra de Baza, southeastern Iberian Peninsula. They concluded that the higher diversity of the scarabaeoid dung beetle at high altitudes was a result of fewer disturbances of the habitats by human activities such as deforestation. Conversely, in another study, the diversity of butterfly communities across the Mediterranean mountain in Spain did not differ significantly at different altitudinal zones (Sanchez-rodriguez and Baz, 1995).

Climatic factors also contribute significantly to the diversity and abundance of insect communities. In a study to evaluate the seasonal variability of insects in Cerrado region, Brazil, higher diversity and abundance of insects were observed in the rainy season as compared to the dry season (Silva et al., 2006). Similar pattern was observed in a study conducted in gaps Panama whereby higher arthropod diversity was observed in the rainy season compared to the dry season (Richards and Windsor, 2005).

To our knowledge, a nationalwide study for the major pests of African nightshades in Kenya and their associated natural enemies has not been conducted. In the present study, we were interested in assessing the diversity and abundances of arthropod pests infesting African nightshades across different attitudinal zones and seasons in the major nightshade production areas in Kenya. We also evaluated the damage caused by these arthropod pests on African nightshades and assessed the natural enemies for these pests occurring naturally in these areas.

4.3 Methodology

4.3.1 Field assessment methodology

The first season study was done between the months of February to May 2014, while the second season study was done in October to November 2014. The first season was characterized by dry and hot weather conditions while the second season witnessed heavy rains. In the high altitude zone, nightshade farms in Kiambu, Kirinyaga, Nyamira, and Transzoia counties were screened for pest of nightshades and associated natural enemies. In the mid altitude zone, nightshade farms in Machakos, Embu, Kajiado, Kisii, Narok, Kisumu, Kakamega, Busia, Nyamira, Kirinyaga, and Tranzoia Counties were studied. In the low altitude zone, nightshade farms in Mombasa, Kilifi, Lamu and some parts of Embu County were considered. The altitude ranges were as follows; low altitude zone (< 1000 m asl), mid altitude zone (1000-1800 m asl) and high altitude zone (> 1800 m asl) (Hassan, 1998).

10 nightshade farms from each of the County (5 farms each from 2 sub-counties) were chosen for the study. 40 plants were examined in each nightshade farm in a 10 x 10 m plot. The plot was sub-divided into 4 quadrants and plants sampled following a diagonal transect across the plot. Two sampling strategies were employed i.e. the beating and the picking methods. For the cryptic insects hiding inside the plants canopy, the beating method was used whereby a 32 x 23.5 cm tray sprayed with a layer of 70% ethyl alcohol was placed below the plant canopy. The plant was then tapped 10 times to dislodge insects that were hiding on the leaves and flowers of nightshade plants. A fine camel brush (No. 1) was used to pick the

insects that fell on the tray and transferred them into insect collection vial containing 70% ethyl alcohol. The vials were labeled appropriately with the details of the location where the sample was obtained. For large insects that were rather docile, hand picking method was used to capture the insects and transfer them into the insect collection vials containing 70% ethyl alcohol. Immature stages of insects, mainly caterpillars and leafminers, were hand-picked and put into lunch boxes (19 cm ×13 cm × 8 cm) lined with paper towel on the inside. Fresh leaves were also added into the lunch boxes to provide food for the immature insects during transportation into the laboratory. Upon arrival into the laboratory, the nutritional supply was continued until insects went into pupation. Suitable pupation sites were provided to enable the insects develop to adulthood. Thereafter, they were collected and preserved in insect collection vials containing 70% ethyl alcohol. A similar procedure as for the live immature stages of insects was followed for the parasitoid mummies. Matures stages collected by either the beating or the picking methods were mounted and taken to the National Museums of Kenya, 1.2740° S, 36.8145° E, for identification.

4.3.2 Insect damage scoring guidelines

It was important to record the damage caused by different pest groups as some pests cause more damage than others. Moreover, information on pest damage complemented the information on pest abundance. The guide on table 4-1 was used for scoring damage from various pests.

Table 4-1: damage guide used to score for various insect orders during the survey to identify the major pests of African nightshades in Kenya.

Insect	**Damage score**	**Description of damage**	**Reference**
Aphids	0	Plant appear healthy, may have small chrotic spots	Webster et al. 1987
	1	Chlorosis and leaf folding 26-50 % of total leaf area	
	2	Chlorosis and leaf folding 26-50 % of total leaf area	
	3	Chlorosis and leaf folding 51-75 % of total leaf area	
	4	Chlorosis and leaf folding > 75% of total leaf area	
Coleopterans	0	No visible damage on the crop	Smith, 2000 modified
	1	1-20% leaf consumed	
	2	21-40% leaf consumed	
	3	41-60% leaf consumed	
	4	61-80% leaf consumed	
	5	81-100% leaf consumed	
Lepidopterans	0	No leaf damage	Said and Itulya, 2003 modified
	1	1-25 % of leaf consumed	
	2	26-50 % of leaf consumed	
	3	51-75 % of leaf consumed	
	4	76-100 % of leaf consumed	
Thysanopterans	1	No leaf damage	Nyasani et al., 2011
	2	Few silvery streaking (≤25%)	
	3	Moderate streaking (26-50%)	
	4	Heavy streaking (51-75%)	

Table 4-1: damage guide used to score for various insect orders during the survey to identify the major pests of African nightshades in Kenya (Continued).

Insect	**Damage score**	**Description of damage**	**Reference**
Thysanoptera	5	Severe streaking and drying of attacked leaves (≥75%)	Nyasani et al., 2011
Mites	1	1-20 % of leaf damaged	Hussey & Parr 1963 modified
	2	21-30 % of leaf damaged	
	3	31-50 % of leaf damaged	
	4	51-70 % of leaf damaged	
	5	71-0 % of leaf damaged	

4.3.3 Data analysis

ANOVA for the pest abundance in the 4 most important orders and for the three most abundant species per order at different altitudinal zones and seasons was done using R. program (R version 3.3.1, 2016). The insect count data was log transformed before the analysis was done. Where significant differences were observed, the means were separated using the Tukey test. All tests were carried out at 5% level of significance. Insect species diversity on nightshade crop across different altitudinal zones and in different seasons were analysed using the Renyi diversity profiles. Biodiversity analyses were done using R (version 3.3.1, 2016). V*egan* (Oksanen et al., 2005) and *BiodiversityR* (Kindt and Coe, 2005) packages were used to calculate the diversity and IBM SPSS was used to plot the graphics.

The Renyi diversity profiles order species in an ecosystem from species richness to species evenness. Other common diversity indices used by ecologist such as the Shannon index or Simpson index are specific cases of Rényi entropy formula. In Renyi diversity profile, the diversity values on the y-axis (H-alpha) are related to the scalar parameter "alpha" on the x-axis. H_0 = species richness, H_1 = Shannon Diversity, H_2 = Simpson Diversity and H_∞ = Berger-Parker Index (Legendre and Legendre, 1998, Kindt et al. 2006). H-alpha is based on the frequency of each component species (proportional abundances "pi" = abundance of species i/ total abundance) and a scale parameter (α) ranging from zero to infinity (Tóthmérész, 1995).

$$H_\alpha = \frac{\ln\left(\sum p_i^\alpha\right)}{1-\alpha}$$

A given insect community X is said to have higher species richness than community Y if it has a higher value at alpha=0 compared to community Y. Similarly, insect community X is said to have higher evenness than community Y if it has a higher value at alpha=∞ than community Y. Species diversity is a combination of species richness and species evenness. A given insect community X is regarded as more diverse than a community Y if its diversity profile is everywhere above that of community Y in the Renyi diversity profiles graph. This

means that community X has higher species richness and higher species evenness than community Y. If the profile lines of different insect communities cross each other, it is not possible to order the diversity of these communities from the most diverse to the least diverse. This is because one community say community X could have higher species richness than another community Y but at the same time community Y could have higher species evenness than community X. In that case, we can only discuss species richness and species evenness separately (Legendre and Legendre). The values of the series for the three altitudinal zones and in the two seasons were calculated for the scales of $\alpha = \{0, 0.25, 0.5, 1, 2, 4, 8, \infty\}$ and plotted as diversity profiles for each altitudinal zone (high, mid, and low) in a single graph or diversity profiles for each of the seasons (Season 1 and season 2) in a single graph.

4.4 Results

4.4.1 Diversity of African nightshade pests across altitudinal zones and seasons

The Renyi diversity profile for the mid altitude zone was everywhere above those of the high and low altitude zones meaning the mid altitude zone had the highest insect diversity. However, the Renyi diversity profiles for the high and the low altitude zones were crossing each other suggesting that there was no difference in nightshade pest species diversity between the two altitudinal zones (Fig 4-1a).

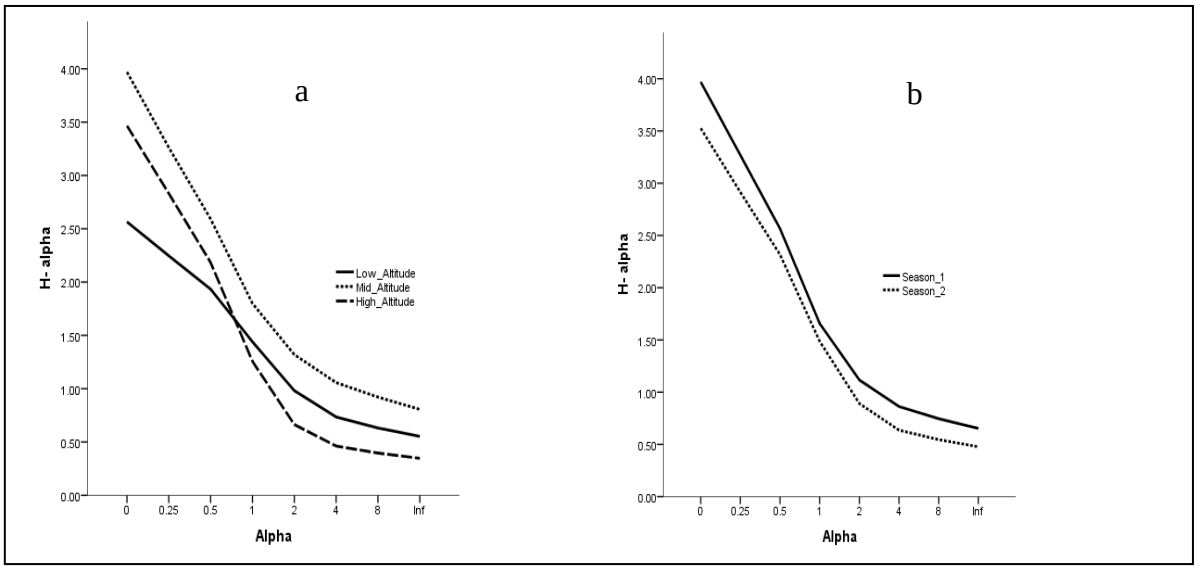

Fig 4-1: Renyi diversity indices for African nightshades pests in a study conducted in Kenya across three agro-ecological zones. These are; high altitude, mid altitude and low altitude zones (a). Renyi diversity indices for African nightshades pests in a survey conducted in Kenya across the six regions producing African nightshades for two seasons of 2014 (b).

When pest species richness was considered separately, i.e. at alpha= 0, the mid altitude had the highest pest species richness followed by the high altitude and low altitude respectively. The H-alpha value at alpha=0 were 3.97 for the mid altitude, 3.47 for the high altitude, and 2.56 for the low altitude (Fig 4-1a). When pest species evenness was considered, the mid altitude had the highest H-alpha value (0.81) at H=∞ value indicating it had the highest pest species evenness. The low altitude zone had higher H-alpha value (0.55) at H=∞ that high altitude (0.35) indicating it had higher pest species evenness than the high altitude. When the two seasons were compared, the diversity profile for season 1 (dry season) was everywhere above that of the second season (rainy season) indicating that there was higher pest species diversity on nightshade farms in the first season (Fig 4-1b). Moreover, there was higher pest species richness in the first season compared to the second season. The species richness index was 3.97 in the first season compared to 3.53 in season 2. There was also higher pest species evenness in the first season compared to the second season. The H- alpha value at H=∞ signifying pest species evenness was 0.65 in the first season compared to 0.48 in the second season (Fig 4-1b).

4.4.2 Major insect orders attacking African nightshades

The major pest groups observed on African nightshades during the study were from insect orders Trombidiformes, Coleoptera, Homoptera, Lepidoptera, and Thysanoptera. There was significantly higher number of Homopterans compared to the other pest groups. Similarly, there were significantly higher numbers of Coleopterans compared to Trombidiformes, Lepidopterans and Thysanopterans. However, there was no significant difference in the abundance of Trombidiformes, Lepidopterans, and Thysanopterans ($F_{4,662}$=46.01; P<0.001)(Fig 4-2).

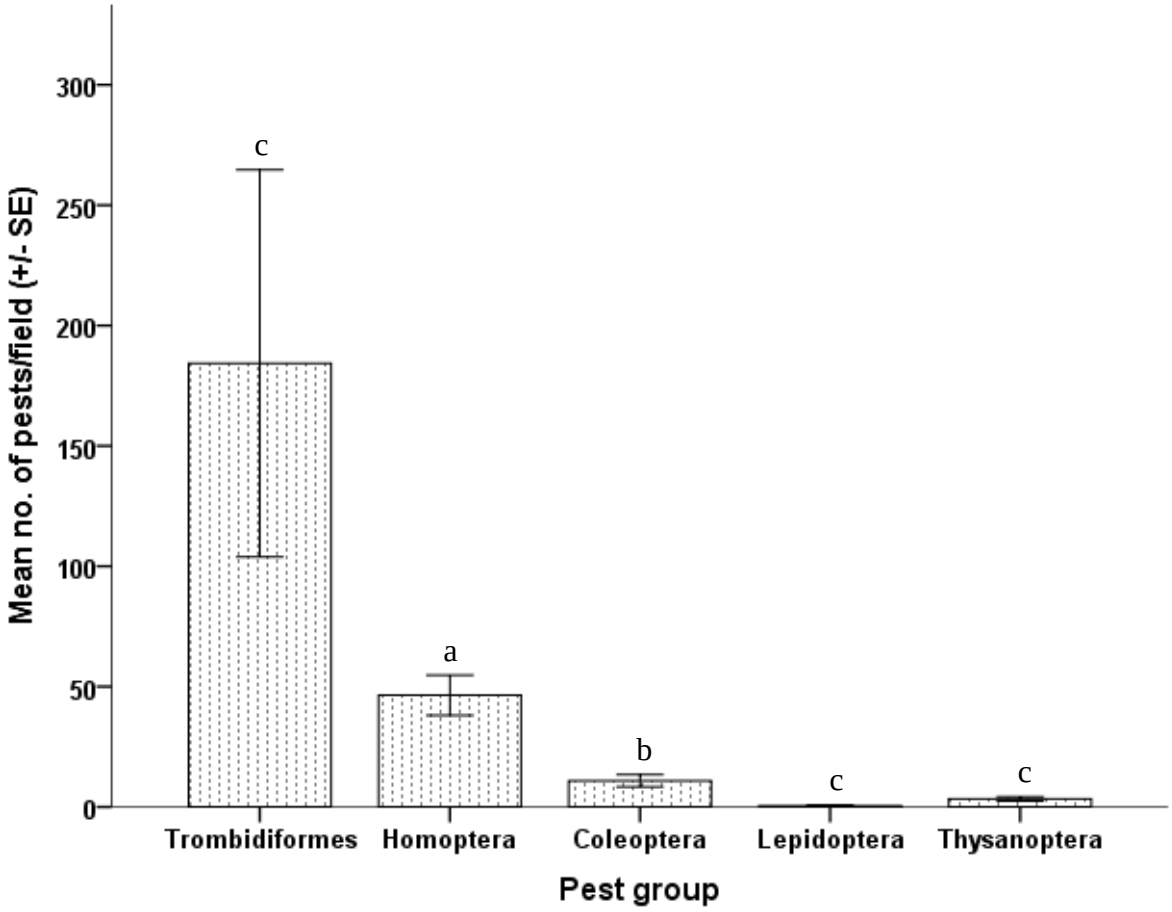

Fig 4-2: Mean number (+/- SE) of insects/ field infesting African nightshades from the major orders in a study conducted in two seasons of year 2014 across the three altitudinal zones (low, mid and high) growing African nightshades in Kenya. The insect count data was log transformed before the analysis. Anova was carried out at P≤0.05. Where significance difference was observed, Tukey test was used to separate the means.

4.4.3 Damage by major insect orders attacking nightshades

With 26.76% damage, Homopterans caused significantly higher damage on African nightshades compared to the other pest groups. Moreover, Coleopterans caused higher damage on the crop (16.5%) compared to the Trombidiformes (spider mites), Lepidopterans, and the Thysanopterans. There was no significant difference in the damage caused by Trombidiformes, Lepidopterans, and Thysanopterans ($F_{4,662}$=91.70; P<0.001) (Fig 4-3).

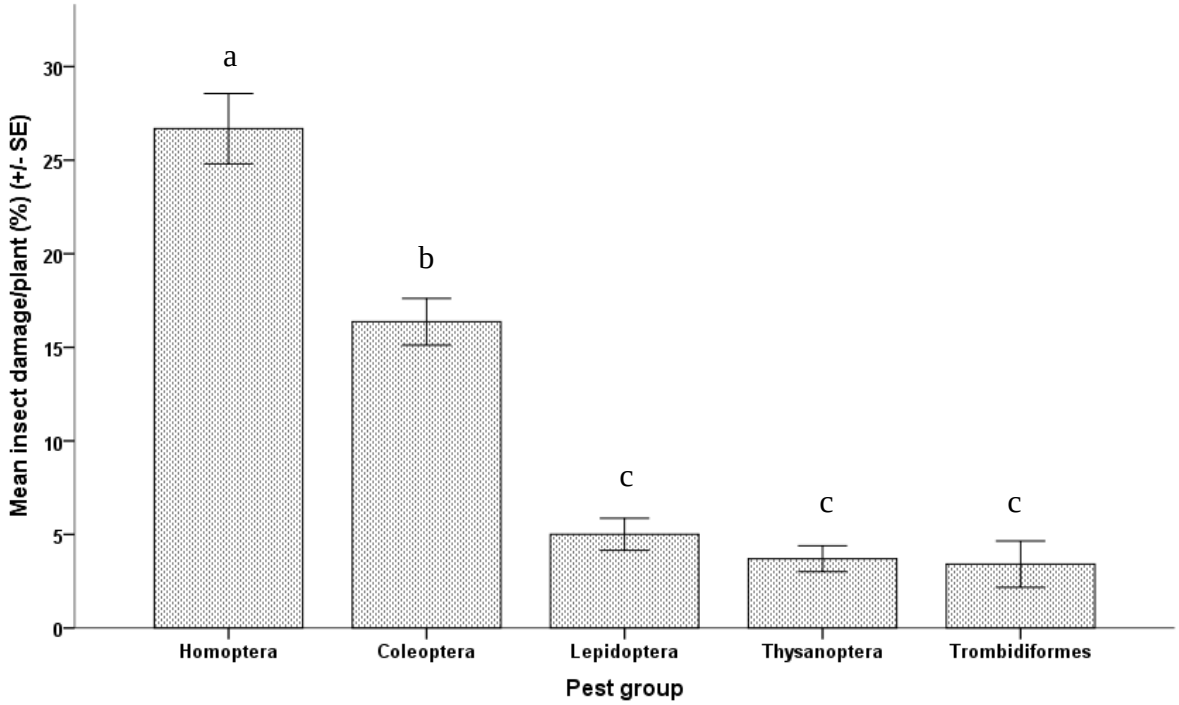

Fig 4-3: Mean insect damage (%) (+/- SE) from the major insect orders infesting African nightshades in a study conducted in two seasons of year 2014 across the three altitudinal zones (low, mid and high) growing African nightshades in Kenya.

The main damage by Homopterans was caused by aphids. They caused chlorosis, curling and twisting of the leaves. Moreover, production of honey dew by the aphids led to the development of black sooty moulds that stained the leaves. Leaves infested by the coleopterans particularly the flea beetles had mainly small shoot holes. However, some Coleopterans made larger holes in or around the leaf margin. The Lepidopteran caterpillars were foraging on the leaves leaving large irregular holes. Thrips damage was mainly through punctures made on the leaves leaving unsightly white scars on the nightshade leaves.

4.4.4 Abundance, distribution and seasonality of major Homopteran species

Six aphid species belonging to 4 genera were observed. All the aphids belonged to Aphididae family. *Aphis craccivora*, *Aphis gossypii* belonged to the Aphis genus while *Myzus persicae* belonged to the Myzus genus. Moreover, *Macrosiphum sp* belonged to Macrosiphum genus while *Toxoptera sp* belonged to Toxoptera genus. The three most abundant aphid species on African nightshades were *A. fabae*, *M. persicae* and *A. craccivora* (Table 4-2).

Table 4-2: Composition of insect species infesting African nightshades in Kenya in a study conducted during the dry and the rainy seasons in the year 2014 across the three altitudinal zones (low, mid and high).

Order	**Family**	**Species**	**Proportion (%)**
Homoptera	Aphididae	***Aphis fabae* (Scopoli)**	**72.29**
		***Myzus persicae* (Sulzer)**	**20.48**
		***Aphis craccivora* (Koch)**	**5.76**
		Macrosiphum sp.	1.01
		Aphis citricola (Van Der Goot)	0.43
		Toxoptera sp.	0.03
Coleoptera	Anthicidae	*Formicomus* sp.	1.05
	Apionidae	*Apion* sp.	0.37
		Cylas brunneus	0.22
	Bruchidae	*Acanthoscelides obtectus* (Say)	0.07
	Carabidae	*Tachys ascendens* (Alluaud)	0.30
	Chrysomelidae	***Epitrix silvicola* (Bryant)**	**59.04**
		***Phyllotreta* sp.**	**19.96**
		***Luperodes quaternus* (Fairmaire)**	**2.99**
		Monolepta cruciata (Guerin De Meneville)	1.05
		Haltica pyritosa (Erichs)	0.67
		Bicolorizea semifulua	0.45
		Monolepta vinosa (Gerstaecker)	0.37
		Exora pusilla (Gerstaecker)	0.22
		Hapsidolema nigroparallela (Crow)	0.22
		Herma insignis (Weise)	0.22
		Mesoplatys ochroptera (Stal)	0.22
		Hapsidolema viridisuturalis (Pic)	0.15
		Pachnephorus sp.	0.15
		Prosmidia pygidialis	0.15
		Leptaulaca basalis (Weise)	0.07
	Coccinelidae	*Epilachna fulvosignata* (Reiche)	0.30
		Epilachna paykulli (Muls)	0.07
	Curculionidae	*Baris* sp.	1.49
		Systates crenatipennis (Fairmaire)	1.12
		Micrelus cruciatus (Schultze)	0.37
		Babaultia sp.	0.30
		Nematocerus lindblomi (Aurivillius)	0.15
		Balanogastris sp.	0.07
	Elateridae	*Candiophorus* sp.	0.52
		Drasterius aethiopicus (Abyss)	0.15
		Paederus riftensis (Fauvel)	0.15
		Heteroderes sp.	0.07
	Meloidae	*Epicauta alborvita* (Gestro)	2.84

Table 4-2: Composition of insect species infesting African nightshades in Kenya in a study conducted during the dry and the rainy seasons in the year 2014 across the three altitudinal zones (low, mid and high) (Continued).

Order	**Family**	**Species**	**Proportion (%)**
Coleoptera	Melyridae	*Hapalochrus similaeuis*	0.07
	Nitidulidae	*Brachypeplus* sp.	0.30
		Carpophilus dimidiatus (Fabricius)	0.07
	Scarabaeidae	*Sisyphus ocellatus* (Reiche)	0.22
		Onthophagus sp.	0.30
	Staphylinidae	*Paederus sabaeus* (Erichson)	2.39
	Tenebrionidae	*Eutochia pulla* (Fabricius)	0.22
		Lagria cuprina (Thomas)	0.22
		Lagria purpurascens (Borchmann)	0.22
		Lagria cyanicollis (Borchmann)	0.15
		Derolagria dermatodes (Fairmaire)	0.07
		Gonocephalum simplex (Fabricius)	0.07
		Lagria sexvitta	0.07
Lepidoptera	Erebidae	*Spilosoma investigatosum*	1.67
	Gelechiidae	*Tuta absoluta* (Meyrick)	1.67
	Noctuidae	***Spodoptera litorralis* (Boisduval)**	**40.00**
		***Helicoverpa armigera* (Hübner)**	**31.67**
		***Spodoptera exigua* (Hübner)**	**15.00**
		Plusia sp	5.00
		Spodoptera exempta (Walker)	3.33
		Timora crofti (Pinhey)	1.67
Thysanoptera	Aeolothripidae	*Franklinothrips megalops* (Trybom)	0.68
	Phlaeothripidae	***Haplothrips gowdeyi* (Franklin)**	**24.94**
	Thripidae	***Megalurothrips sjostedti* (Trybom)**	**42.63**
		***Dendrothrips* sp.**	**15.65**
		Frankliniella schultzei (Trybom)	9.52
		Thrips sp.	4.76
		Thrips pusillus (Bagnall)	1.59
		Frankliniella occidentalis (Pergande)	0.23

There was significantly higher population of *A. fabae* in the high elevation zone compared to the mid and low elevation zone. However, there was no significant difference in the abundance of *A. fabae* between the mid and the low altitude ($F_{2,129}$=15.94; P<0.001) (Fig 4-4).

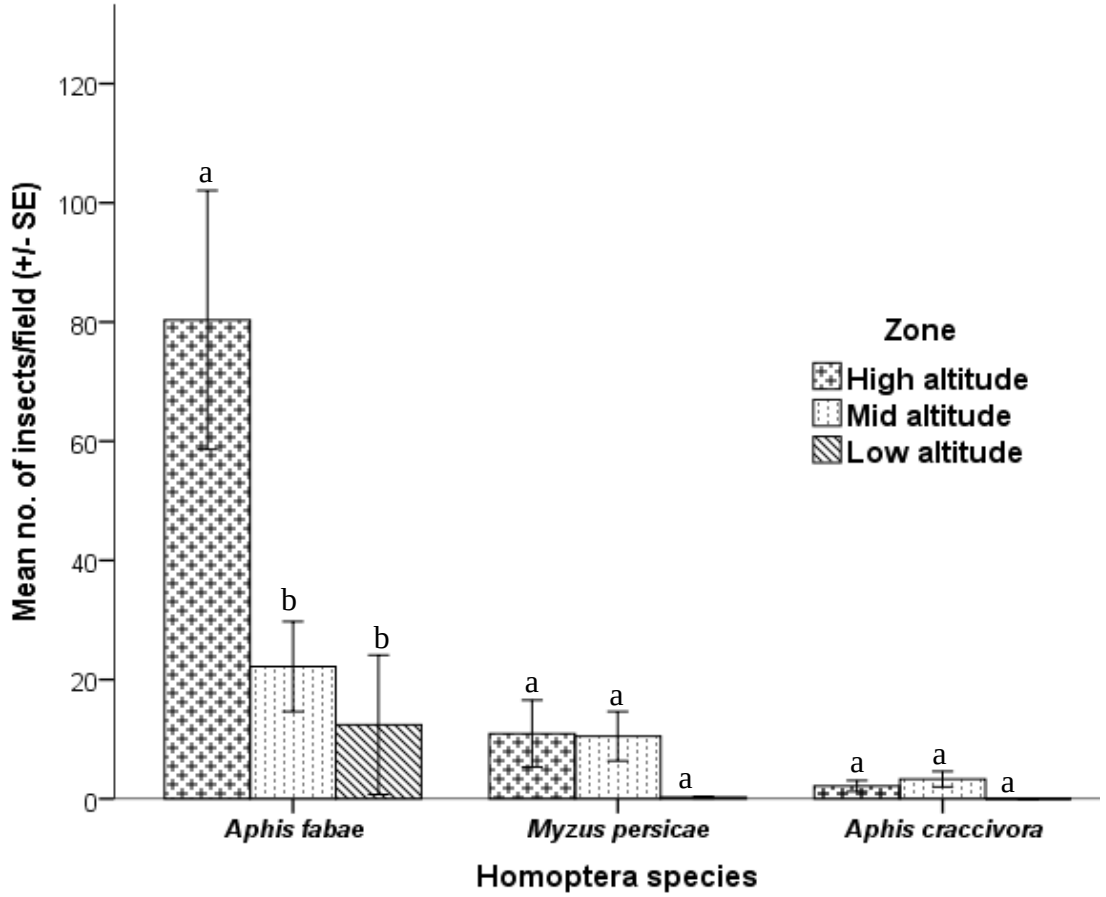

Fig 4-4: Mean number of *Aphis fabae*, *Myzus persicae* and *Aphis craccivora* per field infesting African nightshades from a survey conducted across the high, mid and low altitude zones of Kenya in the year 2014. The zones were drawn from the six regions (Central, Coast, Eastern, Nyanza, Rift, and Western) growing African nightshades in Kenya. Same letter for a given species denotes no significant difference among the zones. The insect count data was log transformed before the analysis. Anova was carried out at P≤0.05. Where significance difference was observed, Tukey test was used to separate the means.

When the abundance of *M. persicae* was evaluated across the elevation zones, no significance difference was observed ($F_{2,129}$=1.19; P=0.309) (Fig 4-4). Moreover, there was no significant difference in the abundance of *A. craccivora* across the three elevation zones ($F_{2,129}$=0.98; P=0.380) (Fig 4-4). When different seasons were considered separately, there was no significant difference in the population of *A. fabae* between the first and the second season ($F_{1,130}$=4.32; P=0.121) (Table 4-3).

Table 4-3: Mean number of insects/ field from the three major aphid species infesting African nightshades in Kenya in a study conducted in the two seasons in the year 2014 across the three altitudinal zones (low, mid and high).

Species	**Season 1 (Dry season)**	**Season 2 (Rainy season)**
Aphis fabae	43.42±12.39a	25.80±8.61a
Myzus persicae	16.52±6.32a	3.59±1.49b
Aphis craccivora	0.85±0.53b	4.39±1.67a

Same lower case letter in a given insect species denotes no significant difference between the seasons

However, there was significantly higher abundance of *M. persicae* in the first season than in the second season ($F_{1,130}$=10.22; P=0.013) (Table 4-3). Conversely, there was significantly higher abundance of *A. craccivora* in the second season compared to the first season ($F_{1,130}$=25.94; P=0.012) (Table 4-3).

4.4.5 Abundance, distribution and seasonality of major Coleopteran species

Forty seven (47) Coleoptera species from 41 genera and 13 families were observed during the study. Chrysomelidae had the largest number of species with 16 species. Majority of these species were flea beetles which cause damage to the crop by puncturing the shoot-holes. Tenebrionidae family was second with 7 species followed by Curculionidae with 6 species. Four species were observed in Elateridae family. Families Apoinidae, Coccinelidae, Nitidulidae, and Scarabaeidae had 2 species each while families Anthicidae, Carabidae, Meloidae, Melyridae, and Staphylinidae had one species each (Table 4-2). *Epitrix silvicola*, *Phyllotreta* spp and *Luperodes quaternus* were the three most abundant Coleoptera species in Kenya (Table 4-2). The three most abundant Coleoptera species i.e. *E. silvicola*, *Phyllotreta* spp, and *L. quaternus* were all absent in the low elevation zone (Fig 4-5).

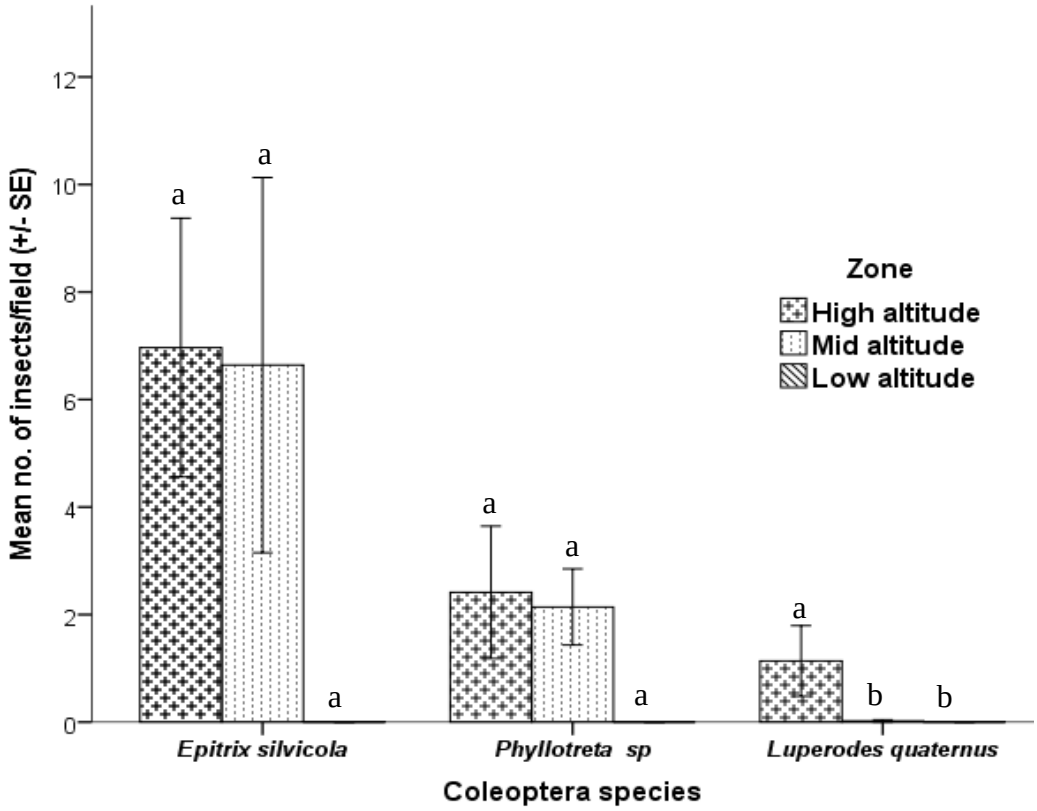

Fig 4-5: Mean number of *Epitrix silvicola*, *Phyllotreta* sp and *Luperodes quaternus* per field infesting African nightshades from a survey conducted across the high, mid and low altitude zones of Kenya in the year 2014. The zones were drawn from the six regions (Central, Coast, Eastern, Nyanza, Rift, and Western) growing African nightshades in Kenya. Same letter for a given species denotes no significant difference among the zones. The

insect count data was log transformed before the analysis. Anova was carried out at P≤0.05. Where significance difference was observed, Tukey test was used to separate the means.

The abundance of *E. silvicola* was not significantly affected by elevation zones ($F_{2,129}$=1.96; P=0.145) (Fig 4-5). Moreover, elevation zone did not have a significant effect on the abundance of *Phyllotreta* sp ($F_{2,129}$=1.38; P=0.257) (Fig 4-5). . However, the abundance of *L. quaternus* was significantly higher in the high elevation zone compared to the other zones ($F_{2,129}$=5.84; P=0.004) (Fig 4-5). . Seasonality did not significantly affect the population of *E. silvicola* ($F_{1,130}$=4.35; P=0.261) (Table 4-4).

Table 4-4: Mean number of insects/ field from the three major Coleopteran species infesting African nightshades in Kenya in a study conducted in the two seasons in the year 2014 across the three altitudinal zones (low, mid and high).

Species	**Season 1 (Dry season)**	**Season 2 (Rainy season)**
Epitrix silvicola	9.79±5.19a	2.94±0.87a
Phyllotreta sp.	3.44±1.06a	0.77±0.46b
Luperodes quaternus	0.55±0.31a	0.01±0.01a

Same lower case letter in a given insect species denotes no significant difference between the seasons

Moreover, there was no significance difference in the abundance of *L. quaternus* between the first and the second season ($F_{1,130}$=16.04; P=0.077) (Table 4-4). . However, there was significantly higher abundance of *Phyllotreta* spp. in the first season compared to the second season ($F_{1,130}$=33.88; P=0.004) (Table 4-4). .

4.4.6 Abundance, distribution and seasonality of major Lepidopteran species

Eight Lepidopteran species belonging to three families and six genera were observed. The dominant family was the Noctuidae with 6 species while the Arctiidae and Gelenchiidae had one species each (Table 4-2). The three most abundant Lepidoptera species were *S. littoralis, H. armigera* and *S. exigua.* (Table 4-2). Different elevation zones did not have a significant effect on the abundance of *S. littoralis*. ($F_{2,129}$=1.36; P=0.260) (Fig 4-6).

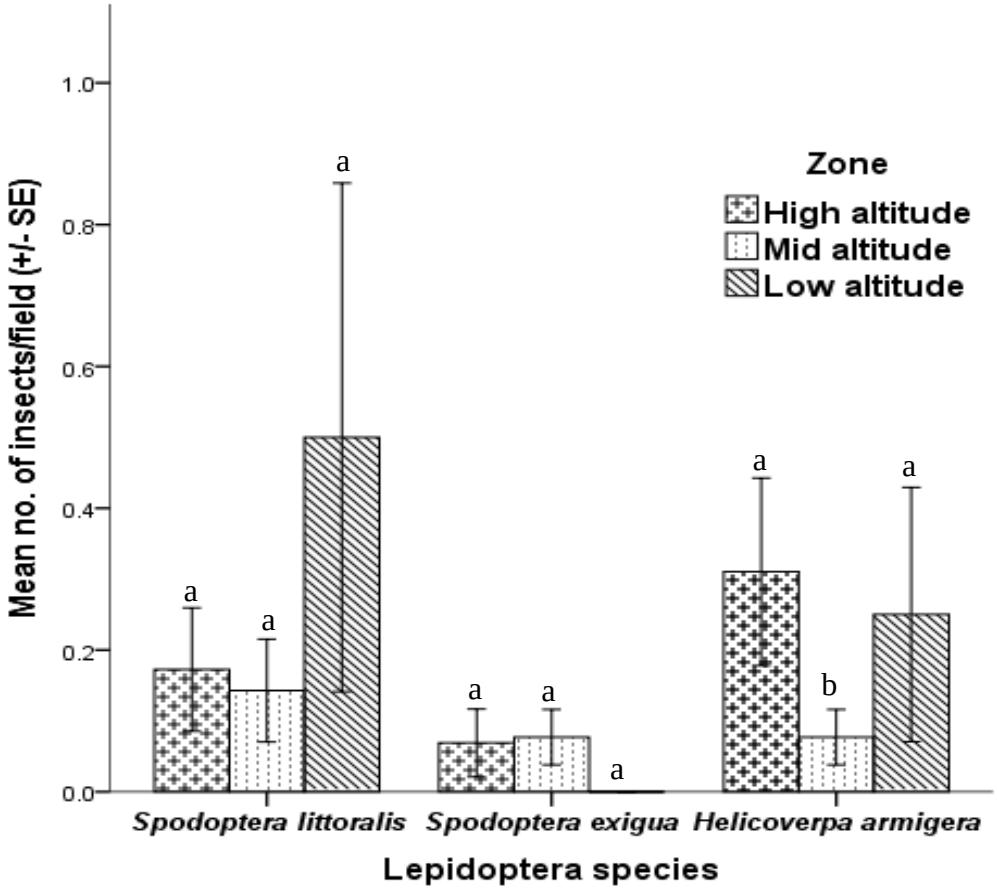

Fig 4-6: Mean number of *Helicoverpa armigera*, *Spodoptera litorralis* and *Spodoptera exigua*/ field infesting African nightshades from a survey conducted across the high, mid and low altitude zones of Kenya in the year 2014. The zones were drawn from the six regions (Central, Coast, Eastern, Nyanza, Rift, and Western) growing African nightshades in Kenya. Same letter for a given species denotes no significant difference among the zones. The insect count data was log transformed before the analysis. Anova was carried out at $P \leq 0.05$. Where significance difference was observed, Tukey test was used to separate the means.

Similarly, there was no significant difference in the population of *S. exigua* across the three elevation zones ($F_{2,129}=0.33$; P=0.719) (Fig 4-6). However, there was significantly higher abundance of *H. armigera* in the high and low altitude zones as compared to the mid elevation zone ($F_{2,129}=0.23$; P=0.043) (Fig 4-6). . Seasonality did not influence significantly the abundance of the three major Lepidoptera species infesting African nightshades. There was no significant difference in the abundance of *S. littoralis* between the first and the second season ($F_{1,130}=0.51$; P=0.701) (Table 4-5).

Table 4-5: Mean number of insects/field from the three major Lepidopteran species infesting African nightshades in Kenya in a study conducted in the two seasons in the year 2014 across the three altitudinal zones (low, mid and high).

Species	**Season 1 (Dry season)**	**Season 2 (Rainy season)**
Spodoptera littoralis	0.21±0.11a	0.16±0.07a
Helicoverpa armigera	0.15±0.05a	0.14±0.07a
Spodoptera exigua	0.08±0.04a	0.06±0.05a

Same lower case letter in a given insect species denotes no significant difference between the seasons

Similarly, seasonality did not affect significantly the population of *H. armigera* ($F_{1,130}$=0.50; P=0.661) (Table 4-5). Moreover, the population of *S. exigua* did not differ significantly between the two seasons ($F_{1,130}$=2.37; P=0.420) (Table 4-5). .

4.4.7 Abundance, distribution and seasonality of major Thysanopteran species

Eight thrips belonging to six genera and three families were observed during the survey. The dominant family was the Thripidae family with six species. One species was observed in the Aeolothripidae and Phlaeothripidae family (Table 4-2). The three most abundant species were *Megalurothrips sjostedti*, *Haplothrips gowdeyi* and *Dendrothrips* sp (Fig 4-2). Elevation zone had a significant effect on the abundance of *M. sjostedti* and *Dendrothrips* sp. but not on *H. gowdeyi*. There was significantly higher abundance of *M. sjostedti* in the high and mid elevation zones as compared to the low elevation zone ($F_{2,129}$=3.85; P=0.024). (Fig 4-7).

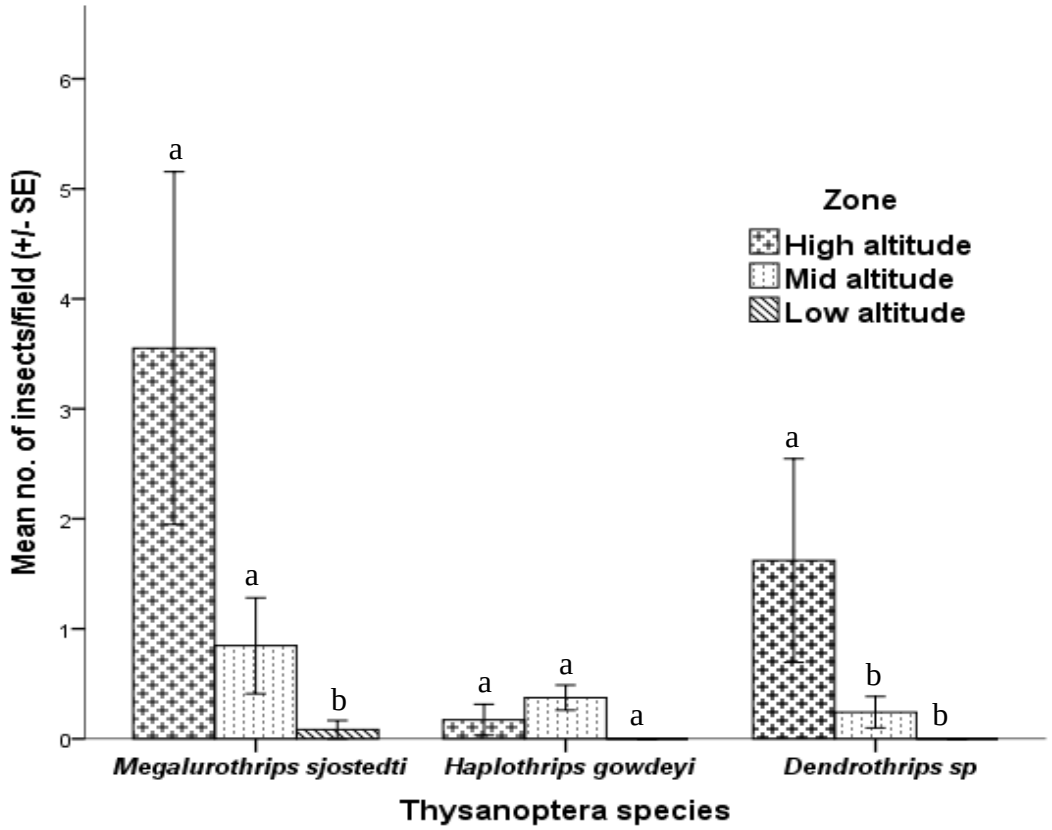

Fig 4-7: Mean number of *Megalurothrips sjostedti*, *Haplothrips gowdeyi* and *Dendrothrips* sp./field infesting African nightshades from a survey conducted across the high, mid and low altitude zones of Kenya in the year 2014. The zones were drawn from the six regions (Central, Coast, Eastern, Nyanza, Rift, and Western) growing African nightshades in Kenya. Same letter for a given species denotes no significant difference among the zones. The insect count data was log transformed before the analysis. Anova was carried out at $P \leq 0.05$. Where significance difference was observed, Tukey test was used to separate the means.

Moreover, the high elevation zone contained significantly higher abundance of *Dendrothrips* sp. compared to the mid and low elevation zones ($F_{2,129}=3.53$; P=0.032) (Fig 4-7). (Fig 4-7). . However, elevation zone did not have a significant effect on the population of *H. gowdeyi* ($F_{2,129}=1.65$; P=0.196) (Fig 4-7). Among the three most abundant thrips species, seasonality had a significant effect on the abundance of *M. sjostedti* only. There was significantly higher abundance of *M. sjostedti* in the first season compared to the second season ($F_{1,130}=26.39$; P=0.023) (Table 4-6).

Table 4-6: Mean number of insects/field from the three major Thysanopteran species infesting African nightshades in Kenya in a study conducted in the two seasons in the year 2014 across the three altitudinal zones (low, mid and high).

Species	**Season 1 (Dry season)**	**Season 2 (Rainy season)**
Megalurothrips sjostedti	2.61±0.98a	0.27±0.08b
Haplothrips gowdeyi	0.44±0.17a	1.31±1.16a
Dendrothrips sp	0.68±0.43a	0.39±0.20a

Same lower case letter in a given insect species denotes no significant difference between the seasons

4.4.8 Natural enemies of African nightshades pests

Different types of predators for pests of African nightshades were identified during the study. Fifteen species of Coccinelids ladybeetles were observed on nightshade plants that were infested with aphids. Moreover, and *Orius* sp. (Hemiptera: Anthocoridae) were observed feeding on aphids. *Aphidius colemani* was also collected from mummified aphids that were sampled from the nightshade crop (Table 4-7).

Table 4-7: Natural enemies of African nightshades pests observed during a study conducted in the two seasons in the year 2014 across the low, mid and high altitudinal zones in Kenya.

Family	**Species**	**Abundance (%)**	**Pest controlled**
Anthocoridae	*Orius* sp.	1.92	Spidermites, thrips, whiteflies
Braconidae	*Aphidius colemani* (Viereck)	13.46	Aphids
Coccinelidae	*Scymnus trepidulus* (Weise)	26.28	
	Cheilomenes sulphurea (Olivier)	14.10	
	Scymnus sp.	12.82	
	Chnotriba similes (Thunberg)	8.01	
	Hippodamia variagata (Goeze)	7.37	
	Platynaspis sp.	2.88	
	Exochomus ventralis (Gerstäcker)	2.56	
	Scymnus scapuliferus (Mulsant)	2.56	
	Stethorus sp.	2.24	
	Micraspis sp.	1.28	
	Scymnus pruinosus (Weise)	1.28	
	Platynaspis vittigera (Weise)	0.96	
	Scymnus morelleti (Muls)	0.96	
	Scymnus kibonotensis (Weise)	0.64	
	Scymnus pruinosus (Weise)	0.64	
	Scymnus casstroemi (Mulsant)	0.64	
	Scymnus luteus (Sicard)	0.64	
	Platynaspis sexguttata	0.32	
	Scymnus usambaricus (Weise)	0.32	

4.5 Discussion

In our study, we observed the highest pest diversity in the mid altitude zone. The moderately warm climate in the mid altitude zone could not only have been favourable for the survival and development of many pest species but also ideal for growth of various vegetation types that serve as food resources for the insects. Lawton et al. (1987) observed that an increase in food resources contributes to the diversity of insect communities in a given ecosystem. In our

study, we also observed that most of the farmers in the high altitude zone did production of African nightshade mainly as an income generation enterprise as opposed to most of the farmers in the mid altitude zone who produced the African nightshades at substance scale. The farmers in the high altitude zone applied heavy inputs in their production particularly insecticides and this might have lead to a decline in diversity of insect communities in that zone. This is in concurrence with Wold, (1987) who reported that disturbances of the environment by human being activities could lead to decrease in insect community in an ecosystem. Moreover, the farmers in the high altitude zone mainly practiced monoculture in the production of African nightshades as opposed to the farmers in the mid altitude zone who either intercrop African nightshades with other food crops or grew several other crops adjacent to the nightshade crop. Altieri and Letourneau (1982) made similar observation when they reported less insect species diversity in monocultures compared to polycultures.

In our study, there was higher insect diversity in the dry season than in the wet season. Moreover, except for the Lepidopterans, there was more abundance of major insect group in the dry season than in the rainy season. Contrary to our findings, greater insect abundance was observed in the wet season than in the dry season in studies done in other tropical regions of Brazil and Panama (Richards and Windsor, 2007; Silva et al., 2006). In these studies done in Brazil and Panama, they associated greater insect abundance to increased availability of food resources as a result of new growth of vegetation during the rainy season. However in our study, higher abundance and diversity of insects in the dry season might have been driven by the warmer temperatures that are within the thermal tolerance of many arthropod pest species. Moreover, food resources was available throughout the dry season as supplemental irrigation in most of the farms during the dry season supported the growth of African nightshades that provided food resources necessary for survival, growth and reproduction of the pests.

The higher abundance of Homopterans in season 1 could have resulted from the dry and hot weather conditions which were prevailing during the first season. Similar observation were made in the Kimaru et al., 2015 study where there were more pests observed in the dry season than in the wet season. Higher temperatures shorten the insect development time and therefore higher temperatures in the dry season could have lead to an increase in the population of aphids on the nightshade crop. Furthermore, 80% of the lifecycle of flea beetles is in the soil. It could also be that the wet soils during the wet season were unsuitable for survival and development of soil stages (eggs and larval) of flea beetles leading to the low population of flea beetles in the wet season.

Overall, the Homopterans and Coleopterans particularly the aphids and flea beetles respectively were the major pests of African nightshades during our study. These groups of pests could be having a higher preference for nightshades compared to the other insects present in the African nightshade growing regions. Similar observations were made in a related study conducted in Nairobi region of Kenya where the aphids were among the dominant species observed on African nightshades (Kimaru et al., 2015). However, contrary to our findings, the study only recorded one species of aphid i.e. *Aphis fabae*, where in our study, we observed six different aphid species. During our study, we studied nightshade pests in all the major production areas in Kenya as opposed to Kimaru et.al. (2015) study, which was done in only one location. This might explain why we observed more aphid species as some aphid species were only present in certain areas only.

In conclusion, this study has demonstrated the dry season in Kenya poses the highest pest challenge for production of African nightshades. During the dry season, there is high proliferation of the African nightshade pests particularly the aphids and flea beetles which bring significant damage to the nightshade crop. Moreover, our study has revealed that nightshade farmers in the mid altitude zone are faced with the highest diversity of pest compared to the farmers in the other altitudinal zones. This information is critical in future development of effective integrated pest management (IPM) measures for African nightshade pests particularly during the time the pest pressure is high such as dry the season and in the mid altitude zone. This will eventually contribute to minimizing the production losses associated with pests thereby increasing the production and incomes for the African nightshades farmers.

4.6 References

Abukutsa - Onyango M.O. 2007. The diversity of cultivated African leafy vegetables in three communities in western Kenya. *African Journal of Food, Agriculture, Nutrition and Development* **7(3)**:1-15.

Altieri MA, Letourneau DK. 1982. Vegetation management and biological control in agroecosystems. *Crop Protection* **1**: 405–430.

Hassan RM. 1998. Maize technology development and transfer. A GIS application for research planning in Kenya. CAB International, Wallingford, United Kingdom.

IPGRI. 2003. Rediscovering a forgotten treasure. IPGRI public awareness. Rome, Italy. http:// ipgr.pa.grinfo.net/index.php. Hemiol. 101.

Kamga RT, Kouamé C, Atangana AR, Chagomoka T, Ndango R. 2013. Nutritional evaluation of five African indigenous vegetables. *Journal of Horticultural Research*, **21(1)**: 99-106

Kindt R, Coe R. 2005. Tree diversity analysis. A manual and software for common statistical methods for ecological and biodiversity studies. World Agroforestry Centre (ICRAF), Nairobi (Kenya).

Lawton JH, Maccarvin M, Heads PA. 1987. Effects of altitude on the abundance and species richness of insect hervibores on bracken. *J. Anim. BioI.* **56**: 147-160.

Legendre L, Legendre P. 1998. Numerical ecology, 2 edn. Elsevier.

Mbugua G, Gitonga LN, Mureithi E, Karoga J, Manyeki L. 2006. Farmer-Participatory prioritization and development of agronomic practices for African Leafy Vegetables. Proceedings of 6th Horticultural Seminar on Sustainable Horticultural production in the Tropics held in Njoro, Kenya.

Mureithi DM, Fiaboe KKM, Ekesi S, Meyhöfer R. 2017. Important arthropod pests on leafy Amaranth (*Amaranthus viridis, A. tricolor* and *A.* 1 *blitum*) and broad-leafed African nightshade (*Solanum scabrum*) with a special focus on 2 host-plant ranges. *African Journal of Horticultural science* **11**:1-17

Oksanen M. Pietarinen J. 2004. Philosophy and biodiversity. Cambridge University Press, Edinburgh, UK.

Richards LA, Windsor DM. 2007. Seasonal variation of arthropod abundance in gaps and in the understorey of a lowland moist forest in Panama. *Journal of tropical ecology* **23**: 169-176

Romero A, Avila JM. 2000. Effect of elevation and type of habitat on the abundance and diversity of Scarabaeoid dung beetle (Scarabaeoidae) assemblage in a Mediterranean area from southern Peninsula. *Zoological studies* **39(4)**: 351-359

Sanchez-Rodriguez JF, Baz A. 1995. The effects of elevation on the butterfly communities of a Mediterranean mountain, Sierra de ja v alambre, central Spain. *Journal of the Lepidopterists' Society* **49(3)**: 192-207

Silva NA, Frizzas MR, Oliveira CM. 2006. Seasonality in insect abundance in the "Cerrado" of Goiás State, Brazil. *Revista Brasileira de Entomologia* **55(1)**: 79-87

Sithanantham S, Matoka CM, Maundu M, Jakari M, Agong SG. 2003. Integrated crop protection research for sustainable production of Indigenous vegetable crops in Eastern Africa. Proceedings of 4th Horticultural Seminar on Sustainable Horticultural production in the Tropics held in Njoro, Kenya.

Torres, FR, Madi-Ravazzi L. 2006. Seasonal variation in natural populations of *Drosophila* spp. (Diptera) in two woodlands in the State of São Paulo, Brazil. *Iheringia, Série Zoologia* **96**: 437-444.

Tóthmérész B. 1995. Comparison of different methods for diversity ordering. *Journal of Vegetation Science* **6**: 283-290.

Varela AN, Seif A. 2004. A Guide to IPM and Hygiene Standards in okra Production in Kenya. ICIPE Science Press, Nairobi, ICIPE.

Wolda, H. 1987. Altitude, habitat and tropical insect diversity. *BioI. J. Linn. Soc.* **30**: 313-323.

5 Seasonal abundance of *Aphis fabae, Epitrix silvicola* and Lepidopteran pests on African nightshades (*Solanum* spp.) in different agro-ecological zones in Kenya

5.1 Abstract

African nightshades (*Solanum scabrum* and *S. villosum*) are important vegetables in addressing minerals and vitamin deficiency in Africa. However, their production is constrained by damage from *Aphis gossypii* Glower (Homoptera: Aphididae), *Epitrix silvicola* Bryant (Coleoptera: Chrysomelidae) and Lepidopteran pests. Seasonal variations of *Aphis gossypii, Epitrix silvicola* and Lepidopteran pests on African nightshades at mid and high altitudes of Kenya in two dry (1st and 3rd growing seasons) and two wet (2nd and 4th growing seasons) seasons in the year 2015 and 2016 were studied. We show for the first time that the peak abundance of these pests occur in different seasons at the mid and high altitude zone. The highest abundance of *A. fabae* was observed in the 2nd growing season at the mid altitude zone and in the 3rd growing season in the high altitude zone. For the *Epitrix silvicola*, the highest abundance was observed in the 4th growing season at the mid altitude zone and in the 3rd growing season at the high altitude zone. For the Lepidopteran pests, the peak abundance was recorded in the 1st growing season at the mid altitude zone and 4th growing season at the high altitude zone. Infestation on nightshade by these pests started at the seedling stage and their population increased up to the flowering stage in most of the seasons. The parasitoid *Aphidius colemani* Viereck (Hymenoptera: Braconidae) recorded the highest abundance at the 2nd growing season at the mid altitude zone and 3rd growing season at the high altitude zone. These findings are useful for development and implementation of effective integrated pest management (IPM) measures for these pests.

Key words: *Solanum scabrum; Solanum villosum; Phenological stages; insect population*

5.2 Introduction

Although African indigenous vegetables (AIVs) were part of the old-age farming tradition in Africa, they were largely abandoned after the introduction of exotic vegetables. However, in the recent past, these vegetables have gained prominence as they are a rich source of important minerals such as potassium, calcium, iron and vitamins (Orech et al., 2007; Uusiku et al., 2010). African nightshades (*Solanum* spp.) are among the most widely grown AIVs in Kenya (AVRDC, 2003; HCDA, 2013). The two most popular species of African nightshades are *Solanum scabrum* and *S. villosum* although *S. villosum* is more popular (Kamga et al., 2013).

Among the factors constraining production of African nightshades is attack by insect pests resulting in yield losses of 36-100% (Sithanantham et al., 2003; Mureithi et al., 2017). African nightshades have many insect pests attacking them including aphids, flea beetles, and caterpillars (Kimaru et al., 2015; Mureithi et al., 2017; Mureithi et al., unpublished). Aphids cause damage by sucking the phloem sap on nightshade leaves leading to curling and distorted growth. The aphids also produce honeydew that subsequently is colonized by sooty mould that contaminates the produce leading to market rejection (AVRDC, 2003; Varela and Seif, 2004). Among the aphid species that infest African nightshades are *A. craccivora* Koch, *A. fabae* Scopoli, and *Myzus persiae* Sulzer (Ashilenje *et al.,* 2011; Suganthy and Sakthivel, 2012; Singh *et al.,* 2014: Kimaru et al., 2015; 4.4.3). Flea beetles particularly *Epitrix silvicola* Bryant puncture shoot-holes on nightshade leaves making them undesirable by the consumers. Lepidopteran pests are also voracious feeders on nightshade leaves reducing the yields. In the tropics, aphids undergo anholocyclic life cycle, reproducing parthenogenetically throughout the entire year on different hosts (Eastop 1957, Blackman 1974). However, their populations fluctuate as a result of changes in temperature, rainfall and relative humidity (Michaud and Browning, 1999).

Despite the knowledge on damage caused by aphids, flea beetles and Lepidopterans on African nightshades, there are few reports on their seasonal abundance on African nightshades. Little is also know with regard to the host range of these pests in African nightshade production areas in Kenya. In particular, *A. fabae*, *E. silvicola* and Lepidopteran pests were abundant and caused significant damage to African nightshade in a survey conducted in Kenya in the year 2014 (4.4.3; 4.4.4: 4.4.5; 4.4.6) The present study was designed to provide data on seasonal abundances of *A. fabae*, *A. colemani*, *Epitrix silvicola* and Lepidopterans on African nightshades in Kenya in different growing seasons and at different phenological stages of African nightshades. This information is needed for

development and implementation of effective integrated pest management (IPM) measures for these pests. The role of neighbourhood crops and weed species as potential refuge for aphids, flea beetles and the Lepidopteran pests was also studied.

5.3 Methodology

5.3.1 Description of field sites

The study was conducted in two agro-ecological zones; the mid and the high altitude zones in Kenya. The mid altitude was located between 1000 – 1800 m above sea level (asl) while the high altitude zone was located above 1800 m asl. The study sites for the mid altitude zone were Yatta, Machakos County for the 1st growing season and KALRO – Kandara in Muranga County for the 2nd, 3rd, and 4th growing season. The high altitude site was located at KALRO-Tigoni for the four growing seasons. The study was done in the following growing seasons; 1st growing season- February – May 2015, 2nd growing season – August- November 2015, 3rd growing season - February – May 2016, 4th growing season August- November 2016. The 1st and the 3rd growing seasons were generally dry and hot, with rainfall in the month April. The 2nd and the 4th growing seasons were generally rainy but having a dry spell in the month of August.

5.3.2 Crop establishment

African nightshades *Solanum scabrum* (variety Giant Nightshade from Kenya Seed Company) and *Solanum villosum* (from Kenya Seed Company) were used in this trial. 10 plots measuring 10 x 10 m were prepared in each altitudinal zone except for the 1st growing season where only three plots were established. The path of 2m was left between the nightshade plots while a distance of at least 5 m was observed between the other neighbouring crops. African nightshade seeds were sown and raised in a nursery bed for one month before being transplanted in the field at a spacing of 45 x 30 cm. Well decomposed cattle manure was applied to the field at a rate of 40 tonnes /hectare at the planting hole during transplanting. The seedlings were irrigated after transplanting to enhance their establishment. Thereafter, the crop was managed in accordance to the normal farmer's practices. However, no crop protection measures were applied. During the dry spell, supplemental irrigation was applied. The fields were kept weed free throughout the experimental period.

5.3.3 Sampling for pests and natural enemies

In each of the growing season the sampling was done at the following phenological stages of crop growth; seedling (3 weeks after transplanting), pre-flowering (5 weeks after transplanting), flowering (7 weeks after transplanting), fruit formation (9 weeks after transplanting), fruit ripening(11 weeks after transplanting), senescence (13 weeks after transplanting). The plots were divided into 4 equal quadrants for the purpose of sampling. The population of pests of interest were monitored by sampling 10 plants per quadrant following a diagonal transect from one corner of the quadrant to the opposite one.

Sampling of *A. fabae* was done by randomly selecting 3 leaves each at the top, middle, and bottom of the selected plants and counting all the alate and apterous aphids present. Aphid parasitoids, mainly *A. colemani*, were sampled by counting the number of aphid mummies that were present on the leaves where aphids were sampled. The mummies were collected in lunch boxes lined with paper towel for identification of the parasitoid upon emergence. Sampling of *Epitrix silvicola* was done using the beating method where the selected plants were rapidly tapped by hand for 15 times over a tray smeared with 70% alcohol and counting the number of flea beetles that fall on the tray. The flea beetles were then collected in plastic vials containing 70% ethanol for identification in the laboratory. Lepidopteran pests on the crops were sampled by visually examining the top, middle and lower leaves of the selected plants, counting the caterpillars that were observed on upper and on the underside of the leaves. The caterpillars were then collected and put in lunchboxes lined with paper towel on the inner side and putting a leaf of African nightshade for the caterpillar to continue feeding before covering the containers. The caterpillars were reared to adulthood at *icipe* laboratories before being identified. The infection pathways of *A. fabae, Epitrix silvicola* and Lepidopteran pests were also monitored by sampling from crops/weeds neighbouring the experimental plots. Crop/weeds within the radius of 30 m from the experimental field were examined for the presence of *A. fabae, Epitrix silvicola* and Lepidopterans.

5.3.4 Data analysis

The count data was log-transformed before analysis by a three way anova with agro-ecological zone, growing season, and crop phenological stage of African nightshade as the factors was performed. Where significance difference was observed, Tukey´s HSD test was done to separate the means. The number of *A. fabae, Epitrix silvicola*, and Lepidopterans observed per plant at each phenological stage of African nightshade was analysed using

repeated measures analysis of variance (RM-ANOVA). All tests were done at 0.05 level of significance.

5.4 Results

5.4.1 Effect of seasonality and crop phenology on abundance of A. fabae

During the study, *A. fabae* was the major aphid species on the African nightshade crop representing 94 % of all the aphids observed in the study. Other minor aphid species observed were M. persicae (5 %), Aphis craccivora (1%). A three way interaction between agro-ecological zone (AEZ), growing season and crop phenological stage had a significant effect on the abundance of *A. fabae* on African nightshades (F = 19.94; df = 15,348; P = <0.001). Moreover, the single factors had significant effect (Table 5-1).

Table 5-1: ANOVA table for main effects (Agro-ecological zone, seasonality and crop phenology) and interactions for abundance of *Aphis fabae*, *Aphidius colemani*, *Epitrix silvicola* and Lepidopteran pest attacking African nightshades during a study conducted in 2015 and 2016 in mid and high altitude zones in Kenya (total df=396).

Source	df	Species							
		Aphis fabae		*Aphidius colemani*		*Epitrix silvicola*		Lepidopterans	
		F	P	F	P	F	P	F	P
Agro-ecological zone	1	101.50	< 0.001	215.090	< 0.001	4.71	< 0.001	65.15	< 0.001
Season	3	19.03	< 0.001	1.900	0.130	3.24	< 0.001	48.24	< 0.001
Crop phenology	5	14.14	< 0.001	12.620	< 0.001	1.68	0.138	8.38	< 0.001
Agro-ecologal zone x Season	3	132.31	< 0.001	78.48	< 0.001	7.38	< 0.001	34.13	< 0.001
Agro-ecological zone x Crop phenology	5	11.39	< 0.001	11.76	< 0.001	2.55	0.028	7.15	< 0.001
Season x Crop phenology	15	54.48	< 0.001	46.30	< 0.001	3.89	< 0.001	8.44	< 0.001
Agro-ecological zone x Season x Crop phenology	15	19.94	< 0.001	42.530	< 0.001	0.46	< 0.001	11.11	< 0.001

In the mid altitude zone, there was significantly higher population of *A. fabae* in the 2nd growing season compared to the other growing seasons (F = 7.03; df = 3,194; P = <0.001). Moreover in the high altitude zone, there was significant difference on the population of *A. fabae* with the highest abundance observed in the 3rd growing season (F = 58.74; df = 3,194; P = <0.001) (Fig 5-1).

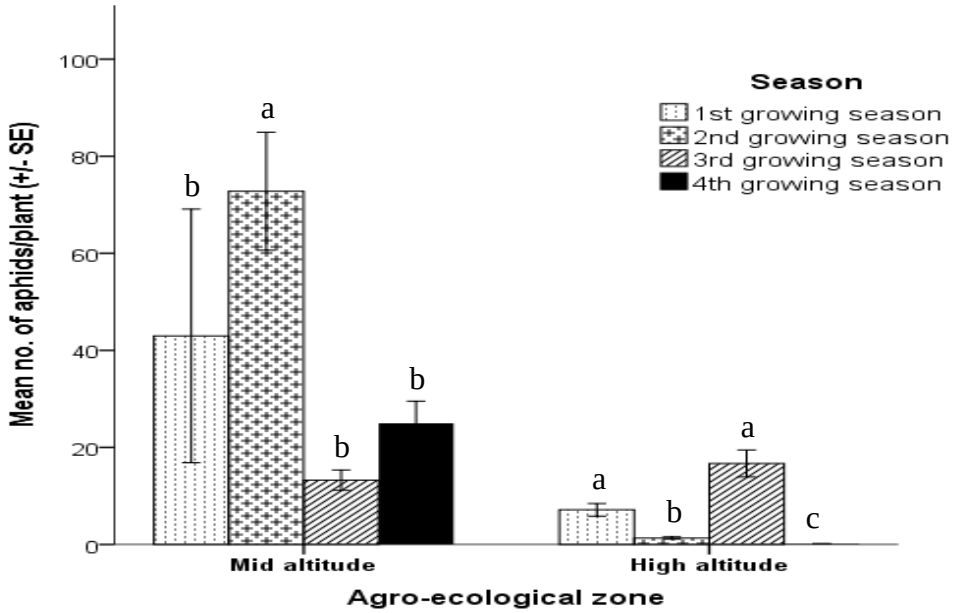

Fig 5-1: Mean number (+/- SE) of *Aphis fabae/* African nightshade plant/field in different growing seasons of African nightshades in 2015 (1st & 2nd) and 2016 (3rd & 4th) in mid and high altitude zones in Kenya. The 1st and 3rd season were dry while the 2nd and 4th season were wet. In each of the agro-ecological zone, ten African nightshades fields measuring 10 x 10 m were sampled except in the 1st season where three fields were sampled in each agro-ecological zone. The insect count data was log transformed before anova was carried out at $P \leq 0.05$. Where significance difference was observed, Tukey HSD test was used to separate the means. Same letter for a given agro-ecological zone signify no significant difference

When the population density of *A. fabae* was considered at different phenological stages of plant growth in the mid altitude zone, the population increased from seedling stage to the pre flowering stage before declining sharply at the other stages of crop growth in 1st season. During the 2nd growing season, a similar trend was observed, except that the population reached its peak at the flowering stage before declining at the later stages of plant development. However, in the 3rd and 4th growing season, few *A. fabae* were observed on the crop during the vegetative phases (seedling and pre-flowering stages), with the population rising in the generative phases (flowering, fruiting and senescence stages) (Fig 5-2).

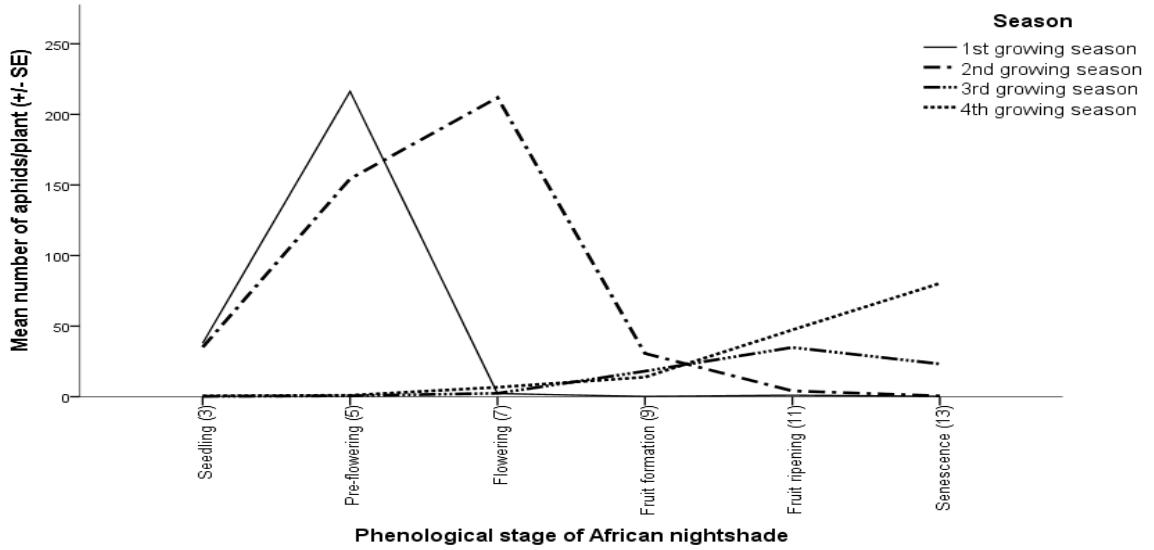

Fig 5-2: Mean number (+/- SE) of *Aphis fabae*/African nightshade plant/field at different phenological stages of crop growth in different growing seasons in 2015 (1st & 2nd) and 2016 (3rd & 4th) in mid altitude zones in Kenya. The 1st and 3rd season were dry while the 2nd and 4th season were wet. Numbers in brackets represent weeks after transplanting. At each of the phenological stage, , ten African nightshades fields measuring 10 x 10 m were sampled except in the 1st season where three fields were sampled per phenological stage

At the high altitude zone, higher *A. fabae* densities were observed in the generative phases of plant growth compared to the vegetative phases in the 1st, 2nd and 3rd growing seasons. However, very few aphids were observed in the 4th growing season in all the development phases of crop growth (Fig 5-3).

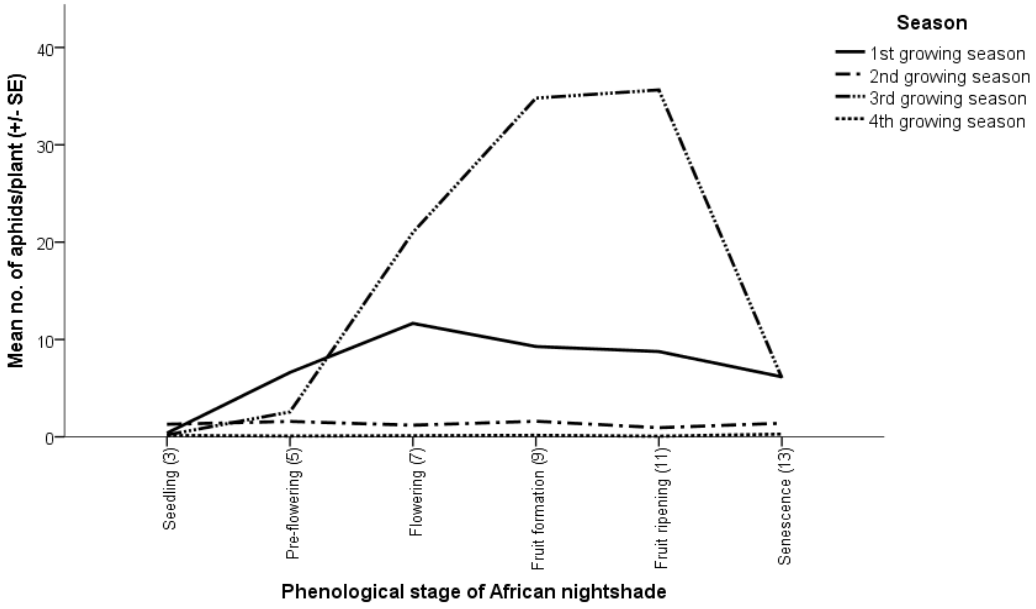

Fig 5-3: Mean number (+/- SE) of *Aphis fabae*/African nightshade plant/field at different phenological stages of crop growth in different growing seasons in 2015 (1st & 2nd) and 2016 (3rd & 4th) in high altitude zones in Kenya. The 1st and 3rd season were dry while the 2nd and 4th season were wet. Numbers in brackets represent weeks after transplanting. At each of the phenological stage, , ten African nightshades fields measuring 10 x 10 m were sampled except in the 1st season where three fields were sampled per phenological stage

5.4.2 Effect of seasonality and crop phenology on abundance of A. colemani

In general, the parasitism rate of *A. fabae* by parasitoid *A. colemani* was very low. There was a significant interaction in the abundance of aphid parasitoid *A. colemani* among the three factors, agro-ecological zone (AEZ), growing season and crop phenology (F = 42.53; df = 15,348; P = <0.001). Moreover, the agro-ecological zone and crop phenology individually also affected the population of *A. colemani* (Table 5-1). When each agro-ecological zone was considered separately, there was significantly higher population of *A. colemani* in 2nd growing season compared to the 3rd growing season at the mid altitude zone. However, there was no significant difference in the abundance of *A. colemani* between the 1st, 2nd, and 4th growing season in the mid agro-ecological zone (F = 4.52; f = 3,194; P = 0.004). At the high altitude zone, significantly higher population of *A. colemani* was recorded at the 3rd growing season compared to the other growing seasons. However, there was no significant difference in the abundance of *A. colemani* between the second and the 4th growing season (F = 32.10; df = 3,194; P = <0.001) (Fig 5-4).

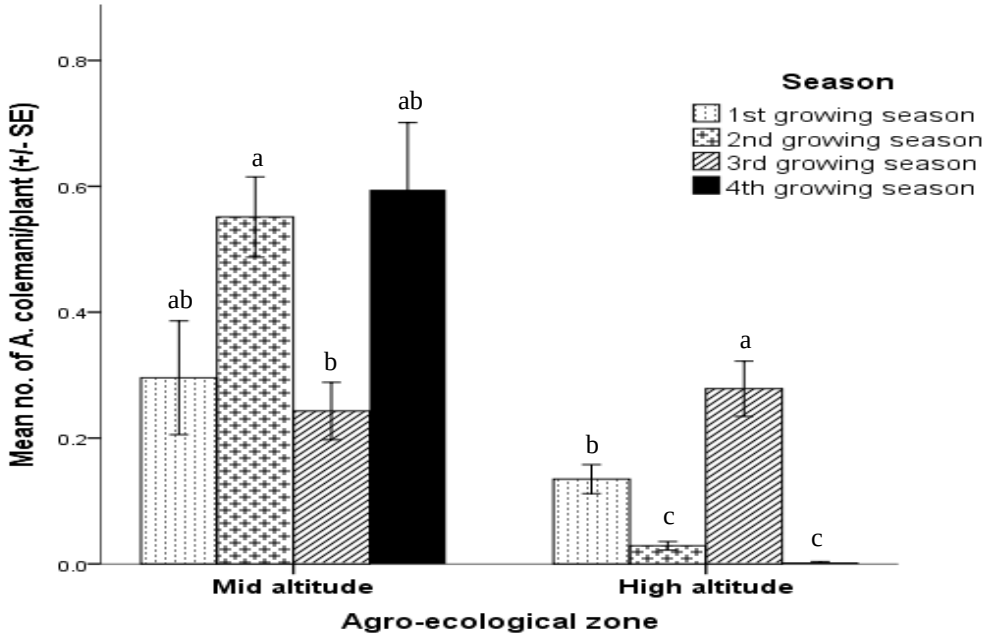

Fig 5-4: Mean number (+/- SE) of *Aphidius colemani*/African nightshade plant/field in different growing seasons of African nightshades in 2015 (1st & 2nd) and 2016 (3rd & 4th) in mid and high altitude zones in Kenya. In each of the agro-ecological zone, ten African nightshades fields measuring 10 x 10 m were sampled except in the 1st season where three fields were sampled in each agro-ecological zone. The 1st and 3rd season were dry while the 2nd and 4th season were wet. The insect count data was log transformed before anova was carried out at P≤0.05. Analysis was done separately for each agro-ecological zone. Where significance difference was observed, Tukey HSD test was used to separate the means. Same letter for a given agro-ecological zone signify no significant difference.

The abundance of the parasitoid *A. colemani* was also different at different phenological stages of crop development. When the mid altitude zone was considered, the abundance of *A.*

colemani increased during the vegetative stages of crop development before declining at the generative phases of crop development in the 1st and 2nd growing season. The scenario was different in the 3rd and 4th growing season where higher populations of *A. colemani* were observed in the generative phases of crop development (Fig 5-5).

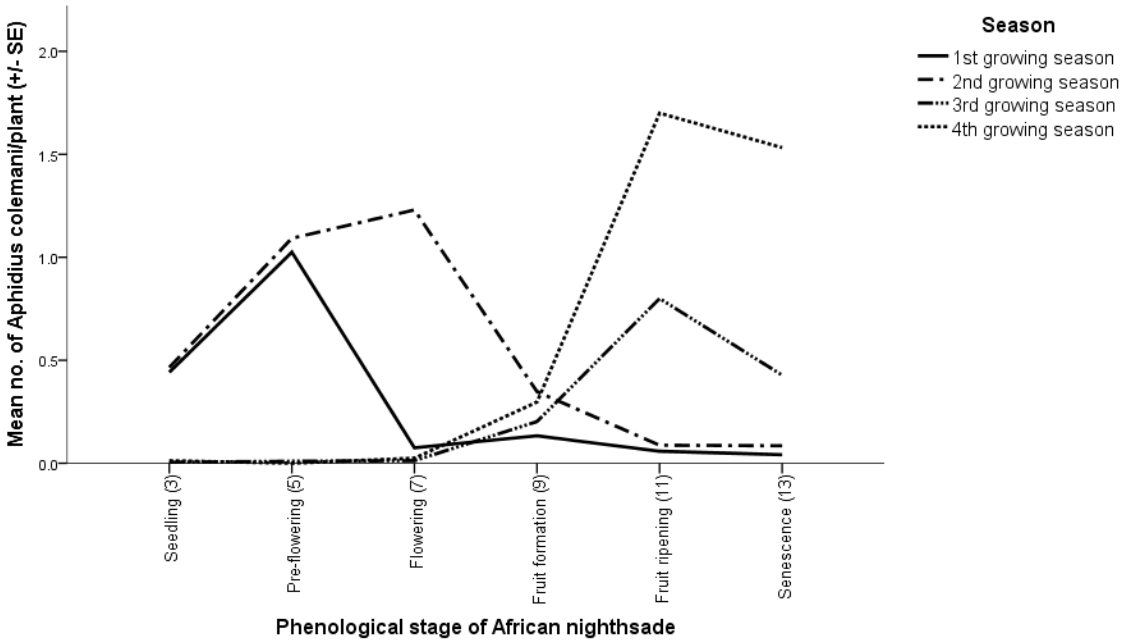

Fig 5-5: Mean number (+/- SE) of *Aphidius colemani*/African nightshade plant/field at different phenological stages of crop growth in different growing seasons in 2015 (1st & 2nd) and 2016 (3rd & 4th) in mid altitude zones in Kenya. The 1st and 3rd season were dry while the 2nd and 4th season were wet. Numbers in brackets represent weeks after transplanting. At each of the phenological stage, ten African nightshades fields measuring 10 x 10 m were sampled except in the 1st season where three fields were sampled per phenological stage

In the high altitude zone, low population densities were observed at the vegetative phases for all the four growing seasons. However, whereas the population increased at the generative phases in the 1st and the 3rd growing seasons, it decreased in the 2nd and the 4th growing seasons (Fig 5-6).

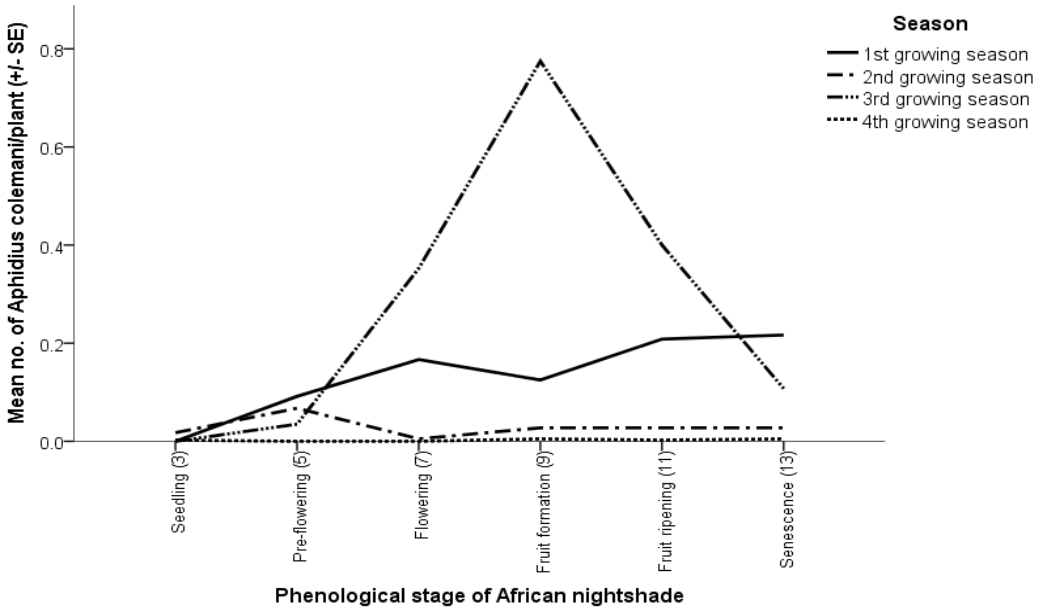

Fig 5-6: Mean number (+/- SE) of *Aphidius colemani*/African nightshade plant/field at different phenological stages of crop growth in different growing seasons in 2015 (1st & 2nd) and 2016 (3rd & 4th) in high altitude zones in Kenya. The 1st and 3rd season were dry while the 2nd and 4th season were wet. Numbers in brackets represent weeks after transplanting. At each of the phenological stage, ten African nightshades fields measuring 10 x 10 m were sampled except in the 1st season where three fields were sampled per phenological stage

5.4.3 Effect of seasonality and crop phenology in abundance of Epitrix silvicola

Majority (~90%) of the flea beetles observed in the African nightshade crop were *Epitrix silvicola*. A three way interaction between the agro-ecological zone, growing season, and crop phenological stage on the abundance of flea beetles was significant (F = 3.21; df = 15,348; P = <0.001). Agro-ecological zone and seasonality also significantly affected the *E. silvicola* abundance (Table 5-1). In the mid altitude zone, significantly higher population of *Epitrix silvicola* was observed in the 4th growing season compared to the 1st growing season. However, there was no significant difference in the abundance of *Epitrix silvicola* in the 2nd, 3rd, and 4th growing season zone (F = 4.57; df = 3,194; P = 0.004) (Fig 5-7).

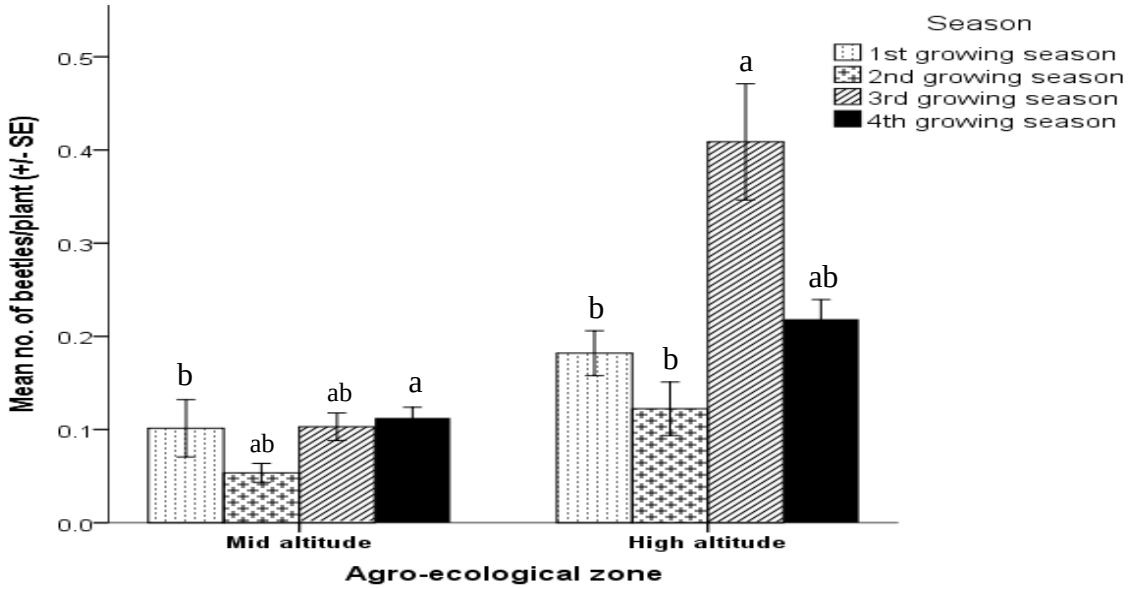

Fig 5-7: Mean number (+/- SE) of *Epitrix silvicola*/African nightshade plant/field in different growing seasons of African nightshades in 2015 (1st & 2nd) and 2016 (3rd & 4th) in mid and high altitude zones in Kenya. In each of the agro-ecological zone, ten African nightshades fields measuring 10 x 10 m were sampled except in the 1st season where three fields were sampled in each agro-ecological zone. The 1st and 3rd season were dry while the 2nd and 4th season were wet. The insect count data was log transformed before anova was carried out at P≤0.05. Analysis was done separately for each agro-ecological zone. Where significance difference was observed, Tukey HSD test was used to separate the means. Same letter for a given agro-ecological zone signify no significant difference

In the high altitude zone, there was significantly higher population of flea beetles in the 3rd growing season compared to the 1st and the 2nd growing seasons. However, there was no significant difference in population of *Epitrix silvicola* between the 1st and 2nd growing season or from the 3rd and 4th growing season ($F = 10.42$; $df = 3,194$; $P = <0.001$) (Fig 5-7). There was a steady increase in the population of *Epitrix silvicola* from the seedling stage up to the flowering stage for all the seasons in the mid altitude zone. Thereafter the population either increased or decreased in different seasons (Fig 5-8).

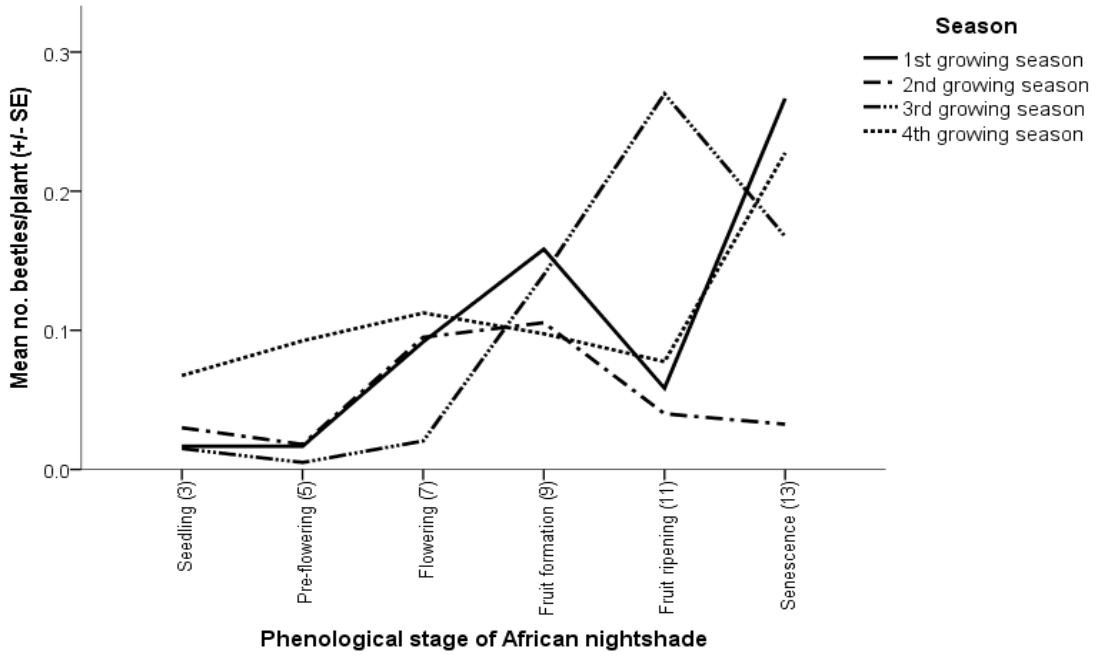

Fig 5-8: Mean number (+/- SE) of *Epitrix silvicola*/African nightshade plant/field at different phenological stages of crop growth in different growing seasons in 2015 (1st & 2nd) and 2016 (3rd & 4th) in mid altitude zones in Kenya. The 1st and 3rd season were dry while the 2nd and 4th season were wet. Numbers in brackets represent weeks after transplanting. At each of the phenological stage, ten African nightshades fields measuring 10 x 10 m were sampled except in the 1st season where three fields were sampled per phenological stage

There was no clear trend in the population of flea beetles at different phenological stages of plant growth for all of the growing seasons in the high altitude zone. The population kept on rising and fluctuating (Fig 5-9).

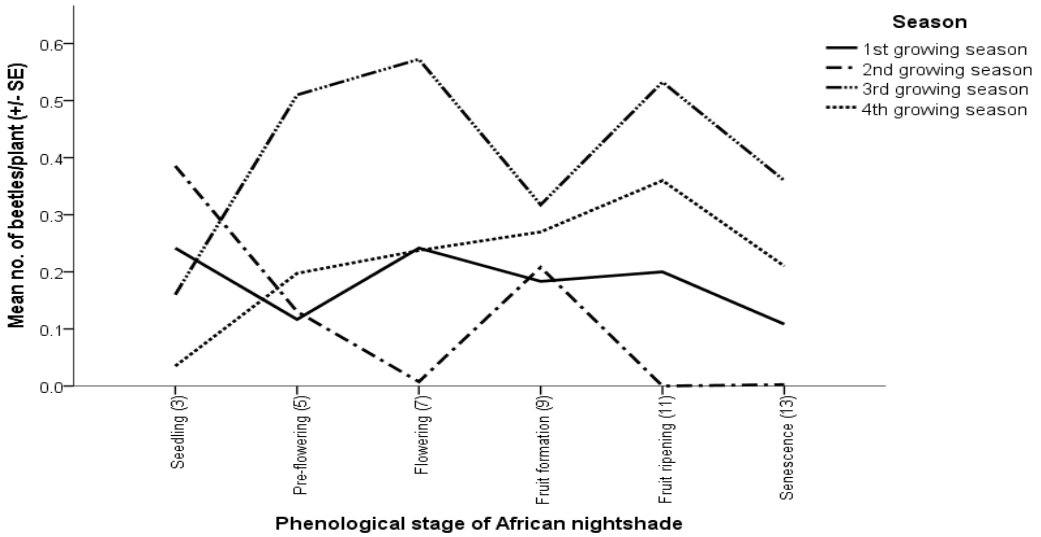

Fig 5-9: Mean number (+/- SE) of *Epitrix silvicola*/African nightshade plant/field at different phenological stages of crop growth in different growing seasons in 2015 (1st & 2nd) and 2016 (3rd & 4th) in high altitude zones in Kenya. The 1st and 3rd season were dry while the 2nd and 4th season were wet. Numbers in brackets represent weeks after transplanting. At each of the phenological stage, ten African nightshades fields measuring 10 x 10 m were sampled except in the 1st season where three fields were sampled per phenological stage

5.4.4 Effect of seasonality and crop phenology in abundance of Lepidopterans

The major Lepidopterans infesting African nightshades observed during the trial were *Spodoptera exigua*, *S. littoralis*, *Tuta absoluta* and *Plusia* sp. The interaction between agro-ecological zone, growing season and crop phenology had a significant effect on the population of the Lepidopterans (F = 11.11; df = 15,348; P = <0.001). Each of the three factors significantly affected the abundance of the Lepidopterans (Table 5-1). Significantly higher population of Lepidopterans were observed in the 1st growing season compared to the other growing seasons in the mid altitude zones. However, there was no significant difference in the abundance of the Lepidopterans in the other growing seasons at the mid altitude zone (F = 8.70; df = 3,194; P = <0.001) (Fig 5-10).

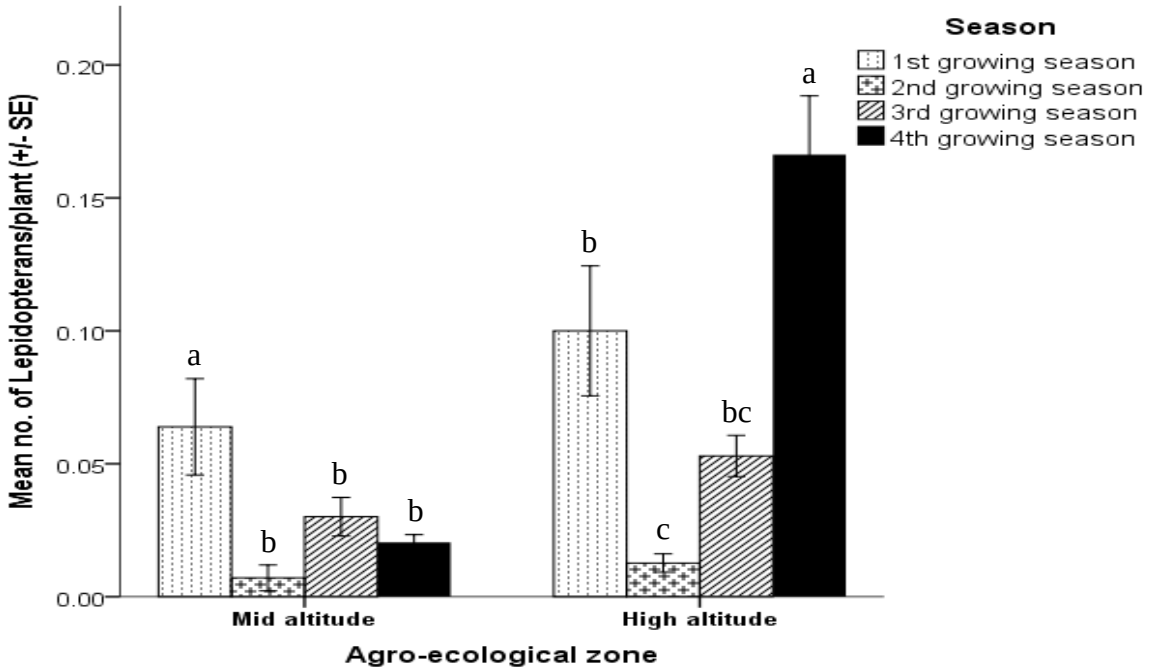

Fig 5-10: Mean number (+/- SE) of Lepidopterans/African nightshade plant/field in different growing seasons of African nightshades in 2015 (1st & 2nd) and 2016 (3rd & 4th) in mid and high altitude zones in Kenya. In each of the agro-ecological zone, ten African nightshades fields measuring 10 x 10 m were sampled except in the 1st season where three fields were sampled in each agro-ecological zone. The 1st and 3rd season were dry while the 2nd and 4th season were wet. The insect count data was log transformed before anova was carried out at P≤0.05. Analysis was done separately for each agro-ecological zone. Where significance difference was observed, Tukey HSD test was used to separate the means. Same letter for a given agro-ecological zone signify no significant difference

Conversely, there was significantly higher population of Lepidopterans in the 4th growing season compared to the other growing seasons at the high altitude zone. Although there was significant difference in the abundance of Lepidopterans between the 1st and the 2nd growing season, no such difference was observed between the 2nd and the 3rd growing season in the high altitude zone (F = 24.13; df = 3,194; P = <0.001) (Fig 5-10).

In the mid altitude zone, the abundance of Lepidopterans increased in the vegetative phase in 1st, 3rd, and 4th growing season. Although the population continued to increase for the 3rd growing season up to the fruiting stage, the population decreased and stabilized for the 4th growing season while it decreased towards fruit maturation stage and later decreased as the crop approached senescence for the 1st growing season. In the 2nd growing season, the Lepidoptera population increased steadily from the seedling stage up to the flowering stage, after which the population decreased dramatically at the fruit formation stage after which no Lepidopteran was observed on the crop (Fig 5-11).

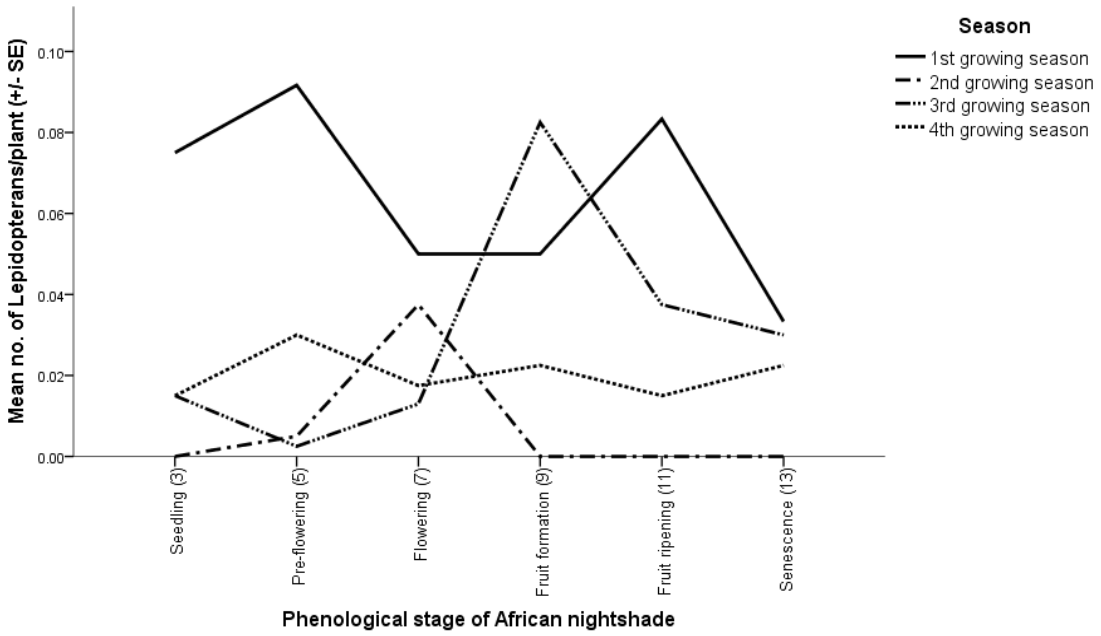

Fig 5-11: Mean number (+/- SE) of *Lepidopteran*/African nightshade plant/field at different phenological stages of crop growth in different growing seasons in 2015 (1st & 2nd) and 2016 (3rd & 4th) in mid altitude zones in Kenya. The 1st and 3rd season were dry while the 2nd and 4th season were wet. Numbers in brackets represent weeks after transplanting. At each of the phenological stage, ten African nightshades fields measuring 10 x 10 m were sampled except in the 1st season where three fields were sampled per phenological stage

In the high altitude zone, higher abundance of Lepidopterans were observed in the generative phases of nightshade plant in the 1st, 3rd, and 4th growing cycle compared to the vegetative phases. However, in the 2nd growing season, the population of Lepidopterans decreased as the crop developed from the vegetative to the generative phases of plant growth and the pest was absent as from the flowering phase until senescence (Fig 5-12).

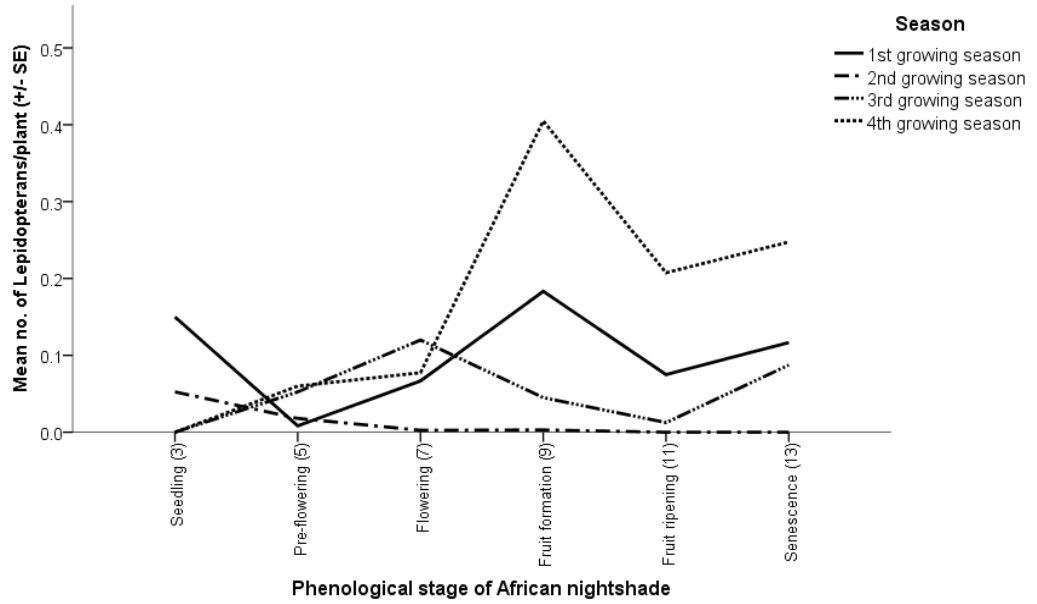

Fig 5-12: Mean number (+/- SE) of *Lepidopteran*/African nightshade plant/field at different phenological stages of crop growth in different growing seasons in 2015 (1st & 2nd) and 2016 (3rd & 4th) in high altitude zones in Kenya. The 1st and 3rd season were dry while the 2nd and 4th season were wet. Numbers in brackets represent weeks after transplanting. At each of the phenological stage, ten African nightshades fields measuring 10 x 10 m were sampled except in the 1st season where three fields were sampled per phenological stage

5.4.5 Host range for the major pests of African nightshades in Kenya

Pests infesting African nightshades were also observed to attack other cultivated and wild plants growing in or around the nightshade fields. At the mid altitude zone, the alternative hosts for *A. fabae* observed were primarily wild plants. However the alternative hosts for the *E. silvicola* and Lepidopterans were cultivated crops (Table 5-1). At the high altitude zone, wild African nightshade plants growing on the hedges of the nightshade fields were also colonized by *A. fabae*. The wild African nightshade plants were also found to host *Epitrix silvicola*. Potato crop which was cultivated adjacent to the African nightshade experimental plot at the high altitude zone was also infested by *Tuta absoluta* (Table 5-2).

Table 5-2: Alternative host plants of major pests infesting African nightshades in Kenya in a study conducted in different growing seasons of African nightshades in 2015 (1st & 2nd) and 2016 (3rd & 4th) at the mid altitude (KALRO-Kandara and Yatta) and high altitude (KALRO-Tigoni) zones of Kenya.

Agro-ecological zone	**African nightshade pest**	**Alternative host plants**	**Plant Family**	**Abundance**
Mid altitude zone (1000 - 1800 m)	*Aphis fabae*	*Bidens pilosa* *Euphobia* sp. *Leonotis leonurus* *Eupatorium* sp.	*Asteraceae* *Euphobiaceae* *Lamiaceae* *Asteracea*	Moderate Low Low Low
	Epitrix silvicola	*Amaranthus dubious* *Amaranthus cruentes*	*Amaranthaceae*	Low
	Spodoptera littoralis	*Amaranthus dubious* *Amaranthus cruentes*		
	Spodoptera exigua	*Amaranthus* sp. (wild amaranth) *Amaranthus cruentes*		
	Tuta absoluta	*Solanum lycopersicon*	*Solanaceae*	High

Table 5-2: Alternative host plants of major pests infesting African nightshades in Kenya in a study conducted in different growing seasons of African nightshades in 2015 (1st & 2nd) and 2016 (3rd & 4th) at the mid altitude (KALRO-Kandara and Yatta) and high altitude (KALRO-Tigoni) zones of Kenya (Continued).

Agro-ecological zone	**African nightshade pest**	**Alternative host plants**	**Plant Family**	**Abundance**
High altitude zone (above 1800m)	*Aphis fabae*	*Solanum* sp. (wild nightshades) *Eupatorium* sp.	*Solanaceae* *Asteraceae*	Moderate Low
	Epitrix silvicola	*Solanum* sp. (wild nightshades) *Solanum tuberosum*	*Solanaceae*	Moderate Low
	Spodoptera littoralis	*Amaranthus dubious* *Amaranthus cruentes*	*Amaranthaceae*	Low Low
	Spodoptera exigua	*Amaranthus dubious* *Amaranthus cruentes*		Low Low
	Tuta absoluta	*Solanum tuberosum*		High

Keys for abundance scoring: *A. fabae* (low=less than 10 insects/plant; moderate=10-20 insects/plant; high= more than 20 insects/plant). *E. silvicola* (low=less than 5 insects/plant; moderate=5-10 insects/plant; high= more than 10 insects/plant). Lepidoptrans (low=less than 5 insect/plant; moderate=5-10 insects/plant; high= more than 10 insects/plant).

5.5 Discussion

Presence of aphids on African nightshades is particularly injurious to the crop and populations of between 10 - 80 aphids/plant can result to high yield losses of up to 26% (4.4.3). From the present study, we observed populations of up to 80 aphids/plant particularly in the mid altitude zone. This could be a concern for the African nightshade farmers as such high population could result to huge economic losses. Agro-ecological zone seemed to have played role on the abundance of *A. fabae* whereby the mid altitude zone recorded higher populations of *A. fabae* compared to the high altitude zone. We observed higher temperatures at the mid altitude zone compared to the high altitude zone. Temperatures in the mid altitude zone can go as high as 32˚C (Hassan, 1998). Therefore, the high temperatures at the mid altitude zone could have shortened the development time of *A. fabae* at the mid altitude zone leading to more generations of this pests in that zone.

We also observed higher abundance of *A. fabae* in the year 2015 compared to year 2016 particularly in the mid altitude zone. It is probable that the other weather conditions such as relative humidity and rainfall, affected the population of the aphids negatively in the year 2016 as more rains were experienced in the year 2016 compared to 2015 (data not shown). There was strong seasonal effect particularly in the second season particularly in the second growing season. This could be attributed to the prolonged dry and hot spell in the mid altitude zone and the delay in the onset of the rains. In the high altitude zone, the low population of *A. fabae* in the 2nd and 4th growing season could have resulted from more rainfall and low temperatures that were experienced in those seasons. The rains have the potential of displacing aphids from the plants and it is probable that the rains washed off the aphids from

the African nightshade plants in our study during the rainy season. Moreover, the lower temperatures during the rainy season could have slowed down the development of *A. fabae* leading to low population densities. Jones (1979) reported that the abundance of cereal aphids were positively correlated to temperature and negatively correlated to rainfall. Nakata (1995) also noted higher abundance of *A. fabae* with increase in temperatures. In our study, we observed highest population density of *A. fabae* in the 2nd growing season at the mid altitude zone.

We also observed that while there was low colonization of African nightshades by *A. fabae* at the seedling stage, the population densities increased with time up to the fruit formation stage for most of the cases. This is particularly significant to the farmers since the crop is harvested between the pre-flowering and early flowering stage. Therefore, the pest will have inflicted the damage to the crop by the time is ready for harvesting. It would therefore be important to institute aphid management practices at an early stage of crop development to prevent the population build-up that may lead to yield losses.

Successful parasitism by a parasitoid depends on habitat location, host location, host acceptability and host suitability host regulation (Vinson, 1976). In the present study, very low parasitism rate of *A. fabae* by *A. colemani* was observed in all the growing seasons in the two agro-ecological zones. This was the case even when there was a high aphid density on the crop suggesting that there could be low parasitoid density in the fields. Takasu and Lewis, (1984) reported that when parasitoids are adequately fed, their search activities for a host to parasitize is increased. It could also be that the African nightshades are not a preferred host plant for *A. colemani*. Abiotic factors also affect the performance of parasitoids. *A. colemani* larvae cease to develop when the temperatures of above 30˚C (Goh et al., 2001; Bueno et al., 2006). Moreover, wind speed of 2 m/s has been reported to lower the oviposition rate of the parasitoid *Aphidius rosae* (Fink and Volkl, 1995). High temperatures and windy conditions which prevailed at some times during our trial could also have negatively affected survival of *A. colemani* and subsequent parasitism of *A. fabae*. In the present study, very low parasitism rate of *A. fabae* by *A. colemani* was observed in all the growing seasons in the two agro-ecological zones. Low parasitiod population could also result from high application of pesticides in the neighbouring farms. It is probable that there was heavy application of pesticides in in the farms neighbouring our experimental sites that could have resulted to low parasitoid abundance observed in the present study. Conservation and augmentative biocontrol strategies for *A. colemani* could be used by farmers growing African nightshades for the control of *A. fabae* in their fields.

Flea beetles such as *E. silvicola* cause immense damages the nightshade plant by making small holes on the leaves (Mureithi et al., 2017; Mureithi et al., unpublished). This is disadvantageous to the farmer as leaves having the holes are discarded from the harvested produce before taking it to the market. *E. silvicola* might be more tolerant to lower temperatures than high temperatures more populations was observed in the high altitude zone which is characterized by low temperatures. It might also be that *E. silvicola* was out-competed by *A. fabae* in the mid altitude zone since high infestation by aphids on African nightshades was observed in the mid altitude zone compared to the high altitude zone.

Our results also reveal that at the high altitude zone, infestation by *E. silvicola* occur early in the season and continues throughout the crop cycle unlike in the mid altitude zone where colonization occur occurs early and is sustained at almost all stages of crop phenology. Mani and Pal (2013) also observed early colonization of okra by the flea beetle *Nisotra chrysomeloides* starting from the seedling stage. Therefore farmers at the high altitude are likely to face higher damage on their nightshade crop from *E. silvicola* damage considering that the crop is normally harvested between pre-flowering and flowering stages and by that time, the pest will have damaged the crop. Early detection and control of *E. silvicola* in African nightshade fields particularly at the high altitude zone could be useful in order to curtail the pest damage. Lamb (1983) reported that the *Phyllotreta* flea beetles are capable of flying long distances from the overwintering sites to colonise canola fields. In our study, the flea beetle *E. silvicola* could have migrated from the mid altitude to high altitude zone particularly in the early stages of crop development. The cool weather conditions in the high altitude zone could have driven this migration. High colonisation of African nightshades with E. silvicola even at the later stages of crop development at the mid altitude zone suggest that farmers in the mid altitude zone could reduce *E. silvicola* population build-up by removing the older nightshade crop from the field. The older crop is rarely harvested and removing it from the field could aid in lowering the incidences of flea beetles in the subsequent season. The use of entomopathogenic nematodes (EPNs) and zero tillage are other strategies that have been reported to lower the population of this pest and future studies could investigate their potential on management of E. silvicola in African nightshades production. (Dosdall et al., 1999; Trdan et al., 2008; Xu et al., 2010).

Our study further revealed low infestation of African nightshades by the Lepidopteran pests in all the growing seasons for both agro-ecological zones. The Lepidopterans could have preferred other hosts such as amaranths which were grown in close proximity to African nightshades. In the year 2015, higher abundance of Lepidopterans were observed in the dry

season (1st growing season), compared to the wet season (2nd growing season). The flowering plants during the 1st growing season might have provided the adults Lepidopterans with abundant source of pollen and nectar necessary for reproduction. In the year 2016, we did not observe differences in the abundance of Lepidopteran pests in both growing seasons at the mid altitude zone. However, more Lepidopterans were observed in the wet (4th growing season) compared to the dry season (3rd growing season) in the high altitude zone. It might be that the high temperatures that prevailed in the 3rd growing season may have been unsuitable for the Lepidopterans.

The major pests attacking African nightshades are polyphagous and have a wide host range among the cultivated and wild plants (Mureithi et al., 2017). For instance, *A. fabae* has a wide host range in the families Solanaceae, Amaranthaceae, Chenopodiaceae, Brassicaceae, Cucurbitaceae, and Fabaceae (Cammell and Way 1983; Fernandez-Quintanilla *et al.*, 2002). However, in our study we found higher *A. fabae* colonization on African nightshades compared to the other hosts present in the neighbourhood. However farmers should not ignore alternative hosts of *A. fabae* such as *Bidens pilosa*, a common weed species in and around African nightshade farms since they can be a source of new infestation for a newly planted nightshade crop.

Moreover, higher *E. silvicola* colonization on African nightshades was observed relative to the other hosts presents in the trial sites. Preference of flea beetles to particular hosts has been reported. In their study, Altieri and Schmidt (1986) observed higher attractiveness of flea beetle *Phyllotreta cruciferi* to wild mustard compared to cultivated collards. When the collards were sprayed with extract from the mustard plant, they became more attractive than the unsprayed collards. They concluded that the wild mustard could be having higher concentration of volatiles that attract flea beetles than collards. In our study, there was higher colonization by flea beetle *E. silvicola* on African nightshades compared to the other neighbouring hosts. African nightshade plants might be having stronger volatiles that attract *E. silvicola* more than the alternative hosts present in the neighbourhood.

In conclusion, our study has shown that while the abundance of *A. fabae* and *A. colemani* were higher in the mid altitude zone than in the high altitude zone, more *E. silvicola*, and Lepidopterans were observed in the high altitude zone. Moreover, infestation of African nightshades by *A. fabae*, *E. silvicola* and Lepidopteran pests starts at an early stage of nightshade development. However, their populations peaks and fluctuates at different phenological stages of nightshade growth. Interventions for pest management should be instituted at an early stage of crop development when the pests are first observed. Parasitiod

A. colemani which was observed occurring naturally in nightshade fields could be exploited as a biological control of *A. fabae*. However, studies aimed at improving its performance should be conducted since low parasitism rate was observed in the present study. This study provides important information needed for development and implementation of integrated pest management strategies for nightshade pests at different agro-ecological zones in Kenya and in various seasons of the year. Additionally, the findings on the alternative hosts of the nightshade pests are critical in informing the cultural methods that are required to control these pests.

5.6 References

Altieri MA and Schmidt LL. 1986. Population trends and feeding preferences of flea beetles (*Phyllotreta cruciferae* Goeze) in collard- wild mustard mixtures. *Crop protection* **5 (3)**: 170-175

Ashilenje DS, Omunyin ME, and Okalebo JR. 2011. Influence of potassium on aphid incidence and yield of vegetable African nightshades (*Solanum* L. section *Solanum*). *Annals of Biological Research* **2 (3)**:317-326

AVRDC. 2003. Broad-leaved Nightshade, International Cooperators Fact Sheet 8, Arusha, Tanzania.

Blackman RL. 1974**.** Lifecycle variation of *Myzus persicae* (Sulz.) (Hom., Aphididae) in different parts of the world, in relation to genotype and environment. *Bull. Entomol. Res*. **63**: 595-607.

Bueno VHP, Sampaio MV, van Lenteren JC, De Conti BF, Silva RJ, Rodrigues SMM, Carnevale AB. 2006. Evaluation of two aphid parasitoids as candidates for biocontrol of aphid pests in protected cultivation in Brazil. In *Integrated Control in Protected Crops, Mediterranean Climate*; Castañé, C.; Sanchez, J.A., Eds.; IOBC/WPRS Bulletin: Darmstadt, Germany **29**: 175–180.

Cammell ME, Way MJ. 1983. Aphid Pests. *In* P. D. Hebblethwaite [ed.], The Faba Bean (*Vicia faba* L.), A Basis for Improvement. Butterworths, London. pp. 315-346.

Dosdall LM, Dolinski MG, Cowle NT, Conway PM. 1999. The effect of tillage, row spacing, and seeding rate on feeding injury by flea beetles, *Phyllotreta* spp. (Coleoptera: Chrysomelidae) in canola in central Alberta, Canada. *Crop Prot.* **18**:217-224.

Eastop VF. 1957. The periodicity of aphid flight in East Africa. *Bull. Entomol. Res*. **48**: 305-310.

Ebert TA, Cartwright B. 1997. Biology and ecology of Aphis gossypii Glover (Homoptera: Aphididae). *Southwestern Entomologist* **22(1)**:116-153

Fernandez-Quintanilla C, Fereres A, Godfrey L, Norris RF. 2002. Development and reproduction of *Myzus persicae* and *Aphis fabae* (Hom., Aphididae) on selected weed species surrounding sugar beet fields. *J. Appl. Ent.* **126**:198–202

Fink U, Volkl W. 1995. The effect of abiotic factors on foraging and oviposition success of the aphid parasitoid, *Aphidius rosae*. *Oecologia* **103**: 371–378.

Goh HG, Kim JH, Han MW. 2001. Application of *Aphidius colemani* viereck for control of the aphid in greenhouse. *J. Asia. Pac. Entomol.* **4:** 171–174.

Lamb RJ. 1983. Phenology of flea beetle (Coleoptera: Chrysomelidae) flight in relation to their invasion of canola fields in Manitoba. *The Canadian Entomologist* **115**: 1493–1502.

Leite GLD, Picanço M, Zanuncio JC, Gusmão MR. 2007. Factors affecting colonization and abundance of *Aphis gossypii* Glover (Hemiptera: Aphididae) on okra plantations. *Ciência e Agrotecnologia.* **31 (2)**: 337-343

Hassan RM. 1998. Maize technology development and transfer. A GIS application for research planning in Kenya. CAB International,Wallingford, United Kingdom.

HCDA. 2013. Horticulture Validated report- Ministry of Agriculture & Horticultural Crops Development Authority.

Jones, MG. 1979. Abundance of aphids on cereals from before 1973 to 1977. *J. Appl. Ecol*. **16**: 1-22

Kamga, RT, Kouamé C, Atangana CA, Chagomoka T, Ndango R. 2013. Nutritional evaluation of five African indigenous vegetables. *Journal of Horticultural Research*, **21(1)**: 99-106

Kimaru, SL, Onyango CM , Kimenju JW and Kilalo DC. 2015. Potential of intercropping for management of some arthropod and nematode pests of leafy vegetables in Kenya. *Journal of Agricultural Sciences*, **60(3)**:301-314

Mani JK, Pal S. 2013. Influence of weather factors on the abundance of flea beetle (*Nisotra chrysomeloides* Jacoby) infesting okra. *Crop Res.* **46** (1, 2 and 3): 208-211

Michaud, JP, Browning HW. 1999. Seasonal abundance of the brown citrus aphid, *Toxoptera citricida* (Homoptera: Aphididae), and its natural enemies in Puerto Rico. *Fl. Entomol*. **82**: 424-447.

Mureithi, DM, Fiaboe KKM, Ekesi S, Meyhöfer R. 2017. Important arthropod pests on leafy Amaranth (*Amaranthus viridis*, *A. tricolor* and *A.* 1 *blitum*) and broad-leafed African nightshade (*Solanum scabrum*) with a special focus on 2 host-plant ranges. *African Journal of Horticultural science* **11**: 1-17

Nakata, T. 1995. Population fluctuations of aphids and their natural enemies on potato in Hokkaido, Japan. *Appl. Entomol. Zool*. **30(1)**: 129-138

Orech FO, Christensen DL, Larsen T, Friis H, Aagaard-Hansen J, Estambale BA. 2007. Mineral content of traditional leafy vegetables from western Kenya. Int. J. Food Sci. Nutr., **58(8)**:595-602.

Sithanantham S, Matoka CM, Maundu M, Jakari M, Agong SG. 2003. Integrated crop protection research for sustainable production of Indigenous vegetable crops in Eastern Africa. Proceedings of 4th Horticultural Seminar on Sustainable Horticultural production in the Tropics held in Njoro, Kenya.

Singh G, Singh NP, Singh R. 2014. Food plants of a major agricultural pest *Aphis gossypii* glover (homoptera: aphididae) from india: an updated checklist. *Int. J. LifeSc. Bt and Pharm*. **3**: 2

Suganthy M, Sakthivel P. 2012. Efficacy of botanical pesticides against major pests of black nightshade, solanum nigrum. *Int j pharm bio sci* **3(3)**:220 – 228

Takasu K. Lewis WJ. 1994. Importance of adult food sources to host searching of a larval parasitoid Microplitis croceipes. *Biological control* **5**: 25.30

Trdan S, Vidrih M, Valic N, Laznik Z. 2008. Impact of entomopathogenic nematodes on adults of Phyllotreta spp. (Coleoptera: Chrysomelidae) under laboratory conditions. *Acta Agriculturae Scandinavica Section B _ Soil and Plant Science* **58**: 169-175

Uusiku NP, Oelofse A, Duodu KG, Bester MJ, Faber M. 2010. Nutritional value of leafy vegetables of sub-Saharan Africa and their potential contribution to human health: A review. J. Food Comp. Anal., **23**:499-509.

Varela AN, Seif A. 2004. A guide to IPM and Hygiene Standards in Okra Production in Kenya. ICIPE Science Press, Nairobi ICIPE.

Vinson SB. 1976: Host selection by insect parasitoids. *Annu. Rev. Entomol.* **21**: 109–133.

Xu, C, De Clercq P, Miens M, Chen S, Han R. 2010. Efficacy of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) against the striped flea beetle, *Phyllotreta striolata*. *BioControl* **55**:789-797

6 Effects of host and host-plants on acceptability and suitability of *Aphis fabae* and *Myzus persicae* by *Aphidius colemani* in African nightshades production system

6.1 Abstract

The current study evaluated the acceptability and suitability of *Aphis fabae* Scopoli, and *Myzus persicae* Sulzer, as host aphids and *Solanum scabrum* and *Solanum villosum* as tested host plants for *Aphidius colemani* Viereck. A 24 hr old mated *A. colemani* reared on *A. fabae* feeding on either *S. scabrum* or *S. villosum* was allowed to parasitize separately twenty second instar larvae of *A. fabae* or *Myzus persicae*. The host aphids were each reared separately on *S. scabrum* and *S. villosum*. We show for the first time that *A. colemani* has higher acceptance for *M. persicae* compared to *A. fabae* regardless of the rearing host plant. Whereas significantly higher parasitism occurs on *A. fabae* when the test host plant was *S. villosum* (62.50±1.06%) compared to *S. scabrum* (55.25±1.13%), the inverse was the case for *M. persicae*, whereby significantly higher parasitism occur when the test host plant is *S. scabrum* (11.12±2.17%) compared to *S. villosum* (3.37±0.99%). However, higher parasitoid emergence occurs on *A. fabae* (85.31±1.10%) compared to *M. persicae* (17.42±3.89%). *A. fabae* feeding on *S. villosum* is therefore the most preferable and suitable host for *A. colemani* in nightshade production system. *A. colemani* presents a promising alternative in management of aphids in nightshade farms which currently is over-reliant on synthetic pesticides.

Key words: *Parasitiod; aphids; African nightshades*

6.2 Introduction

Sucking insects are among the most important pest problem in the production of African nightshades (Schippers, 2000; Sithanantham *et al*., 2003; Mureithi et al., 2017). In particular, aphids are a menace in African nightshades production causing up to 26.76 % damage to the crop (Mbugua et al., 2006; 4.4.3). Among the most prevalent aphid species infesting African nightshades are *Aphis fabae* (Scopoli) and *Myzus persicae* (Sulzer) (Kimaru et al., 2015; 4.4.3). Currently, the control of *A. fabae* and *M. persicae* in African nightshade farms is largely through application of synthetic insecticides. However, these aphids develop fast resistance to the application of synthetic insecticides (Kung et al., 1964; Furk et al., 1980; Zil'bermints and Zhuravleva, 1984; Devonshire et al., 1998; Herron and Wilson, 2011). Biological control of aphids is considered a good alternative to the use of synthetic chemical pesticides.

For decades, parasitoids have been incorporated in IPM programs for the control of aphids (Boivin et. al., 2012). For instance, parasitiod *Aphidius rhopalosiphi* (Hymenoptera: Aphidiinae) is reported to reduce the population of wheat aphid *Sitobion avenae* in wheat fields below economic injury point (Levie et al., 2005). *Aphidius colemani*, a generalist parasitoid that parasitizes many aphid species including *A. fabae* and *M. persicae* is well established in many programs for the management of aphids in vegetables and ornamental plants (Stary, 1975; Messing and Rabasse, 1995; Jones et al. 2003; Vásquez et al., 2006). Moreover, parasitiod *A. colemani* takes a short time to apply compared to the chemical pesticides, has great dispersal distance, and is easy to produce cost effectively (Van Lenteren et al., 1988; Van Steenis et al., 1995; Van Schelt et al., 2011).

Factors influencing parasitism by a parasitoid include habitat location, host location, host acceptance and host suitability (Vinson, 1976, 1984; Hatano et al., 2008; Rasekh et al., 2010). Host acceptance and host suitability are affected by the insect host and the host-plant the host was feeding on before being parasitized (Chau & Mackauer, 2001). The acceptance of a host is influenced by the pre-imaginal learning whereby a parasitoid has higher acceptance to the host used in its rearing (van Emden et al., 2008). The semio-chemicals cues present on the insect host body aid the parasitoid in selecting an acceptable host during antennae contacts or ovipositor probing. Moreover, the plant volatiles released when the plant is attacked by insects and the visual cues of the host plant also guide the parasitoids in finding its host (Powell and Wright, 1988; Turlings et al., 1990; Mattiacci et al., 1994; Du et al., 1998; Dicke et al., 2003 Muratori et al., 2006; Larocca et al., 2007). The colour of the host aphid also

influences preference of the parasitoid as with the case of parasitoid *Lysiphlebus fabarum* which show strong preference for dark coloured aphids (Tregubenko, 1980).

Parasitoid *A. colemani* reared on *M. persicae* as host and pepper as host plant had higher parasitism on *M. persicae*-pepper combination than on other plants. However, in the same study, *A. colemani* reared on *M. persicae* as host and radish as host plant had lower parasitism on *M. persicae*-radish combination than when the host plant was pepper (Bilu et al, 2006). In a related study, there was higher host acceptance of parasitoid *Lysiphlebus testaceipes* (Cresson) on *A. fabae* when the host plant of the aphid was bean as compared to sugarbeet. Conversely, no difference was observed in host acceptance of *A. colemani* to *M. persicae* or *A. fabae* when the host plant was sugarbeet or beans. (Albittar et al., 2016). These studies suggest that host acceptance is mediated by combined effects of the host aphid and the host plant of the host aphid Pre-imaginal conditioning of the parasitoids and herbivore induced volatiles play a significant role in host acceptance and suitability by the parasitoid.

To our knowledge, no study on the performance of *A. colemani* in the management *A. fabae* and *M. persicae* in African nightshades has been done. In the current study, we investigated the acceptability and suitability to *A. colemani* two aphid species, *A. fabae* and *M. persicae* which were reared on *S. scabrum* or *S. villosum*.

6.3 Materials and methods

6.3.1 Aphid colonies

Both aphid species, *A. fabae* and *M. persicae*, were collected from nightshade farms in Kiambu County, Kenya and continuously reared on potted nightshade plants placed inside plexiglass cages at ICIPE laboratories. Colonies of *A. fabae* and *M. persicae* were reared in separate plexiglass cages. Prior to rearing, the aphid sample from the collection was taken to the National Museums of Kenya for confirmation of the species identity. Three potted *S. scabrum* or *S. villosum* were placed in each of the plexiglass cages. Five mature *A. fabae* or *M. persicae* were placed onto the leaves of each plant in the cage. The cages were then placed on top of a bench inside the rearing room. The aphids were left to reproduce for several generations before being used in the experiments. Withered or senescing plants were replaced with fresh plants once or twice per week depending on the level of infestation. During replacement of new plants, the old plants were retained in the cages until all the aphids had migrated naturally from the old plants to the new plants. Thereafter, the old plants were removed and discarded. When the population of aphids became high in a particular cage, they

were redistributed into new cages to avoiding over-crowding and completion for the food resource. The climatic conditions in the rearing room were maintained at 23^0C±2.

6.3.2 Rearing of A. colemani

Mummies of *A. colemani* were collected from African nightshade plants in Kiambu County, Kenya and brought to ICIPE laboratory for rearing. After the emergence of the parasitoids, they were first identified at ICIPE laboratories before rearing them continuously for several generations on *A. fabae* as host and potted African nightshades as the host plant at ICIPE laboratories. Stock cultures of *A. colemani* were maintained in plexiglass cages (30 x 30 x 30 cm) where 3 nightshade plants (*S. scabrum* or *S. villosum*) infested with *A. fabae* were exposed to the parasitoid for parasitism. The climatic conditions in the rearing room were maintained at 23±2^0C and 60% RH. For use in experiments, African nightshade leaves with mummies developing from the parasitized *A. fabae* from the stock culture were plucked from the plant and put in a new plexiglass cage (30 x 30 x 30 cm). The bottom of the cage was lined with paper towel to absorb the excess moisture in the cage and cotton wool balls soaked with honey solution were placed on the inside walls of the cage to nourish the emerging parasitoids. The emerged adults were left together in the cage for 24 hours for the purpose of mating and female maturation. Thereafter, the mated and naïve females were collected and used in the experiments.

6.3.3 Preparation of the Petri-dishes for experiment

A layer of 1% solution agar/water was poured into a petri dish (9 cm diameter, 1.5 cm height) until half-full and left overnight to solidify. An African nightshade leaf (*S. scabrum/ Solanum villosum*) that covered almost the entire bottom of the petri dish was placed upside down on top of the agar. The petiole of the leaf passed through a small hole drilled on the side of the petri dish to the outside of the petri dish. The base of the petiole was then covered by a wet cotton wool to sustain the freshness of the leaf throughout the experimental period. In all cases in the experiments, the leaf placed at the bottom of the petri dish corresponded with the host plant the test aphid was feeding on before the experiment.

6.3.4 Effect of rearing host plant, host aphid and test host plant on acceptability and suitability of A. fabae and M. persicae by A. colemani

The parasitoids used for the experiment were obtained from two sources; **rearing host plant 1**, where the rearing host plant for the parasitoid was *S. scabrum*, and **rearing host plant 2**,

where the rearing host plant for the parasitoid was *S. villosum*. The parasitoids were tested on two host aphid species, *A. fabae* (mass rearing host) and *M. persicae* (alternative host). Each aphid species was reared separately on each of the rearing host plants. In the first experiment, 20 2nd instar nymphs of *A. fabae* which were reared on *S. scabrum* as the test host plant were gently picked from the stock culture with the camel brush (size No. 1) and placed on top of the leaf in a petri dish and allowed 10 minutes to settle on the leaf. A 24 hr-old-mated female parasitoid and without previous oviposition experience obtained from rearing host plant 1, was exposed to the aphids for a period of 10 minutes. Pre-experiments done prior to this experiment showed that the parasitoid was not actively searching for the host after the 10 minutes exposure period.

During the 10 minutes exposure period, the following observations were made; the number of "contacts" the parasitoid made on the aphid host using the antennae, the number of "oviposition attempts" (bending of the parasitoid ovipositor beneath the aphid host and thrusting it in the aphid body) and the number of "kicks" (aphid defending itself by kicking the approaching *A. colemani* with its hind leg). Thereafter, the petri-dishes containing the parasitized aphids were transferred to a climate chamber (23±2^{0}C, RH 65%, 12 hrs light, 12 hrs darkness) to await mummification of parasitized aphids and parasitoid emergence. To maintain freshness of the leaves placed at the bottom of the petri dish, drops of water were added on the cotton wool wrapping the base of the petiole. This enabled continued survival of the aphids before the parasitized ones became mummified. The parasitized aphids were checked daily to determine parasitism rate, emergence rate, host size and sex ratio.

In the second experiment, the parasitoid was obtained from *A. fabae* from rearing host plant 1, and tested on *A. fabae* feeding on *S. villosum* as the tet host plant. In the third experiment, the parasitoid was obtained from rearing host plant 1and tested on M. persicae feeding on *S. scabrum* as the test host plant. In the fourth experiment, the parasitoid was obtained from rearing host plant 1 but the tested aphid was *M. persicae* reared on *S. villosum* as the test host plant. In experiment 5 to 8, the parasitoid was obtained from rearing host plant 2. The parasitoid was tested on *A. fabae* on *S. scabrum*, *A. fabae* on *S. villosum*, *M. persicae* on *S. scabrum*, and *M. persicae* on *S. villosum* in experiment 5, 6, 7, and 8 respectively (Fig 6-1).

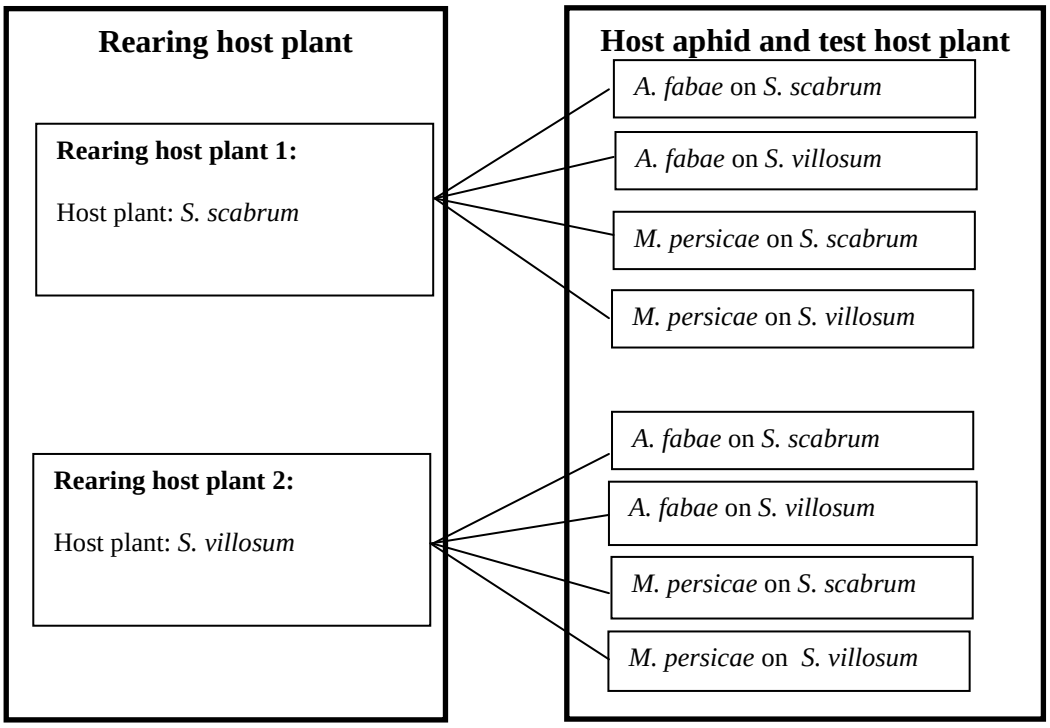

Fig 6-1: Schematic diagram showing the various combinations of rearing host plants, aphid hosts, and tested host plants.

6.3.5 Data analysis

The number of antennae contacts, the number of oviposition attempts, parasitism rate (%), and emergence rate (%) was subjected to a three way ANOVA with rearing host plant, host aphid and tested host plant included as factors in the analysis. The number of antennae contacts and oviposition attempts made by the parasitoids as well as the number of kicks made by the aphids were log transformed before analysis. Parasitism rate was calculated as a percentage of number of mummies formed compared to the total number of host aphids exposed to the parasitoid. The emergence rate was calculated as a percentage of emerged F1 parasitoids from the total number of mummies formed. The percentage data was arcsine-square root transformed before analysis. Host size was determined by measuring the length of the forewing of the female parasitoids emerging from the parasitized aphids Chi-square was used to analyse the sex ratio of the emerging parasitoids from parasitized aphids.

6.4 Results

6.4.1 Effect of rearing host plant, host aphid, and test host plant on antennae contacts by A. colemani

The parasitoid *A. colemani* made between 17.78 ± 0.37 to 26.28 ± 0.89 antennae contacts on the aphids during the 10 minutes exposure period (Fig 6-2).

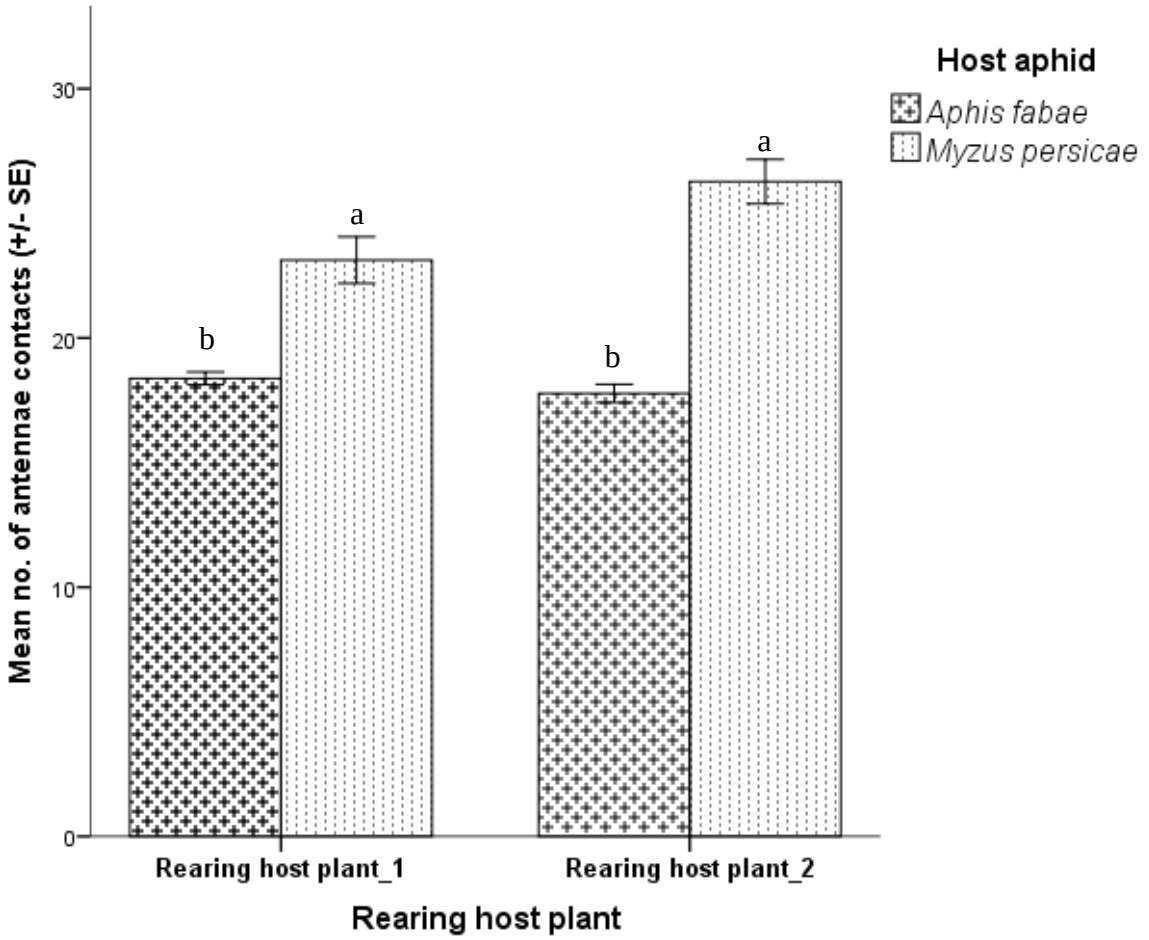

Fig 6-2: Mean number (+/- SE) of antennae contacts made on *Aphis fabae* and *Myzus persicae* by *Aphidius colemani* which was reared from two different sources. Rearing host plant_1Rearing host plant_1- the rearing host plant for *A. colemani* was *Solanum scabrum*; Rearing host plant_2Rearing host plant_2- the rearing host plant for the parasitoid was *Solanum villosum*. The count data was log transformed before the analysis. T-test was carried out at P≤0.05. Where significance difference was observed, Tukey test was used to separate the means. Same letter for a given rearing host plant signify no significant difference.

Interaction between rearing host plant and host aphid was observed when the number of antennae contacts were evaluated (F = 9.034; P = 0.003). Regardless of the rearing host plant, there was significantly higher number of antennae contacts made on *M. persicae* compared to *A. fabae*. When the parasitoids were reared on *S. scabrum* as the rearing host plant, there were significantly higher number of antennae contacts on *M. persicae* than on *A. fabae* (t = -4.83; df = 78; p< 0.001) (Fig 6-2). Similarly, there were significantly higher number of antennae contacts on *M. persicae* than on *A. fabae* when therearing host plant was *S. villosum*

for both of the host aphids (t = -9.35; df = 78; p < 0.001). There was no interaction between the rearing host plantrearing host plant and the test host plant (F = 0.478; P = 0.490). In both aphid species, the test host plant did not have a significant effect on the number of antennae contacts made by the parasitoids (t = -1.34; df = 58; p = 0.894). The mean number of contacts made by parasitoids on aphids feeding on S. *scabrum* was 21.35±0.63 while the one made by parasitoids on aphids feeding on *S. villosum* was 21.43±0.61.

6.4.2 Effect of rearing host plant, host aphid, and test host plant on oviposition attempts by A. colemani

The parasitoid made between 15.10 ± 0.26 and 17.33 ± 0.53 oviposition attempts on both aphid species in the 10 minutes the parasitoid was exposed to the host aphid. Interaction of rearing host plant and host aphid was observed (F = 6.99: P =0.009). *A. colemani* made significantly more oviposition attempts on *M. persicae* than in *A. fabae* when the parasitoid came from rearing host plant_2 (t= -3.35; df = 78; p = 0.001) (Fig 6-3).

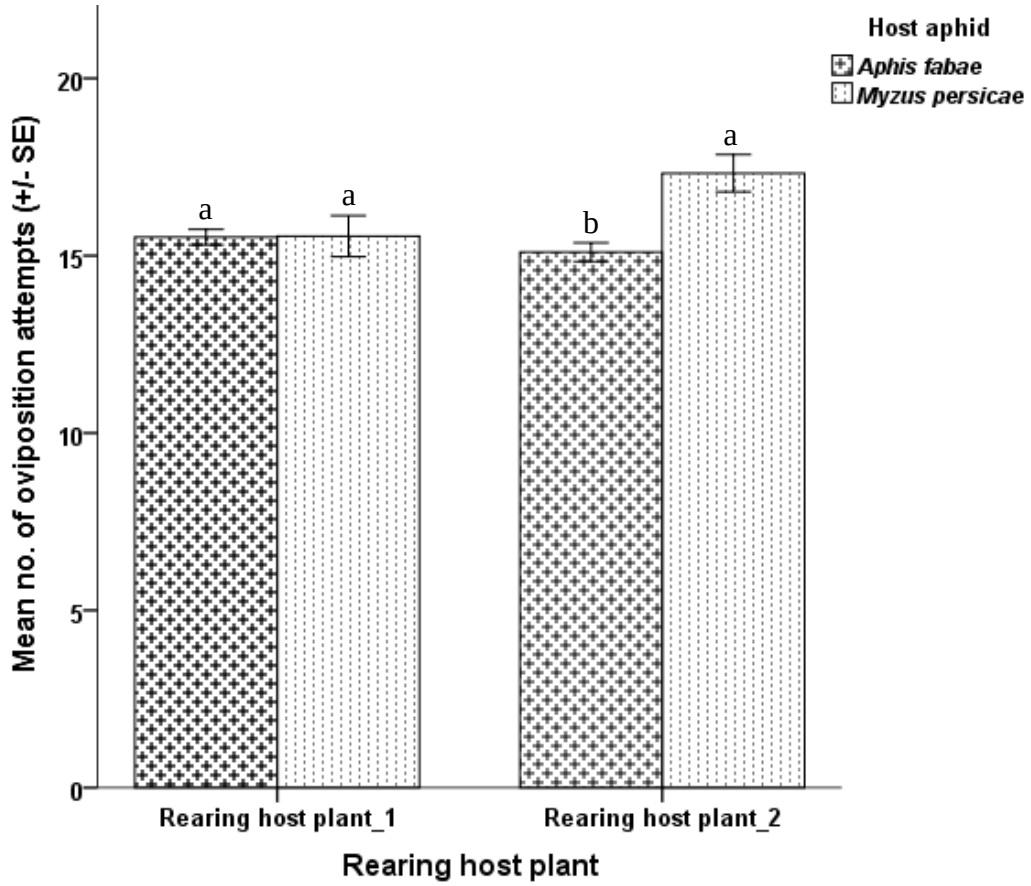

Fig 6-3: Mean number (+/- SE) of oviposition attempts made on *Aphis fabae* and *Myzus persicae* by *Aphidius colemani* which was reared from two different sources. Rearing host plant_1- the rearing host plant for *A. colemani* was *Solanum scabrum*; Rearing host plant_2- the rearing host plant for the parasitoid was *Solanum villosum*. The count data was log transformed before the analysis. T-test was carried out at P≤0.05. Where significance difference was observed, Tukey test was used to separate the means. Same letter for a given rearing host plant signify no significant difference.

However, the number of oviposition attempts was not significantly different on the two aphid species when the parasitoid came from rearing host plant 1 (T= 0.51; df = 78; p=0.613) (Fig 3). There was also interaction between the host aphid and the test host plant for rearing the aphid (F = 6.68; p = 0.011). Significantly higher number of oviposition attempts were made on *M. persicae* that was reared on *S. scabrum* as compared to the one reared on *S. villosum* (T = 0.21; df = 78; p = 0.040) (Fig 3). However, no significant difference on oviposition attempts was observed on *A. fabae* regardless of whether the test host plat was *S. scabrum* or *S. villosum* (T = -1.65; df = 78; p = 0.103) (Fig 6-4).

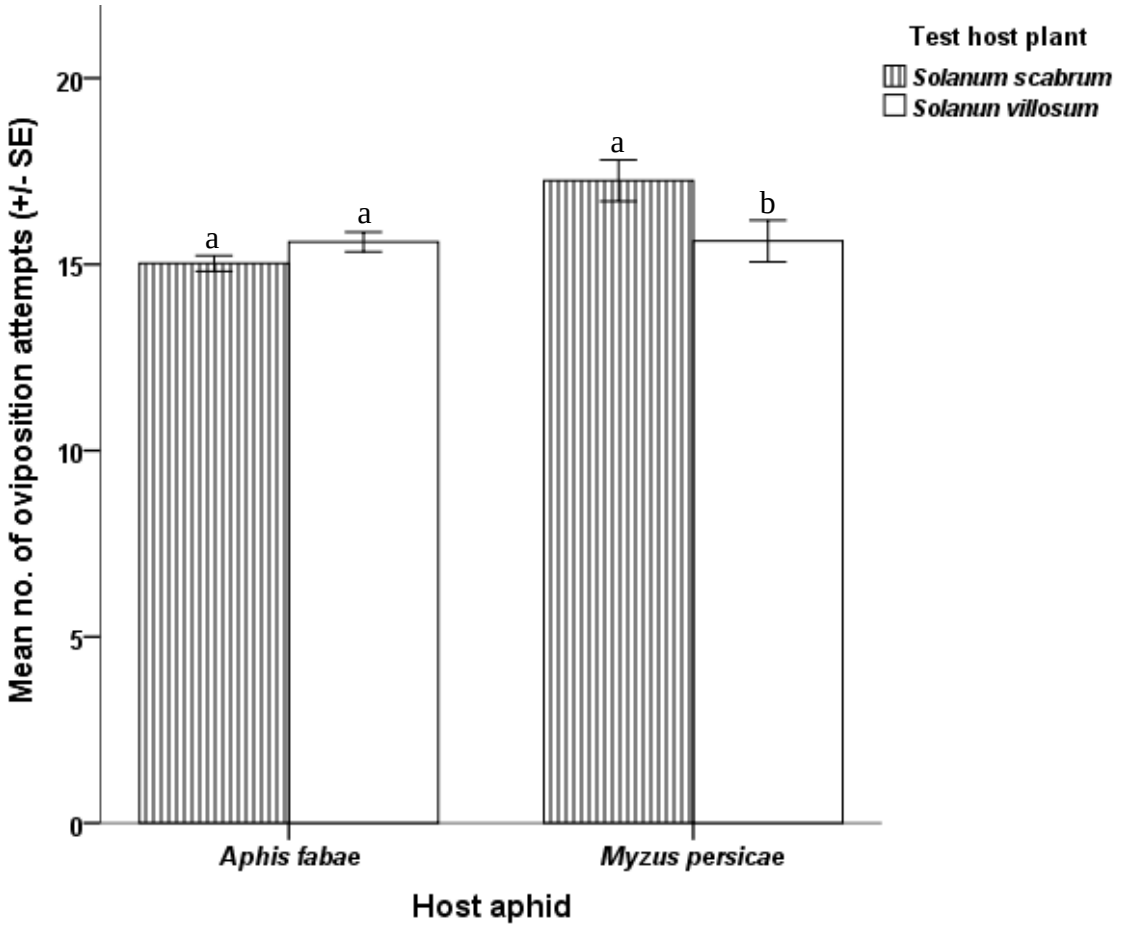

Fig 6-4: Mean number (+/- SE) of oviposition attempts made by *Aphidius colemani* on *Aphis fabae* and *Myzus persicae*. Each aphid was reared separately on *Solanum scabrum* and *Solanum villosum*. The count data was log transformed before the analysis. T-test was carried out at P≤0.05. Where significance difference was observed, Tukey test was used to separate the means. Same letter for a given aphid species signify no significant difference.

6.4.3 Effect of rearing host plant, host aphid, and test host plant on parasitism by A. colemani

Interaction between the host aphid and test host plant was observed when the parasitism rate of the aphids by *A. colemani* was considered (F = 21.24; p < 0.001). Whereas there was significantly higher parasitism on *A. fabae* reared on *S. villosum* than on *S. scabrum* (T = -

4.67; df = 78; p = < 0.001), the opposite was true for the parasitism rate on *M. persicae* (T = 3.34; df = 78; p=0.001) (Fig 6-5).

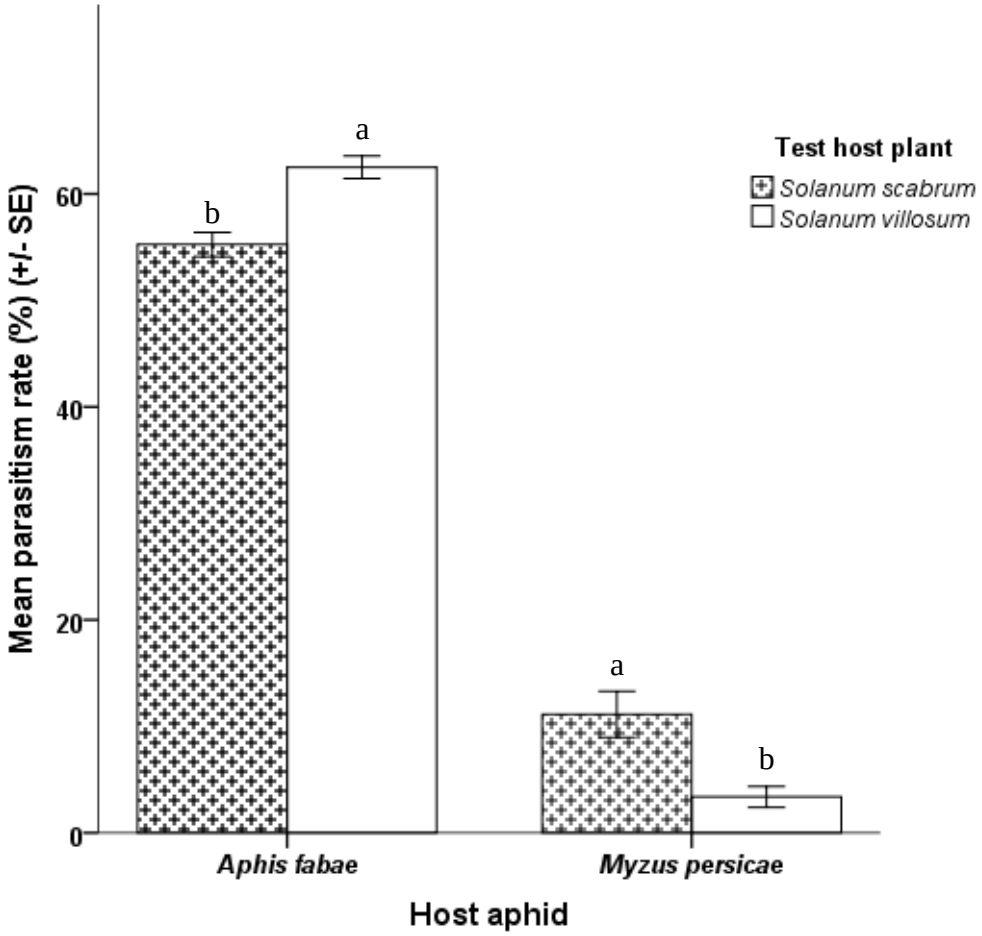

Fig 6-5: Parasitism rate (+/- SE) of *Aphis fabae* and *Myzus persicae* by *Aphidius colemani*. Each aphid species was reared separately on *Solanum scabrum* and *Solanum villosum*. The percentage parasitism data was arcsine square root transformed before the analysis. T-test was carried out at P≤0.05. Where significance difference was observed, Tukey test was used to separate the means. Same letter for a given aphid species signify no significant difference.

However, there was no interaction between the rearing host plant and the host aphid (F = 0.10; p = 0.754) or the host plant (F = 0.13; p = 0.716). The parasitism rates of the aphids were not significantly affected by the rearing host plant (T = 0.13; df = 158; p = 0.897). Parasitism rate by *A. colemani* from rearing host plant 1 was 33.12±3.08 whereas the parasitism rate from rearing host plant 2 was 33.00±3.12

6.4.4 Effect of rearing host plant, host aphid, and test host plant on emergence of A. colemani

The three factors did not interact when the emergence rate of *A. colemani* was considered (F = 0.97; p = 0.33). When the host aphid was considered, significantly higher number of

parasitoids emerged from *A. fabae* compared to *M. persicae* (T= 14.86; df = 158; p < 0.001) (Fig 6-6).

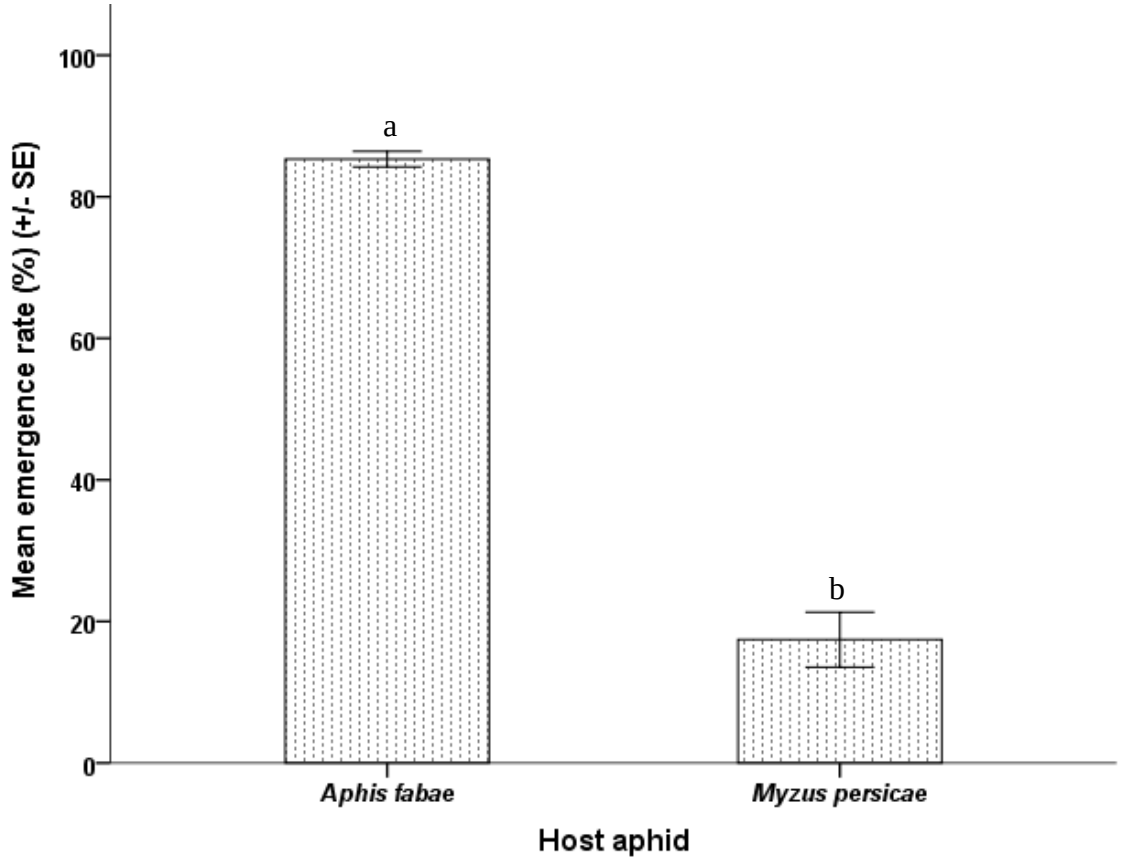

Fig 6-6: Mean emergence rate (+/- SE) of *Aphidius colemani* from *Aphis fabae* and *Myzus persicae*. The percentage emergence data was arcsine square root transformed before the analysis. T-test was carried out at P≤0.05. Where significance difference was observed, Tukey test was used to separate the means. Same letter for a given aphid species signify no significant difference.

However, the rearing host plant (T= -1.13; df = 158; p = 0.261) and test host plant (T= 0.55; df = 158; p = 0.581) did not have a significant effect on the emergence of the parasitoids. Emergence rate of 48.01±4.75% was observed on aphids parasitized by *A. colemani* from rearing host plant 1 while 54.73±4.75% was recorded from aphids parasitized by *A. colemani* from rearing host plant 2. Furthermore, the emergence rate of parasitoids from aphids reared on *S. scabrum* as the test host plant was 49.48±4.75% while those from aphids reared on *S. villosum* as the test host plant were 53.26±4.78%.

6.4.5 Defense of A. fabae and M. persicae against the attack by A. colemani

M. persicae defended itself more aggressively against *A. colemani* compared to *A. fabae*. There were significantly higher number of kicks made by *M. persicae* to wade off the attack of the parasitoid as compared to those made by *A. fabae* (T= -3.84; df = 158; p < 0.001). The

mean number of kicks made by *M. persicae* against the parasitoid during the exposure period was 6.99±0.46 as compared to 4.11±0.09 made by *A. fabae*.

6.4.6 The effect of host and host plant on the size of A. colemani

Interaction between the host and host plant was observed when the size of the emerged parasitoid was evaluated ($F = 11.07$; $p = 0.001$). The parasitoids emerging from *A. fabae* that was reared on *S. villosum* were significantly larger than those reared on *S. scabrum* ($T= -5.40$; $df = 78$; $p < 0.001$). The mean wing length of female parasitoids emerging from *A. fabae* reared on *S. villosum* was 1.61±0.012 mm as compared to 1.48±0.02 mm for those reared on *S. scabrum*. However, there was no significant difference in the size of the parasitoid emerging from *M. persicae* regardless of the rearing host plant ($T= 1.75$; $df = 78$; $p = 0.105$). The wing length of the parasitoids that emerged from *M. persicae* reared on *S. scabrum* was 1.65±0.03 mm as compared to 1.57±0.03 mm for those that emerged from *S. villosum*.

6.4.7 Sex ratio of emerging parasitoids

The sex ratio of the emerged parasitoids from *A. fabae* was male biased (X-squared = 4.357; $df = 1$; $p = 0.03686$). 53.7 % of all the emerged parasitoids from *A. fabae* were males while 46.3% were females. Although 57.1% of the parasitiods that emerged from *M. persicae* were females, the sex ratio of females to males parasitoids.was not statistically different(X-squared = 0.714; $df = 1$; $p = 0.398$).

6.5 Discussion

Antennae contacts and oviposition attempts are some of the parameters used to measure host acceptability by a parasitoid. A higher number of antennae contacts and oviposition attempts would be translated to mean higher acceptance of a particular host to the parasitoid. Although our study revealed higher acceptance of parasitoid *A. colemani* to *M. persicae* compared to *A. fabae* in the nightshade production system, Messing and Rabasse (1995) in their study using eggplant and cucumber as host plant for *M. persicae* and *A. gossypii* respectively, observed higher acceptance of *A. colemani* on *A. gossypii* relative to *M. persicae*. In our study, parasitoid *A. colemani* made more antennae contacts and *oviposition attempts* on *M. persicae* compared to *A. fabae* suggesting that *M. persicae* is a more acceptable host for the parasitoid. *M. persicae* defended itself more aggressively through kicks compared to *A. fabae* and this

might have contributed to the parasitoid making more oviposition attempts in an effort to oviposit its eggs on the host.

Host acceptability does not always translate to higher host parasitism and parasitoid emergence rate as the parasitoid may fail to deposit or deposit fewer eggs on less acceptable hosts. In our study, although *M. persicae* was more acceptable host, higher parasitism rate was observed on *A. fabae*. In a related study, Sampaio et al. (2008) observed higher parasitism rate on *A. gossypii* than *M. persicae* in laboratory trials. These results were further demonstrated in greenhouse trials done with *A. colemani* on both *A. gossypii* and *M. persicae* (van Driesche et al., 2008). *M. persicae* is very aggressive in defending itself by kicking away the attacking parasitoid and this could have lead to fewer successful ovipositions. *A. colemani* may succeed in depositing its egg in the aphid host but the eggs may fail to hatch due to encapsulation of the eggs by chemical substances inside the host body or the parasitoid larvae may die inside the host body before it kills the host. A study by Vorburger et al., (2009) found out that a secondary endosymbiont, *Regiella insecticola*, present on *M. persicae* confers resistance to the aphid against *A. colemani*. Similarly, Oliver et al., (2003) reported that secondary endosymbionts present in *Acyrthosiphon pisum* (the pea aphid) body confer resistance against *A. ervi* leading to death of developing parasitoid larvae inside the host body. It is therefore probable that secondary endosymbionts present in *M. persicae* confer higher resistance against the parasitoid leading to low parasitism by *A. colemani* and also leading to low parasitoid emergence. However, further studies on this aspect are required. Studies with *A. ervi* revealed that the parasitoid does not discriminate between resistant and susceptible aphids and their oviposition behaviour is similar on *M. persicae* having endosymbionts or not (Oliver et al., 2003). If *A. colemani* is unable to distinguish *M. persicae* having endosymbionts from the ones that do not have as is the case with *A. ervi*, the contribution of *A. colemani* in biologically based IPM programs for *M. persicae* control particularly in classical bio-control could be adversely affected.

Similar to our findings, it has been demonstrated that host plant influences the preference of *A. colemani* to particular host (Messing and Rabasse, 1995; Storeck et al., 2000; Martinou and Wright, 2007). *A. rhopalosiphi*, a parasitoid of wheat aphid, was even able to show higher preferences to volatiles from some wheat varieties in a Y-tube olfactometer experiments (Wickremasinghe and van Emden, 1992). The chemical cues from these host plants were revealed to be present on mummy cases and parasitoids picks them while emerging from the mummies, a process called preimaginal conditioning. The preimaginal conditioning of a parasitoid influences the acceptability on the subsequent host (Emden et al.,

1996). Storeck et al. (2000) demonstrated that when the mummies were dissected and the parasitoid pupae removed to prevent any contact of the emerging parasitoid with the mummy cases, *A. colemani* did not show preference for the rearing host plant and have to quickly adopt to the new host plant. In our study, we speculate that the aphid mummy cases bear chemical cues from the nightshade species. It might be that *S. scabrum* has stronger chemical cues than *S. villosum* and consequently, higher residual effect on the aphid mummy cases. Therefore the emerging *A. colemani* from aphids feeding on *S. scabrum* would pick more chemical cues from the mummy cases resulting in higher acceptance to the subsequent host feeding on the same host plant.

Moreover, aphid parasitoids may also learn plant odours from the environment they emerge in resulting to higher preferences for hosts feeding on those particular host plants (Van Emden et al., 2002). In the present study, *S. scabrum* could have been having stronger volatile compounds than *S. villosum* and consequently influencing the foraging behaviour of the emerging parasitoids towards aphids that were feeding on it. Therefore the emerging *A. colemani* could have been more attracted to volatiles from *S. scabrum l*eading to a higher acceptance for *M. persicae* feeding on that host plant. Further studies to investigate the effect of African nightshade species odours on parasitiod A. colemani should be carried out.

Our study has demonstrated that the host aphid and the nightshade host plant play a critical role in host acceptance and host suitability for *A. colemani*. These findings clearly indicate that the performance of *A. colemani* in nightshades is not only dictated by the particular aphid species attacking the nightshade, but also by the African nightshade species. Producers of parasitoid should therefore take into account these factors when producing parasitoids for management of aphids attacking African nightshades. Future studies could also investigate the performance of *A. colemani* reared on *M. persicae* as host aphid and tested on both *A. fabae* and *M. persicae*. These findings are particularly important to nightshade farmers in East Africa who are faced with a major challenge in control of *A. fabae*. Parasitiod *A. colemani* presents a promising alternative to the current practice which is heavily relying on synthetic pesticides. However, further studies at semi-field and field level are required to validate and improve on the performance of *A. colemani* in nightshade fields before the full potential of this parasitoid can be realized by farmers. Studies on how *A. colemani* could also be integrated with other IPM measures which focus on reduction in the use of synthetic pesticides are required for sustainable production of African nightshade.

6.7 References

Albittar L, Ismail M, Bragard C, Hance T. 2016. Host plants and aphid hosts influence the selection behaviour of three aphid parasitoids (Hymenoptera: Braconidae: Aphidiinae). *European Journal Of Entomology*, **113**: 516–522.

Bilu E, Hopper KR, Coll M. 2006. Host choice by Aphidius colemani: effects of plants, plant–aphid combinations and the presence of intraguild predators. *Ecological Entomology*, **31**: 331–336

Boivin G, Hance T, Brodeur J. 2012. Aphid parasitoids in biological control. *Canadian Journal of Plant Science*, ***92*(1)**:1-12.

Chau A, Mackauer M. 2001: Preference of the aphid parasitoid *Monoctonus paulensis* (Hymenoptera: Braconidae, Aphidiinae) for different aphid species: Female choice and offspring survival. *Biol. Contr.* **20**: 30-38.

Dicke M, De Boer JG, Hofte M, Rocha-Granados MC. 2003: Mixed blends of herbivore-induced plant volatiles and foraging success of carnivorous arthropods. *Oikos* **101**: 38−48.

Du YJ, Poppy GM, Powell W, Pickett JA, Wadhams LJ, Woodcock CM. 1998: Identifi cation of semiochemicals released during aphid feeding that attract parasitoid *Aphidius ervi*. *J. Chem. Ecol.* **24**: 1355−1368.

Emden HF, Sponagl B, Wagner E, Baker T, Ganguly S, Douloumpaka S. 1996. Hopkins' 'host selection principle', another nail in its coffin. *Physiological Entomology*, **21**:325–328.

Furk C, Powell DF, Heyd S. 1980. Pirimicarb resistance in the melon and cotton aphid, Aphis gossypii Glover. *Plant Pathology*, **29**: 191-196.

Hatano E, Kunert G, Michaud JP, Weisser WW. 2008. Chemical cues mediating aphid location by natural enemies. *Eur. J. Entomol.* **105**: 797−806.

Herron GA, Wilson LJ. 2011. Neonicotinoid resistance in Aphis gossypii Glover (Aphididae: Hemiptera) from Australian cotton. *Australian Journal of Entomology*, **50**:93-98.

Jones DB, Giles KL, Berberet RC, Royer TA, Elliott NC, Payton ME. 2003. Functional responses of an introduced parasitoid and an indigenous parasitoid on Greenbug at four temperatures. *Environmental Entomology*, **32(3)**: 425-432.

Kimaru, SL, Onyango CM , Kimenju JW and Kilalo DC. 2015. Potential of intercropping for management of some arthropod and nematode pests of leafy vegetables in Kenya. *Journal of Agricultural Sciences*, **60(3)**:301-314

Kung KY, Chang KL, Chai KY. 1964. Detecting and measuring the resistance of cotton aphids to systox. *Acta Entomologica Sinica*, **13**: 1-9.

Larocca A, Fanti P, Romano VA, Marsicovetere E, Isidoro N, Romani R, Ruschioni S, Pennacchio F, Battaglia D. 2007: Functional bases of host-acceptance behaviour in the aphid parasitoid *Aphidius ervi*. *Physiol. Entomol.* **32**: 305- 312.

Levie A, Legrand MA, Dogot P, Pels C, Baret PV, Hance T. 2005. Mass releases of Aphidius rhopalosiphi (Hymenoptera: Aphidiinae), and strip management to control of wheat aphids. *Agriculture, Ecosystems and Environment*, **105**:17–21.

Mattiacci L, Dicke M, Posthumus MA. 1994: Induction of parasitoid attracting synomone in brussels sprouts plants by feeding of *Pieris brassicae* larvae: role of mechanical damage and herbivore elicitor. *J. Chem. Ecol*. **20**: 2229−2247.

Mbugua G, Gitonga LN, Mureithi E, Karoga J, Manyeki L. 2006. Farmer-Participatory prioritization and development of agronomic practices for African Leafy Vegetables. Proceedings of 6th Horticultural Seminar on Sustainable Horticultural production in the Tropics held in Njoro, Kenya.

Messing R, Rabasse J.M. 1995. Oviposition behaviour of the polyphagous aphid parasitoid *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae). *Agriculture, Ecosystems and Environment*, **52**:13–17.

Muratori F, Le Ralec A, Lognay G, Hance T. 2006: Epicuticular factors involved in host recognition for the aphid parasitoid *Aphidius rhopalosiphi*. *J. Chem. Ecol*. **32**: 579–593.

Mureithi, DM, Fiaboe KKM, Ekesi S, Meyhöfer R. 2017. Important arthropod pests on leafy Amaranth (*Amaranthus viridis*, *A. tricolor* and *A.* 1 *blitum*) and broad-leafed African nightshade (*Solanum scabrum*) with a special focus on 2 host-plant ranges. *African Journal of Horticultural science*, **11**:1-17

Oliver KM, Russell J, Moran N, Hunter MS. 2003. Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. *Proceedings of the National Academy of Sciences*, **100**:1803–1807.

Powell W, Wright AF. 1992. The influence of host food plants on host recognition by four aphidiine parasitoids (Hymenoptera: Braconidae). *Bulletin of Entomological Research* 81: 449–453.

Rasekh A, Michaud JP, Allahyari H, Sabahi Q. 2010: The foraging behavior of *Lysiphlebus fabarum* (Marshall), a thelytokous parasitoid of the black bean aphid in Iran. *J. Insect Behav.* **23**: 165–179.

Schippers RR. 2000**.** African Indigenous Vegetables. An Overview of the Cultivated Species. Natural Resources Institute/ACP-EU Technical Centre for Agricultural and Rural Cooperation, Chatham, UK. Pp 214.

Sithanantham S, Matoka CM, Maundu M, Jakari M, Agong SG. 2003. Integrated crop protection research for sustainable production of Indigenous vegetable crops in Eastern Africa. Proceedings of 4th Horticultural Seminar on Sustainable Horticultural production in the Tropics held in Njoro, Kenya.

Starý P. 1975. *Aphidius colemani* Viereck: its taxonomy, distribution and host range (Hymenoptera, Aphidiidae). *Acta Entomologica Bohemoslovaca*, **72**:156–163.

Storeck A, Poppy GM, van Emden HF, Powell W. 2000. The role of plant chemical cues in determining host preference in the generalist aphid parasitoid *Aphidius colemani*. *Entomologia Experimentalis et Applicata*, 97:41–46.

Tregubenko ES. 1980: On the ethology of aphids and aphidophagous insects. *Zashch. Rastenii* **4**: 38−39.

Turlings TCJ, Tumlinson JH, Lewis WJ. 1990: Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* **250**: 1251−1253.

Van Driesche RG, Lyon S, Sanderson JP, Bennett KC, Stanek EJ, Ruitao ZR. 2008. Greenhouse Trials of Aphidius Colemani (Hymenoptera: Braconidae) Banker Plants for Control of Aphids (Hemiptera: Aphididae) in Greenhouse Spring Floral Crops. *Florida Entomologist*, **91(4):**583-591.

Van Emden HF, Eletherianos I, Rose J, Douloumpaka S, Pettersson J. 2002. Aphid parasitoids detect that an alien plant was present nearby during their development. *Physiological Entomology*, **27**:199–205.

Van Lenteren J. 1988. Biological and integrated pest control in greenhouses. *Annu. Rev. Entomol.* **33:** 239–269.

Van Schelt J, Hoogerbrugge H, Becker N, Messelink GJ, Blockmans K. 2011. Comparing *Aphidius colemani* and *Aphidius matricariae* on *Myzus persicae* ssp. nicotianae in sweet pepper. *IOBC/WPRS Bull.* **68** 169–172.

Vásquez GM, Orr DB, Baker JR. 2006. Efficacy assessment of *Aphidius colemani* (Hymenoptera: Braconidae) for suppression of *Aphis gossypii* (Homoptera: Aphididae) in greenhouse-grown chrysanthemum. *J. Econ. Entomol.* **99**: 1104–1111.

Vinson SB. 1976: Host selection by insect parasitoids. *Annu. Rev. Entomol.* **21**: 109–133.

Vinson SB. 1984. Parasitoid-host relationships. In Bell W.J. & Cardes R. (eds): The Chemical Ecology of Insects. Chapman and Hall, New York, pp. 205–233.

Vorburger C, Sandrock C, Gouskov A, Castaneda LE, Ferrari J. 2009. Genotypic variation and the role of defensive endosymbionts in an all-parthenogenetic hostparasitoid interaction. *Evolution*, **63**:1439–1450.

Wickremasinghe, MGV, & van Emden HF. 1992. Reactions of adult female parasitoids, particularly *Aphidius rhopalosiphi*, to volatile chemical cues from the host plants of their aphid prey. *Physiological Entomology*, **17**: 297–304.

Zil'bermints IV, Zhuravleva LM. 1984. Response of melon and greenhouse aphids to Ambush and Actellic. *Khhniva v Sel'skom Khozyaistva*, **3**:37-40.

7 Occurrence, host range, host resistance and seed transmission of a novel potyvirus, tentatively named nightshade veinal mottle virus, infecting nightshades in Kenya

7.1 Abstract

Production of African nightshades faces several challenges, among a new virus disease, tentatively named nightshade veinal mottle virus (NsVMV). In the current study the incidence, host range, host resistance and seed transmission of NsVMV was investigated. For this purpose field surveys were done at the nightshade growing regions of mid and high altitudes. Moreover, mechanical inoculation of NsVMV was performed in greenhouse trials followed by ELISA with a heterologous antiserum in laboratory tests for virus identification. NsVMV was present in both mid and high elevation zones of Kenya. *Solanum lycopersicum*, *Nicotiana occidentalis*, *Nicotiana.hesperis*, *Nicotiana debneyi*, *Nicotiana tabacum* cv. Samsun and *Nicandra sp* were hosts of the virus. *Capsicum sp, Solanum tuberosum, Solanum melongena, Physalis angulata*, *Amaranthus dubious, Lactuca sp, Conyza sp, Bidens pilosa, Chenopodium murale, Chenopodium nubrum*, *Cleome gynandra* and *Leonotis leonurus* were non-hosts of the virus. None of the tested nightshade species/line were resistant to the virus. In addition, 1000 seeds from NsVMV infected plants were germinated and found visually free from symptoms, indicating that the virus is if at all only to very low percentages seed-borne. Management of solanaceous weed plants containing the virus and serving as alternative hosts is critical in disease control. Intervention measures that involve barrier cropping with non-host crops could reduce the severity of the disease since many of the cultivated vegetables tested were not susceptible to the virus. Such measures might reduce losses associated with the virus thereby contributing significantly to poverty alleviation in Africa

Key words: *Plant viruses: host range: host resistance: seed transmission*

7.2 Introduction

Poverty and malnutrition continues to be a threat to many African countries. Africa indigenous vegetables could play an important role in alleviation of these challenges owing to their high nutritional value and higher prices in the market (Uusikua, 2010; Muhanji et al., 2011). African nightshades (*Solanum scabrum* and *Solanum villosum* (Solanaceae) are among the most popular indigenous vegetable species produced in Kenya. Leaves of African nightshades are a rich source of proteins, carbohydrates and vitamins. One of the factors limiting the production of African nightshades (*S. scabrum* and *S. villosum*), is the damage by insect pests (Schippers, 2000; Sithanantham *et al*., 2003). Aphids are particularly injurious to the African nightshades (Mbugua et al., 2006; Kimaru et al., 2015; Mureithi et al, unpublished). Among the aphids that have been observed to cause considerable damage to the crop are *A. fabae* and *Myzus persicae* (Kimaru et al., 2015; 4.4.3). Nightshade plants and leaves attacked by aphids show virus-like symptoms, which include: mottling, leaf rolling, folding and stunted growth which are often characteristic for potyvirus infection (Mureithi et.al, unpublished data).

Potyviruses have been reported to infect nightshades. Among the potyviruses infecting nightshades are pepper veinal mottle virus (PVMV) and chilli veinal mottle virus ChiVMV) (AVRDC, 2004; Shah et al., 2009). More recently, potyviruses were reported on African nightshades growing in western parts of Kenya (Wanjohi et al., 2015). A putative new potyvirus infecting African nightshades has also been characterized from samples collected from the nightshade plants growing in Kenya. The virus shows 70-75% sequence similarity to PVMV and ChiVMV, respectively, except for the P1 and P3 cistron suggesting it is a new virus species to the genus *Potyvirus*. The new virus is tentatively named as nightshade veinal mottle virus (NsVMV; Schimmel et al., 2015).

Potyviruses belong to the family *Potyviridae*. They are characterized by a ssRNA with a genome size of about 10 kb. They cause considerable crop losses all over the world on agricultural and horticultural crops (Ward and Shukla, 1991). Potyvirus infections manifest in various symptoms on the plants such as mosaic, mottling, ringspots, necrotic or chlorotic spots, stunting and yield losses (Shukla et al. 1994). Among the economically important plant families, potyviruses infect plants from the *Leguminosae*, *Solanaceae*, and *Cucurbitaceae* (www.ncbi.com 2013). Some potyviruses have a wide host range such as the watermelon mosaic virus whose host range including 23 plant families (Purcifull et al. 1984). PVMV has several hosts mainly from the Solanaceae family including pepper, African nightshades, and

Physalis angulata (AVRDC, 2004). A wide host range of NsVMV could pose a greater challenge in its management.

Host plant resistance and control of insect vectors are strategies used in management of plant viruses (Bragard et al., 2013). The plant may use several strategies such as inhibiting replication of the virus, prevent cell-to-cell movement, systemic infection or restricting an infection in the primary infected cell (Zaitlin and Hull, 1987). Studies done on potato showed that varieties that carry the resistance gene Rysto from the wild potato *Solanum stoloniferum* were not infected by several potyviruses such as potato virus Y (PVY), potato virus V (PVV), potato virus A (PVA) and tobacco etch virus (TEV) (Hinrichs et al., 1997). Presence of NsVMV resistance genes among African nightshades could contribute to the management of this potyvirus.

Among the methods for transmission of plant viruses is via seeds. Approximately 18 % of known plant viruses are transmissible by seed such as barley stripe mosaic virus (BSMV) and pea seedborne mosaic virus (PSbMV) (Johansen et al. 1994). Seed transmission can occur externally when the surface of the seed is contaminated with virus particles or internally when the virus is present in the embryo or the cotyledons. Transmission of viruses by seed enables the viruses to be carried over from one growing season to another (Shepherd, 1972). African nightshades are propagated using seeds. Seeds could therefore play an important role in spread of NsVMV if the virus is found seed transmissible.

To our knowledge, no study has been conducted so far to determine the occurrence, host range, host resistance and seed transmission of NsVMV in Kenya. Therefore, this study has been conducted to clarify the biology of NsVMV. The findings will be pivotal in developing management practices for NsVMV.

7.3 Methodology

7.3.1 Incidence of NsVMV in African nightshades

The survey was conducted between February 2015 to November 2016 in mid (1000- 1800 m) and high altitude (>1800 m) zones of Kenya for 4 seasons. The mid altitude locations were in Yatta (Machakos County) and KALRO-Kandara (Muranga County) while the high altitude location was in KALRO-Tigoni (Kiambu County). The first and the third season were generally dry with rains being witnessed in the month of April. The second and the fourth season were wet, with rains of moderate intensity.

Plots measuring 10 by 10 m were prepared and planted with African nightshades. In the first season (February - May 2015) three nightshade plots were established with *Solanum scabrum*

at each of the two sites, Yatta and Tigoni. In the second (August – November 2015), third (February - May 2016) and fourth (August - November 2016) seasons, 10 nightshade plots (five plots of *S. scabrum* and 5 plots of *S. villosum)* were planted at each site in KALRO-Kandara and KALRO-Tigoni. Normal agronomic practices such as weeding and irrigation were done until visible viral symptoms were seen on the plants. However, in the fourth season, data were not collected in 4 plots at KALRO-Kandara as all the leaves of the nightshade plants were eaten by birds that visited the site.

Twenty nightshade plants/plot were used to score for the virus disease incidence per plot. To achieve this, each plot was divided into 4 quadrants and 5 nightshade plants/quadrant were randomly selected to determine the incidence of NsVMV. The percentage virus incidence was calculated in each plot by counting the number of symptomatic plants divided by the total number of nightshades plants sampled (20 plants) multiplied by 100.

(Disease incidence (%) = Number of diseased plants /Total number of plants examined × 100).

After scoring for viral disease incidence, leaf samples from 10 symptomatic nightshade plants across the plot whether infested or not infested with aphids were obtained for laboratory testing of NsVMV. In instances where no symptoms were observed on all nightshade plants in the plot, leaf samples were collected from asymptomatic plants that were infested with aphids. For each plant sampled, 3 young symptomatic/asypmtomatic leaves were plucked and put in zip-lock polythene bags before keeping them in a cooler box for transportation into the laboratory. The leaf samples were preserved in a refrigerator at -180°C. The samples were tested at the virology laboratory at ICIPE. Some samples were also kept in falcon tubes containing calcium chloride and transported to the virology laboratory at Leibniz University Hannover Germany for further analysis. The plants in which the leaf samples were picked were tagged for the purpose of harvesting their seeds to test seed transmission of NsVMV.

Samples were tested using DAS-ELISA with a PVMV antiserum (DSMZ; AS0123), which was found to react with NsVMV. The ELISA was conducted according to the manufactures recommendations. Briefly, the microtiter wells were coated with 100 µl of the first antibody with a dilution 1:500 in coating buffer followed by incubation overnight at 4 °C. The microtiter wells were washed 3 times with washing buffer (PBS-Tween). This was done by rinsing each well with the wash buffer and leaving the plate for 3 minutes before emptying it to remove excess buffer. The plant samples were grinded in 600 µl sample extraction buffer (PBS-TPO) in a 1.5 ml Eppendorf tube with a mini-pistil. A sample from a healthy nightshade plant was used as a negative control while a sample from an infected plant raised

in the greenhouse was used as a positive control. The sap was centrifuged for 3 min at maximum speed in a bench top centrifuge. 100 µl of the supernatant was loaded into a well and incubated for 4 h at 37 °C. Each sample was tested in 3 wells. The plate was washed 3 times with washing buffer (PBS-Tween). To each loaded well 100 µl of the second antibody prepared in a dilution of 1:500 with sample extraction buffer (PBS-TPO) was added and the plate incubated overnight at 4 °C. After three times washing of the plate the substrate was prepared at a ratio of 1 µg per ml substrate buffer and each well was loaded with 100 µl. The plate was placed on a shaker until a colour change in the wells was observed followed by measuring the absorbance at 405 nm. A sample was considered positive when its absorbance value was higher than two times the mean value of the healthy control.

7.3.2 Host range determination

To determine the host range of NsVMV, a survey of possible hosts in the field and greenhouse experiment with selected plant species was performed. For the field survey, leaf samples showing/not showing viral symptoms and/or infested with aphids from crops and weed species growing in and around the nightshade fields at KALRO-Tigoni (2114 m; 01° 08.9587'S; 036° 41.0171'E), KALRO-Kandara (1508 m; 01° 00.1617'S; 037° 04.715'E) and ICIPE-Duduville (1599 m; 01° 13.214'S; 036° 53.444'E) campus were collected and tested. Samples from *Amaranthus dubious*, *Solanum lycopersicon*, *Eupatorium sp*, *Leonotis leonurus, Datura stramonium*, *Commelina benghalensis* and *Nicandra sp* were obtained from KALRO-Tigoni and KALRO-Kandara. Additionally, samples from African nightshades plants at the experimental fields at ICIPE-Duduville campus and other neighbouring crops such as *Amaranthus cruentus*, *Cucumis sativus*, *Vigna unguiculata* and *Fragaria* × *ananassa* were also collected. For each of the plant species sampled, 3 young leaves were collected. The samples were tested using DAS-ELISA for the presence of NsVMV.

In the greenhouse experiment, seeds from seven solanaceous plants (*Capsicum annum, Solanum lycopersicon, Solanum tuberosum*, *Solanum melongena, Physalis angulata, Nicotiana occidentalis, Nicotiana occidentalis* subsp. hesperis, *Nicotiana debneyi*, *Nicotiana tabacum* cv. Samsun), 3 Asteraceae (*Conyza sp, Lactuca sp*, and *Bidens pilosa*), two Chenopodiaceae (*Chenopodium murale* and *Chenopodium nubrum*), one Amaranthaceae (*Amaranthus dubious*), one Lamiaceae (*Leonotis leonurus*), and one Cleomanaceae (*Cleome gynandra*) were sown in the nursery. Three weeks old seedlings were transplanted into pots and after one week, the seedlings were mechanically inoculated with NsVMV. The mechanical inoculation of nightshade plants was done with approximately 1 g of NsVMV

infected *Nicotiana benthamiana* leaf material grinded in 5 ml of 0.1 M potassium-sodium phosphate buffer with a spatula tip of both celite and activated charcoal. Fingers were dipped into the sap and then rubbed gently on the mid and upper leaves of the healthy plants. After inoculation the plants were rinsed with clean water to remove excess celite and charcoal. The plants were kept in an insect proof greenhouse for 2-3 weeks to observe symptom development before being collected and tested for the presence of NsVMV using DAS-ELISA.

7.3.3 Host resistance of nightshade species/lines to NsVMV

Various germplasms of nightshade varieties/lines/landraces were tested for resistance to NsVMV in a greenhouse trial. The candidates tested were; five *Solanum scabrum* varieties (Kenya seed, Abuk 1, Abuk 2, Olevolosi, RV1), two *Solanum villosum* (Kenya seed, BG), as well as *Solanum americanum, Solanum sarachoides,* and Solanum *tarderemotum.* The seeds of the candidate nightshade lines/species were sown in plastic trays and raised in the nursery for 4 weeks. After 4 weeks, the seedlings were transplanted into plastic pots and hardened off in the nursery for 1 week. The seedlings were mechanically inoculated with NsVMV and grown for 3 weeks to allow symptom development before being tested for NsVMV using DAS-ELISA.

7.3.4 Transmission of NsVMV by seeds and A. fabae

During the third season, nightshade plants in plots showing severe symptoms of NsVMV were tagged for the purpose of collecting their seeds upon maturity. Five plants/plot at KALRO-Tigoni and KALRO- Kandara were tagged for the study. When fruits from the five tagged plants/plot matured from these plants they were harvested and crushed together in order to obtain one seed sample/plot. The extracted seeds were rinsed in fresh water to remove the fruit sap attached to the seeds and dried on top of a bench in the laboratory. The dry seeds were tested for the presence of NsVMV using the standard germination test and grow-out method. The standard germination method tests the potential of the virus to affect the germination of the seed while the grow-out method tests the potential of the virus to be transmitted to the resulting seedling. In the seed standard germination method, 100 seeds from each seed sample were placed in a petri dish lined up with a moistened filter paper. The petridish was covered with a petri dish cap and placed in a germination chamber. Three replicates were performed for each seed sample. A negative control of seeds obtained from a healthy plant was also included in the test.

The climatic conditions in the germination chamber were set as 28^0C±2 and 85% RH. Seeds were considered to have germinated when the seeds produced the radical. The number of seeds germinating were counted, recorded and removed from the petri dish on a daily basis starting from the 3rd day up to the 14th day. During the daily scoring for the germinated seeds, the filter paper was moistened with distilled water to prevent the seeds from drying up. Thereafter, the germination percentage was calculated at the end of the trial. The formula for calculating germination percentage was: (Total number of seeds germinated per petri dish/100) x 100. In the grow-out method, 100 seeds from each seed sample were sown using sterile growing media. The seeds were grown in an insect proof greenhouse. The plants were grown for one month before leaf samples were collected and tested for NsVMV using DAS-ELISA.

Transmission of NsVMV by *A. gossypii* was also tested. In this experiment, *A. gossypii* were reared in a cage using cucumber plants as the host. The temperature in the rearing room was 23˚C. For use in the experiment, the aphids were taken from the cucumber leaf with a fine brush, put in a petri dish and starved for one hour. They were then transferred to a *Nicotiana benthemiana* plant infected with NsVMV and left to feed/probe the plant for 2 minutes in order to acquire the virus. Five aphids were then transferred to an African nightshade seedling and allowed to feed for 24 hrs. The aphids were finally removed and plants left to continue growing in a climate chamber at 23˚C and 65% RH. Leaf samples were then collected from the African nightshade plants after 3 weeks and tested for presence of NsVMV using DAS-ELISA. The experiment was replicated six times.

7.4 Results

7.4.1 Incidence of NsVMV on African nightshades

The symptoms of NsVMV were present on African nightshades in all the experimental sites. Yatta site had the lowest disease incidence while KALRO-Tigoni had the highest disease incidence. At KALRO-Tigoni, the disease incidence increased with time as African nightshades were planted in the subsequent seasons after the first season. A similar scenario was observed at KALRO-Kandara up to the third season, after which the disease incidence declined slightly (Fig 7-1).

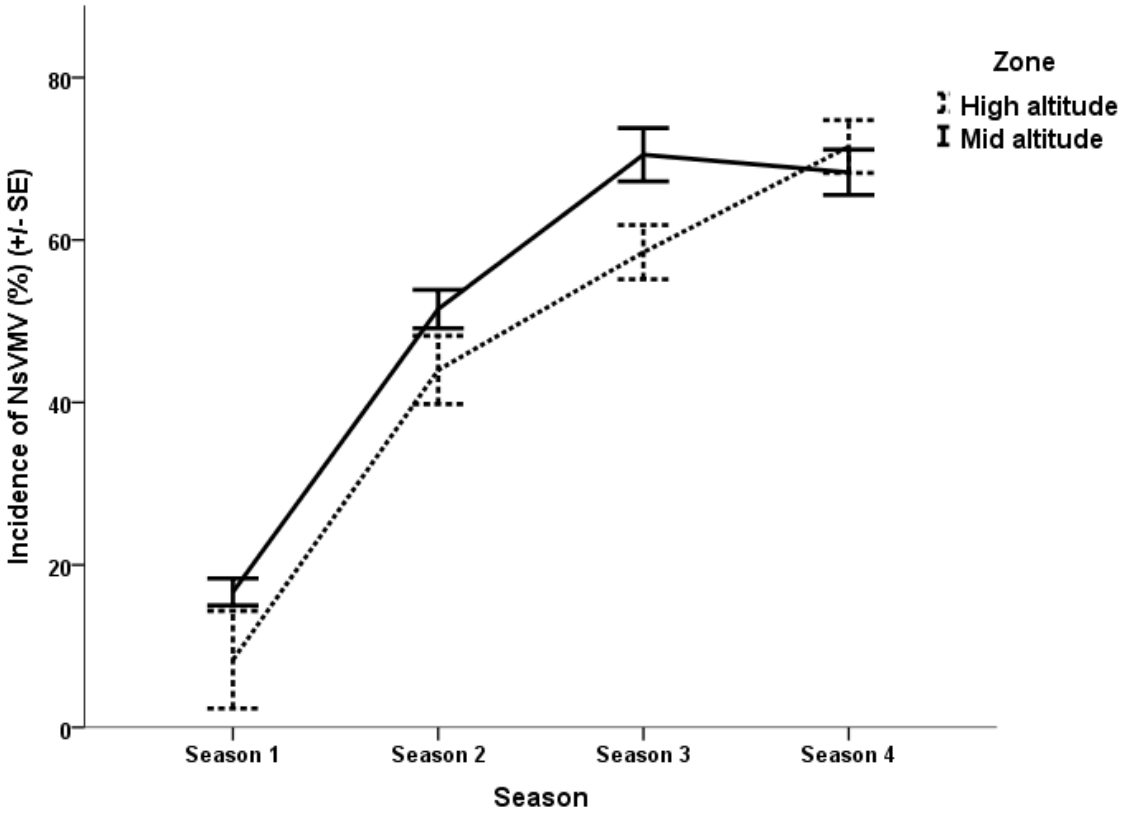

Fig 7-1: Occurrence of NsVMV on African nightshades in nightshade production sites in Kenya. Leaf samples were collected from African nightshade plants in each plot. 20 plants were sampled per plot. The mid altitude sites were located at KALRO-Kandara and Yatta while the high altitude site was situated in KALRO-Tigoni. Leaf samples were tested for presence of NsVMV using DAS-ELISA

When the abundance of aphids on African nightshades was considered at KALRO-Tigoni, it rose and fluctuated through the seasons with the highest abundance observed in the third season. At KALRO-Kandara, the abundance of aphids was highest in the second season and lowest in the third season (Fig 7-2).

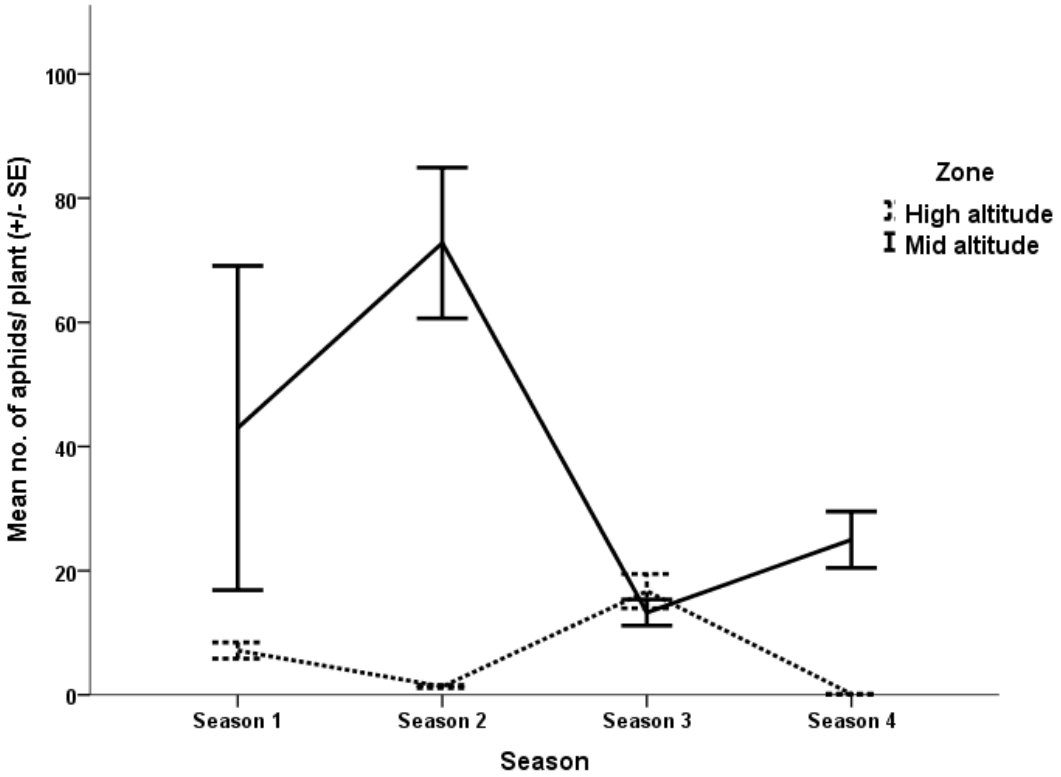

Fig 7-2: Mean number of aphids/plant on African nightshades in nightshade research sites in Kenya. The mid altitude sites were KALRO-Kandara while the high altitude site was situated at KALRO-Tigoni The number of aphids/plant were scored in 40 African nightshade plants per plot.

When serological tests were conducted from symptomatic leaf tissues collected from the nightshade plants at Yatta and at KALRO-Tigoni in season 1 and 2, they gave a negative result for NsVMV. However, samples from the symptomatic plants collected at KALRO-Tigoni during the third and fourth season turned positive for NsVMV. Symptomatic nightshade samples collected from KALRO-Kandara in the three seasons were positive for NsVMV (Table 7-1).

Table 7-1: Incidence of NsVMV on African nightshades in research sites in Kenya. Leaf samples were collected from experimental fields at the three sites (Yatta, KALRO-Tigoni, and KALRO-Kandara) in different seasons. The field sites were located in the mid altitude zone (Yatta and KALRO-Kandara) and high altitude zones (KALRO-Tigoni) of Kenya and tested for presence of NsVMV using DAS-ELISA.

Research site	**Season**	**Remarks**
KALRO-Kandara	2	23 % of samples were +ve for NsVMV
KALRO-Kandara	3	67 % of samples were +ve for NsVMV
KALRO-Kandara	4	60 % of the samples were +ve for NsVMV
KALRO-Tigoni	1	All samples were -ve for NsVMV
KALRO-Tigoni	2	All samples were -ve for NsVMV
KALRO-Tigoni	3	All samples were +ve for NsVMV
KALRO-Tigoni	4	91.7 % of samples were +ve for NsVMV
Yatta	1	All samples were -ve for NsVMV

7.4.2 Host range of NsVMV

ELISA tests show that *Solanum lycopersicum*, at ICIPE-Duduville campus was infected with NsVMV. Moreover, a weed species present at KALRO-Kandara and KALRO-Tigoni field sites, *Nicandra sp*, was also infected with NsVMV. However, all other crops tested which included *Amaranthus sp*, *Solanum tuberosum*, *Vigna unguiculata*, *Cucumis sativus*, and *Fragaria* × *ananassa* were not infected with NsVMV. Moreover, the other weed species present in the trial sites such as *Leonatis sp, Euphobia sp, Commelina benghalensis, Bidens pilosa, Datura stramonium* and *Eupatorium sp* were not infected with the NsVMV. This was despite some of the plant species such as *Amaranthus sp, Vigna unguiculata*, and *Leonatis sp.* showing viral symptoms / being infested with aphids (Table 7-2).

Table 7-2: Natural occurrence of NsVMV on other crops planted in close proximity with the African nightshade and weed species growing naturally around the nightshade fields at the experimental fields in the three sites (KALRO-Tigoni, KALRO-Kandara and ICIPE-Duduville campus). The field sites were located in the mid altitude zone (KALRO-Kandara and ICIPE-Duduville) and high altitude zones (KALRO-Tigoni) of Kenya. Samples were tested for presence of NsVMV using DAS-ELISA

Common Name	**Scientific Name**	**Plant Family**	**Location**	**Serological test**
Tomato	*Solanum esculentus*	Solanaceae	ICIPE-Duduville	+ve
Apple of Peru	*Nicandra sp*	Solanaceae	KALRO-Tigoni/ KALRO-Kandara	+ve
Potato	*Solanum tuberosum*	Solanaceae	KALRO-Tigoni	-ve
Amaranth	*Amaranthus cruentes/ Amaranthus dubious*	Amaranthaceae	KALRO-Tigoni/ KALRO-Kandara	-ve
Cowpea	*Vigna unguiculata*	Fabaceae	ICIPE-Duduville	-ve
Cucumber	*Cucumis sativus*	Cucurbitaceae	ICIPE-Duduville	-ve
Strawberry	*Fragaria x ananassa*	Rosaceae	ICIPE-Duduville	-ve
Lions tail	*Leonotis leonurus*	Lamiaceae	KALRO-Tigoni/ KALRO-Kandara	-ve
	Eupatorium sp	Asteraceae	KALRO-Tigoni	-ve
Datura	*Datura stramonium*	Solanaceae	KALRO-Tigoni	-ve
	Euphobia sp	Euphobiaceae	KALRO-Kandara	-ve
Wondering jew	*Commelina benghalensis*	Commelinaceae	KALRO-Kandara	-ve

Keys: +ve = positive for NsVMV; -ve = Negative for NsVMV; NsVMV – Nightshade veinal mottle virus (genus Potyvirus. Family Potyviridae)

Four out of the fifteen host species experimentally inoculated developed symptoms on their leaves and tested positive for NsVMV. These *include Solanum lycopersicon, Nicotiana occidentalis cv.* hesperis, *Nicotiana debneyi, Nicotiana tabacum cv.* samsun. The NsVMV positive plants were all from the family *Solanaceae*. However, other solanaceous plants such as eggplant, pepper, and potato neither showed symptoms nor tested positive for NsVMV. Furthermore, none of the weeds species and crops from the other plant families tested positive indicating that the virus is hosted only by plants from the family *Solanaceae* (Table 7-3).

Table 7-3: Experimental host range of NsVMV on crops and weed species done through mechanical inoculation. Young plants, 3 weeks old, were mechanically inoculated with NsVMV viroins and tested for NsVMV using DAS-ELISA

Common Name	**Scientific Name**	**Plant Family**	**Symptoms**	**Serological test**
Tomato	*Lycopersicon esculentus*	Solanaceae	C, S, M	+ve
Tobacco	*Nicotiana benthemiana*	Solanaceae	C,S, M,	+ve
Tobacco	*Nicotiana occidentalis cv. hesperis*	Solanaceae	N, C, S	+ve
Tobacco	*Nicotiana debneyi*	Solanaceae	N, C	+ve
Tobacco	*Nicotiana tabacum cv. samsun*	Solanaceae	N, C	+ve
Pepper	*Capsicum sp*	Solanaceae	---	-ve
Potato	*Solanum tuberosum*	Solanaceae	---	-ve
Eggplant	*Solanum melongena*	Solanaceae	---	-ve
Amaranth	*Amaranthus dubious*	Amaranthaceae	---	-ve
Spiderplant	*Cleome gynandra*	Cleomaceae	---	-ve
Horseweed	*Conyza sp*	Asteraceae	---	-ve
Milk thistle	*Lactuca sp*	Asteraceae	C	-ve
Lions tail	*Leonotis leonurus*	Lamiaceae	---	-ve
Blackjack	*Bidens pilosa*	Asteraceae	---	-ve
Nettleleaf goosefoot	*Chenopodium murale*	Chenopodiaceae	---	-ve
Red goosefoot	*Chenopodium nubrum*	Chenopodiaceae	---	-ve

Keys: C = Chlorosis; S = Systemic; N = Necrotic lesion; M = Leaf molting; --- = No symptoms; NsVMV – Nightshade veinal mottle virus (genus Potyvirus. Family Potyviridae)

7.4.3 Host resistance of nightshade species/lines to NsVMV

All the nightshade species tested in this study, i.e. *Solanum scabrum*, *Solanum villosum*, *Solanum americanum*, *Solanum sarachoides* and Solanum *tarderemotum* were susceptible to NsVMV. They developed typical symptoms of NsVMV like interveinal chlorosis and leaf mottling. The symptoms were systemic affecting both the younger and the older leaves. They all turned positive in serological tests.

7.4.4 Transmission of NsVMV by seeds and A. gossypii

The germination of African nightshades seeds was not significantly affected by the presence of NsVMV on the mother plants ($F_{2,27} = 2.48$; $P = 0.103$). Germination percentage of seeds obtained from infected African nightshade plants from KALRO-Tigoni was 85.67±1.61%, while that from KALRO-Kandara was 86.08±2.02%. Seeds obtained from the healthy African nightshade plants had a seed germination of 94.33±1.45%. In the grow-out test, the seedlings did not show any symptoms of NsVMV. Furthermore, the serological test done using DAS-ELISA gave negative results for NsVMV indicating the seeds were free from NsVMV. Moreover, no symptoms were observed on the African nightshade seedlings exposed to *A. gossypii* and the DAS-ELISA test turned negative for these plants.

7.5 Discussion

After harvesting the African nightshades, farmers normally sort and discard the diseased leaves (deformed, curled, mottled, and chlorotic) before taking the product to the market since they are a sign of poor quality of the vegetable. The NsVMV presents itself with similar symptoms on the nightshade leaves. Therefore, the farmers incur yield losses due to infection of their African nightshades by NsVMV. Wanjohi et al., 2015 had reported incidences of potyviruses infecting African nightshades in the western parts of Kenya. However, the specificity of the potyviruses and other biological properties of these viruses had not been documented. To our knowledge, this is the first study to investigate the biological properties of NsVMV.

In our study, we observed that the incidence of NsVMV increased with time in the African nightshade fields. The increase of NsVMV incidence with time particularly in the high altitude zone could have resulted from cooler and humid weather conditions in that area and availability of alternative hosts in the research sites. In a similar study done in Nigeria, the incidence and severity of PVMV was higher in derived savanna and humid forest agro-ecological zones (Fajinmi, 2010). These regions were characterized by warm and humid climate that not only favoured rapid multiplication of the virus and the aphid vector but also supported abundant growth of alternative hosts for the virus (Fajinmi 2006; Fajinmi, 2010). In a related study, Dahal et al. (1997) noted that availability of alternative volunteer/wild hosts which serve as reservoirs of papaya ringspot potyvirus (PRSV) and presence of aphid vectors lead to the high incidence of the disease in Nepal. During our study, there were higher amounts of rainfall experienced in the second, third and fourth season compared to the first season that lead to high proliferation of weed species particularly *Nicandra sp* and wild African nightshades. These are alternative hosts for NsVMV and might have acted as reservoir for the NsVMV and later becoming new sources of NsVMV inoculum in the subsequent seasons. Bosque-Pe´rez and Buddenhagen (1990) also reported that weeds are important sources of new virus infection to the cultivated crop in their study on epidemiology of virus diseases of chickpea.

Aphids are important vectors of plant viruses and nearly half of all plant viruses vectored by invertebrates are transmitted by aphids, majority of which in a non persistent manner (Perring et al., 1999; Hull, 2002). Previous studies have reported the occurrence of aphids on African nightshades in Kenya (Mbugua et al., 2006; Ashilenje *et al*., 2011*;* Kimaru et al., 2015). In our study, we observed aphids, particularly *A. fabae* and *M. persicae* on African nightshades infected with NsVMV. These aphids might have played a role in transmission of NsVMV

even in instances where the aphid infestation was low. Therefore, it was necessary to confirm the role of *A. fabae* in transmission of NsVMV. Schimmel et al. (unpublished) observed transmission of NsVMV by *M. persicae*. However, in our study, transmission of NsVMV by *A. gossypii* was not observed indicating that the virus is not transmissible by *A. gossypii*. However, there were challenges in execution of this experiment as *A. gossypii* died shortly after transferring them to the healthy nightshade plants after being exposed to the infected *N. benthemiana* plants. The use of different host plants for *A. gossypii* in this experiment could have affected the results in our study. *A. gossypii* was mass reared on cucumber plants, before being offered *N. benthemiana* infected with NsVMV, and later offered the healthy African nightshade plants. *A. gossypii* could have rejected to feed on the African nightshades and hence fail to transmit the virus. The aphids could also be allowed more time to settle and aquire the virus from the infected plants before being transferred to the healthy plants for virus transmission. The study on transmission of NsVMV could also be repeated using *N. benthemiana* only in the virus acquisition and inoculation steps.

From our study, there is a strong indication that NsVMV is restricted to a few crops and weeds from the family *Solanaceae*. Apart from tomato and tobacco, no other cultivated solanaceous crop grown in close proximity to African nightshades was infected with the virus. Moreover, other indigenous and exotic vegetables usually cultivated in mixed cropping system together with African nightshades by small scale farmers were not infected by NsVMV. Since NsVMV belongs to the potyvirus family, a group of viruses that are transmitted in a non-persistent manner by insect vectors, biological control of the aphid vectors may not effectively control this virus (Irwin, 1999). A barrier cropping system whereby other indigenous vegetables which are not host of NsVMV are grown around a nightshade crop might be an effective strategy for the management of this disease. The aphid vectors coming to infest African nightshades will lose their virus transmission ability when they probe the other indigenous vegetables planted as barrier crops around the nightshade crop before they reach the nightshade crop. The use of barrier cropping system has been reported to reduce the incidences and spread of non persistent viruses vectored by aphids (Fereres, 2000). For instance, studies by Difonzo et al. (1996) noted decreased incidences of potato virus Y on seed potato that was planted using the barrier cropping system with sorghum, wheat or soybean. This could be practical NsVMV management option for the small scale farmers since no resistant nightshade species/lines were identified in our study. Furthermore, the non-host vegetables are also popular with the consumers who in most cases purchase more than one type of AIVs at the same time. This way, the farmer could not only

manage the disease but also satisfy the consumer needs for AIVs when using the barrier cropping system. However, studies on the use of barrier cropping system for the management of NsVMV in African nightshades need to be undertaken.

An important aspect in effective management of NsVMV could involve management of weeds particularly *Nicandra sp* and wild nightshades in and around the African nightshade farms. Farmers should be particularly watchful on the borders of the nightshade crop and on the hedges where the weed grows. Most farmers tend to ignore such areas, which serve as potential reservoir of the virus when the older crop is uprooted at the end of the season and the land is being prepared for a new nightshade crop.

Our study did not show that NsVMV is seed transmissible. Therefore, the spread of the NsVMV from one nightshade plant to another during the growing season or from one season to another is unlikely to be through the seed. This is particularly good news for the farmers who normally rely on recycling seeds from previous season African nightshades crop for the subsequent season. However, in our study we tested seed transmission using the grow-out method and the germination test, which are indirect methods for testing seed transmission. Future studies are necessary for testing the presence of the virus in the seeds using direct method on the seeds themselves from mechanically inoculated nightshades. This is important before reaching to the conclusion on seed transmission as previous studies have shown that seed transmission incidences as low as 0.1% in lettuce mosaic virus coupled by efficient vector transmitter can lead to high epidemiology of the virus in the crop (Dinant and Lot, 1992).

7.6 References

Ashilenje DS, Omunyin ME, Okalebo JR. 2011. Influence of potassium on aphid incidence and yield of vegetable African nightshades (*Solanum* L. section *Solanum*). *Annals of Biological Research* **2 (3)**:317-326

Bosque-Pe´rez NA, Buddenhagen IW. 1990. Studies on epidemiology of virus disease of chickpea in California. *Phytopathology* **74**:372–378

Dahal G, Lecoq H, Albrechtsen SE. 1997. Occurrence of papaya ringspot potyvirus and cucurbit viruses in Nepal**.** *Ann. appl. Biol.* **130**:491-502

Difonzo CD, Ragsdale DW, Radcliffe EB, Gudmestad NC, Secor GA, Ebesu RH. 1996. Crop borders reduce potato virus Y incidence in seed potato. Ann. Appl. Biol. **129**: 289–302

Dinant S, Lot H. 1992. Lettuce mosaic virus. *Plant Pathology*. **41:** 528—542

Fajinmi AA. 2006. The incidence, spread and possible control strategies of Pepper veinal mottle Potyvirus (PVMV) disease on pepper (*Capsicum annuum.L*.) in Nigeria. Ph. D thesis, University of Ibadan Oyo State Nigeria.

Fajinmi AA. 2010. Agro-ecological incidence and severity of Pepper veinal mottle virus, genus Potyvirus, family Potyviridae, on cultivated pepper (*Capsicum annuum* L.) in Nigeria. *Julius-Kühn-Archiv* **427**: 314-322

Fereres A. 2000. Barrier crops as a cultural control measure of nonpersistently transmitted aphid-borne viruses. *Virus Res.* **71**: 221–231.

Hinrichs J, Berger S, Buchenauer H. 1997. Susceptibility of potato cultivars to the N and O strain of potato virus Y (PVY) and other potyviruses. *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz.* **104**: 193-199.

Hull R. 2002. Matthews' Plant Virology. Academic Press, San Diego, California, p. 1001.

Irwin ME. 1999. Implications of movement in developing and deploying integrated pest management strategies. *Agric. For. Meteorol*. **97**: 235–248.

Kimaru SL, Onyango CM, Kimenju JW, Kilalo DC. 2015. Potential of intercropping for management of some arthropod and nematode pests of leafy vegetables in Kenya. *Journal of Agricultural Sciences* **60 (3)**: 301-314

Mbugua G, Gitonga LN, Mureithi E, Karoga J, Manyeki L. 2006. Farmer-Participatory prioritization and development of agronomic practices for African Leafy Vegetables. Proceedings of 6th Horticultural Seminar on Sustainable Horticultural production in the Tropics held in Njoro, Kenya.

Muhanji G, Roothaert RL, Webo C, Mwangi S. 2011. African indigenous vegetable enterprises and market access for small-scale farmers in East Africa, *International Journal of Agricultural Sustainability* **9:(1)**: 194-202

Perring TM, Gruenhagen NM, Farrar CA. 1999. Management of plant viral diseases through chemical control of insect vectors. *Annu. Rev. Entomol.* **44**: 457–481.

Purcifull D, Hiebert E, Edwardson J. 1984. Watermelon mosaic virus 2. *CMI/AAB Descr*. **293**: 7

Schimmel J, Büttner C, Meyhöfer R, Maiß E. 2016. Nachweis eines neuen Potyvirus: Night shade veinal mottle virus (NSVMV) aus *Solanum nigrum* und *Nicotiana benthamiana*. *Phytomedizin Jahrgang* **46 (2)**: 6

Schippers RR. 2000**.** African Indigenous Vegetables. An Overview of the Cultivated Species. Natural Resources Institute/ACP-EU Technical Centre for Agricultural and Rural Cooperation, Chatham, UK. Pp 214.

Shah H, Yasmin T, Fahim M, Hameed S, Haque MI. 2009. Prevalence, occurrence and distribution of Chili veinal mottle virus in Pakistan. *Pakistan Journal of Botany* **41**: 955-965.

Shukla DD, Ward CW, Brunt AA. 1994. The Potyviridae. Cambridge: CAB International

Uusiku NP, Oelofsea A, Duodub KG, Besterc MJ, Faberd M. 2010. Nutritional value of leafy vegetables of sub-Saharan Africa and their potential contribution to human health: A review. *Journal of Food Composition and Analysis* **23**: 499–509.

Ward CW, Shukla DD. 1991. Taxonomy of Potyviruses-current problems and some solutions. *Intervirology* **32**: 269–296.

Zailtin M, Hull R. 1987. Plant virus-host interactions. *Annual Review of Plant Physiology* **38**: 291-315.

8 General discussion

The role of African Indigenous vegetables (AIVs) in combating malnutrition and fighting poverty cannot be overemphasized. Amaranth and African nightshade being among the most popular AIVs in Kenya play an important nutritional role of providing iron, calcium, potassium, and vitamins A and C to diets of many households in Africa (IPGRI, 2003; Uusikua, 2010; Amicarelli and Camaggio, 2012; Kamga et al., 2013). These vegetables have also been reported to have therapeutic against chronic diseases such as cancers and HIV/AIDS (Abukutsa-Onyango, 2007; Sreelatha et al., 2012). Attack of amaranth and nightshades by arthropod pests is among the challenges growers face in production of this crops.

This study intended to determine the biodiversity, abundance, distribution, and damage of arthropod pests infesting amaranth and African nightshades in the major production areas in Kenya and the natural enemies associated with these pests. This was meant to answer the salient question of the main pests of these vegetables and the natural enemy complex present in the fields to which future research should focus on. After identifying the major pests of African nightshades, the study aimed to find out the effect of seasonality and crop phenology on the abundances of the major pests of African nightshades. The study also investigated the host range of the major pests infesting African nightshades. It further aimed at testing the performance of parasitoid *A. colemani* in management of *A. fabae*, the most abundant aphid species during our study. Information on seasonality, crop phenology, host range and acceptability and suitability of *A. fabae* and *M. persicae* by parasitoid *A. colemani* would be useful in future development of IPM programs for management of the major pests of African nightshades. Finally, this study intended to provide information on the biology of NsVMV, a new virus found to infect African nightshades in Kenya. Understanding the biology of NsVMV will guide in developing effective management practices for it to limit its damage on the African nightshades.

In **chapter 3**, 188 amaranth farms in low, mid and high altitudinal zones of Kenya were surveyed. We found out that amaranth crop in Kenya is attacked by different types of insects. However, the Lepidopterans were the most damaging. Moreover, *Spoladea recurvalis* and *Epicauta albovittata* were found as the major pests of amaranth in the rainy and dry seasons respectively. Although previous studies had also observed higher abundance of *S. recurvalis* in the rainy season (National Research Council, 1984), other reports did not observe significant difference in the abundance of the pest between the dry and the wet season

(Aderolu et al., 2013) suggesting that there might be other factors which determine the abundance of this pest. High abundance of food resources could also lead to an increase of population of this pest as the pest is reported to play host to weed species wild amaranth, *Amaranthus* spp., and the devils horsewhip A*chyranthes aspera* (Kahuthia-Gathu., 2011) which may be abundant during the rainy season. However, this aspects need to be investigated in detail. Nonetheless, farmers of amaranth need to consider these weed species in their management practices for *S. recurvalis*. To our knowledge, this is the first study to report the occurrence of four new pests on amaranth in Kenya i.e. *E. albovittata*, *P. atritermina*, *T. absoluta* and *A. octogueae*. The occurrence of these pests on amaranth crop, majority of which are Lepidopterans indicate that production of amaranth in Kenya is faced with new pest challenge and research should be geared towards seeking ways of managing these pests before they cause huge damage to the crop.

We also profiled the pests of African nightshades in Kenya and their natural enemies in **Chapter 4** during the countrywide survey. Homopterans (mainly aphid species *A. fabae*, *M. persicae* and *A. craccivora*), Coleopterans (flea beetle species *E. silvicola* and *Phyllotreta* sp.) were the most damaging insects on this crop. Several natural enemies for the nightshade pests were also observed among them the parasitoid *A. colemani* which we carried out experiments with in this study (see chapter 6). The highest diversity of African nightshade pests was in the mid altitude zone and during the dry season. Abundance of food resources and minimal human interference with the natural vegetation are factors that lead to increased biodiversity of insect communities (Lawton et al., 1987; Wold, 1987). The warm climate and the abundance of food resources in the mid altitude zone could have had a positive effect on the diversity of insect species in that zone. The frequent application of insecticides by farmers in the high altitude zone could have lowered the diversity of insect species in that region.

During the countrywide survey for the pests of African nightshades and associated natural enemies in chapter 4, we could not study in details the population abundances of the major pests of African nightshades since the area covered during the survey was quite large. Moreover, we did not have much control on the agronomic practices, particularly the crop protection measures the farmers used to manage pests on the amaranth and nightshade crops which could have interfered with our results. In **chapter 5**, we therefore set-up our own field experiments where we planted African nightshades in two agro-ecological zones (mid and high), and monitored closely the natural colonization patterns of *A. fabae*, *E. silvicola* and Lepidopteran pests (*Spodoptera exigua*, *S. littoralis*, *Tuta absoluta* and *Plusia* sp). During the same trial, we also studied the seasonal abundances of parasitoid *A. colemani*. We did not

apply any crop protection measures during the whole trial period in the year 2015 and 2016. Higher abundances of *A. fabae* was observed in the mid attitude zone compared to high altitude zone. Moreover, whereas the highest abundance of *A. fabae* was observed in the 2nd growing season at the mid altitude zone, highest abundance was observed in the 3rd growing season in the high altitude zone. Our findings suggest that nightshade farmers at the mid altitude zone would require to put more effort in management of *A. fabae* compared to the high altitude farmers. Conversely, higher abundance of *E. silvicola* were observed in the high altitude zone compared to the mid altitude zone. *E. silvicola* could have been outcompeted by A. fabae at the mid altitude zone as the infestation by *A. fabae* in the mid altitude zone was high.

Moreover, colonization by *E. silvicola* occurs when the crop is young and continues throughout the other stages of crop phenology. This is unlike in the mid altitude zone where the abundance of *E. silvicola* is mainly at the later stages of crop development.

Lamb (1983) reported that *Phyllotreta* flea beetles are strong flyers and have the potential to migrate to canola crop regardless of the proximity between the overwintering sites and the canola fields. In our study, it might be that the flea beetle *E. silvicola* was able to migrate from the mid altitude zone to the high altitude zone particularly in the early stages of crop development possibly because of the lower temperatures that characterized the high altitude zone. In their study, Mani and Pal (2013) also observed that okra was colonized by the flea beetle *Nisotra chrysomeloides* from the seedling stage and infestation continued throughout the crop phenology. Therefore as the farmers in the mid altitude have to put more emphasis in management of *A. fabae*, the farmers in the high altitude need to put more effort in the management of *E. silvicola*. Farmers in the mid altitude zone could therefore lower the incidences of *E. silvicola* by removing the older nightshade crop from their farms. The use of entomopathogenic nematodes (EPNs) has been reported as a promising strategy in the management of flea beetles. In laboratory trials with adult flea beetles, Trdan et al., (2008) reported at least 74% mortality of the flea beetles with EPNs *Heterorhabditis bacteriophora, Steinernema feltiae,* and *S. carpocapsae* at 25˚C. Moreover, Xu et al., (2010) reported high efficacy of EPNs under field conditions. Therefore studies on the use of these EPNs for the management of flea beetle E. silvicola in African nightshades should be explored. Zero tillage has also been shown to be an effective tool in management of flea beetles on canola as the cool and moist soil conditions are less favourable for the survival of the flea beetles (Dosdall et al., 1999). African nightshade farmers particularly in the high altitude zone could

practice this method in order to lover the infestation of their nightshade crop by the flea beetles.

We also found that some common weed species growing in or around the nightshade farms such as *Bidens pilosa*, *Euphobia* sp., *Leonotis leonurus*, and *Eupatorium* sp are alternative hosts for *A. fabae*. Moreover, wild nightshades growing on the hedges bordering the nightshade crop and cultivated potato and amaranth neighbouring nightshades plots were important alternative hosts for *E. silvicola*. Cultivated amaranth grown adjacent to the nightshades also hosted *Spodoptera exigua*, *S. littoralis*, and *Plusia* sp. that attacked the nightshades. Tomato and potato were also alternative hosts to *Tuta absoluta*. These alternative hosts could serve as refuge for the major pests of nightshades when the nightshade crop is not in the field and later become a source of new infestation to a newly planted nightshade crop. Therefore, IPM measures to control nightshade pests should also include control of alternative wild hosts (weed species) growing in and around the nightshade crop. Moreover farmers should also aim to control key nightshade pests on the other cultivated hosts grown in close proximity to the nightshade crop such as amaranth, potato and tomato, to break-up the infestation pathway between these crops.

One of the most important parasitoid species that we found occurring naturally in African nightshades farms in Kenya is *A. colemani*. The parasitoid *A. colemani* has been reported to be highly effective against aphid species *A. fabae* and *M. persicae* on other crops such as chrysanthemum and pepper, takes short time to apply, has great dispersal distance, easy to rear and cost-effective to produce commercially (Van Lenteren et al., 1988; Van Steenis et al., 1995; Vásquez et al., 2006; Van Schelt et al., 2011). In **chapter 6**, we demonstrated the potential of parasitoid *A. colemani* for the management of *A. fabae* and *M. persicae* in nightshade production systems. Although *A. fabae* and *M. persicae* has been shown to be acceptable and suitable hosts to A. colemani on other crops (Messing and Rabasse, 1995; Jones et al. 2003; Vásquez et al., 2006), we showed for the first time that nightshade species *S. scabrum* and *S. villosum* are also acceptable hosts for *A. colemani*. Our results showed that both aphid host species and host plant species affect the performance of *A. colemani*. Although production of parasitoid *A. colemani* using *A. fabae* as host and *S. scabrum* as the rearing host plant for the aphid gave promising results in control of *A. fabae* and *M. persicae* on nightshades, other rearing systems should also be investigated before full recommendation is made. Moreover, field trials will be required to validate these laboratory findings. Nevertheless, the use of parasitoid *A. colemani* both as for the management of *A. fabae* and *M. persicae* should be encouraged as an alternative to the current situation where farmers

mainly rely on the use synthetic pesticides. Compatibility trials of parasitoid *A. colemani* with other non-chemical methods for control of aphids on African nightshades should also be tested in future.

In **chapter 7**, we carried out tests to determine the occurrence, host range, host resistance and seed transmission of NsVMV. We found out that NsVMV is present in both the mid and high altitude zones of Kenya and the incidence of the disease is increasing with time particularly in the high altitude zone. Cool and humid conditions, availability of alternative hosts and presence of insect vectors increases the incidence of a disease in a given region (Dahal et al. 1997; Fajinmi 2006; Fajinmi, 2010). In our study, the cool weather conditions and high abundance of *Nicandra sp*. as alternative host for the disease could have favoured high incidences of the disease in that region. The use of host genetic resistance and control of insect vectors are two successful strategies employed in management of plant viruses (Bragard et al., 2013). Unfortunately in our study, we did not find a nightshade species/line resistant to NsVMV. Therefore the use of resistance breeding as a strategy for management of NsVMV in African nightshades may not be a feasible strategy unless a resistant nightshade variety/line is identified. Moreover, management of the aphid vector with insecticides or biological control may not be very effective strategy for the management of NsVMV, since the virus is transmitted in a non-persistent manner by *M. persicae*, meaning the aphid vector may already transmit the virus before it is controlled (Irwin, 1999; Perring et al., 1999). Viruses transmitted in a non persistent manner by insect vectors require a very brief period (1-2 minutes) of probing the host to transmit the virus ((Hull, 2002). The disease obviously has a narrow host range and research and the use of barrier cropping system by growing other non-host indigenous vegetables around the African nightshades crop could be an effective way of managing the disease.

In conclusion, the present study has shed more light on the major pest of amaranth and African nightshades in Kenya and their natural enemies, their distribution on different agro-ecological zones, and how their abundances are affected by seasonality and crop phenology. Moreover, the host range of the major African nightshade pests has been highlighted and the potential on the use of parasitoid *A. colemani* in management of *A. fabae* and *M. persicae* has been demonstrated. Biological properties of NsVMV have also been characterized. The findings of this study will guide in future development of management practices both for the major pests of amaranth and African nightshades but also for the management of NsVMV on African nightshades.

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Curriculum vitae

Daniel Mwangi Mureithi was born in Nakuru, Kenya on 20th April 1980. In his earlier years, he sat for his primary school examination at St. Pauls Primary school, Nakuru in 1994. After graduating from Nakuru Day Secondary School in 1998, he enrolled for a Bachelor of science degree in Horticulture at Maseno University, Kisumu, Kenya from 2000 – 2005. After graduating, he worked as a research specialist at Syngenta-Pollen Kenya Ltd where he did trials on different agronomic practices aimed at improving flower and vegetable seed quality from December 2015 – October 2006. From November 2006 – July 2011, he worked as a plant quarantine officer at the Kenya Plant Health Inspectorate Service (KEPHIS). He then benefited from a DAAD scholarship to pursue a Master of Science degree in International Horticulture at Leibniz Universität Hannover from October 2011- September 2013. In his master thesis, he investigated the efficacy of entomopathogenic nematodes and neem in different formulations for the control of the soil stages of western flower thrips. In November 2013, he was awarded a PhD scholarship from BMBF under the HORTINLEA project to characterise key pests of amaranth and African nightshades in Kenya and develop integrated pest management strategies at Leibniz Universität Hannover and the International Centre of Insect Physiology and Ecology, Nairobi. His research interests are in the field of biological plant protection and plant virology. Mr. Mureithi is married to Nancy and together they are blessed with two daughters, Mercy and Precious.