MANAGEMENT OF GREEN PEACH APHID, *MYZUS PERSICAE* IN AMARANTH USING HOST PLANT RESISTANCE AND SEED TREATMENT

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

I dedicate this Ph.D. thesis to, my mother, Nalubowa Kirigwajjo and my father, the late Kirigwajjo Lwanga for their investment in my future. I would also like to dedicate this work to my husband James Lugwana and my children Maria Nambwere, Samuel Ssemanda, Timothy Lule, Mark Kambungu, Daniel Zziwa and my late daughter Ruth Kisakye. I also dedicate this thesis to my siblings Rebecca Nakabungo and Francis Walugembe, my nieces, nephews, cousins and all my friends who supported and encouraged me throughout the study process.

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ABBREVIATIONS AND ACRONYMS

AIV	African Indigenous vegetables
EPA	Environmental Protection Document
ESA	Entomological Society of America
FAO	Food and Agriculture Organization
ICIPE	International Centre of Insect Physiology and Ecology
IPM	Integrated Pest Management
ISO	International Standard Organization
IUPAC	International Union of Pure and Applied Chemistry
LEAP	Leadership Enhancement in Agriculture Program
MDGs	Millennium Development Goals
NACRRI	National Crops Resources Research Institute
NARO	National Agricultural Research Organization
PCPB	Pest Control Products Board
REML	Restricted Maximum Likelihood
USAID	United States Agency for International Development
USDA	United States Department of Agriculture

ABSTRACT

Leafy *Amaranthus* is an important food crop due to its high nutritional value, healthy benefits and improvement of farmers' livelihoods mostly in Sub-Sahara Africa. The green peach aphid, *Myzus persicae* (Sulzer) is a major pest of leafy amaranth Sub-Sahara Africa *Myzus persicae* causes damage through direct feeding and by transmitting plant viruses. Management of the green peach aphids is often based on application of insecticides through conventional spray equipment. However, costs and quantities involved is uneconomical and their application is problematic for small holder farmers. Furthermore, aphids have developed resistance to various chemical groups of insecticides. Aphid-tolerant varieties and seed treatment could offer a potential means of control that would reduce the need for insecticide sprays. However, there is limited knowledge of host resistance mechanisms and seed treatment in new Kenyan amaranth varieties and-aphids interaction This study therefore sought to evaluate the potential of utilizing host plant resistance and seed treatment with three specific objectives namely: (i) to identify farmer practices in the management of aphids; (ii) to determine variation in resistance among new amaranth varieties to the green peach aphids (iii) to evaluate the effect of seed treatment on performance of the new amaranth lines and the green peach aphids. A survey was conducted on 600 randomly selected households in Kisii, Kisumu Vihiga and Kiambu Counties using semi-structured questionnaire to identify farmer practices used in the management of aphids in amaranths. In addition, eight focus group discussions and 16 key informants' interviews were conducted in the four counties. Data collected from the survey were tabulated and analyzed using descriptive statistics to get an overview on farmer's management practices for aphids in amaranth. Data of the focus group discussions and key informants' interviews were discussed basing on management practices used by amaranth farmers to reduce aphid infestation. The survey showed that, 94.3% of the respondents mentioned aphids, (Hemiptera: Aphididae) as a major pest of leafy amaranth, and 96.8% ranked aphids as number one insect pest of leafy amaranth in all the four counties. The most important aphid symptom on leafy amaranth was leaf curling mentioned by 43.2% and 49% in the respondents of Kiambu and Kisumu Counties respectively who also mentioned aphids as the most important insect pest of leafy amaranth. A majority of the farmers (34%) used insecticides for aphid control in leafy amaranth. Non-insecticide methods were used at a lesser extent by 7.6% of the respondents. None of the farmers interviewed mentioned biological control or host plant resistance as aphid control options in leafy amaranth. The effect of *M. persicae* on seven leafy amaranth varieties (Abuku 1-7) was conducted under a high tunnel at Jomo Kenyatta University of Agriculture and Technology (JKUAT). The number of aphids and plant growth parameters including leaf damage score, specific leaf area (SLA) and yield were determined. Data subjected to analysis of variance using R. version 3.43 in the statistical program "R" from the Ime4 package. Tukey's honestly significant difference (HSD) test at 0.05 level of significance was used to separate the means. The number of aphids, leaf damage score, SLA total leaf weight, uninfested and infested leaves were significantly different among leafy amaranth varieties; *A. blitum* selection (Abuku 1 and 2*)* *A. hybridus* selection (Abuku 3,4,5, and

6), *A. hypocandracus* selection (Abuku 8) and within varieties of the same species ($P < 0.001$). Abuku 1 and 2 had the lowest populations of *M. persicae*, while Abuku 5 had the highest population. A significantly greater leaf damage score was noted in Abuku 5 and a lower in Abuku 2. A greater SLA was noted in Abuku 8 and the lowest in Abuku 2. The impact of seed treatment on seed germination was evaluated in the laboratory, while that of *M. persicae* populations on growth and yield of the various varieties were conducted in a high tunnel at JKUAT. A significantly higher germination of seeds was noted with Abuku 1 and 2 seeds treated with a combination of thiamethoxam (20 g/kg), metalaxyl-M (20 g/kg), and difenoconazole (2 g/kg) (1.25ml ai/250g seed). There was significant difference of seeds germinating after 24 h and 3 months of seed treatment ($P < 0.001$). A higher germination of seed was noted with Abuku 1 and 2 seeds of 24 h and 3 months of seed treatment. A significantly higher number of live aphids was noted in Abuku 5. Seeds treated with a combination of thiamethoxam (20 g/kg), metalaxyl-M (20 g/kg), and difenoconazole (2 g/kg) (1.25ml ai/250g seed) had significantly more live aphids. A significantly greater fresh leaf yield was noted with Abuku 3, 4, 5 6 and Terere seeds treated with a combination of thiamethoxam (20 g/kg), metalaxyl-M (20 g/kg), and difenoconazole (2 g/kg) or a combination of imidacloprid (233g/l), pencycuron (50g/l), and thiram (3 ml ai/250 g seed). Abuku 3 untreated and seeds treated with a combination of thiamethoxam (20 g/kg), metalaxyl-M (20 g/kg), and difenoconazole (2 g/kg) (1.25ml ai/250g seed) had a significantly greater fresh leaf yield and SLA respectively. In summary, the population of aphids were more in Abuku 5 than in other Abuku varieties and Terere. The number of germinating seeds was also more effective in Abuku 1 and 2 than other varieties with or without seed treatment. However, the yield was significantly less in Abuku 1 and 2 and more abundant in Abuku 3 with and without seed treatment. In conclusion, utilization of environmentally friendly approaches such as *M. persicae* tolerant varieties and seed treatment can improve management of aphids in amaranth production by small holder farmers.

CHAPTER ONE

INTRODUCTION

1.1 Background

Aphids (Homoptera: Aphididae) are an important group of plant insect pests with some species having more than ten generations in a year (Blackman & Eastop, 2000). The green peach aphid, *Myzus persicae* (Sulzer) damage plants by sucking sap (Louis & Shah, 2013). Extensive feeding by *M. persicae* causes distortion of young leaves and shoots resulting to yield loss (Saljoqi, 2009). In addition, they transmit more than 100 plant viruses (Kennedy *et al.*, 1962; Tagu *et al.*, 2008). The green peach aphid is of Asian origin like its primary host, peach (*Prunus persicae* L.) (Blackman & Eastop, 2000).

The green peach aphid, is a highly polyphagous pest that feeds on many plants' families including Solanaceae, Chenopodiaceae, Compositae, Cruciferae, Cucurbitaceae and Amaranthaceae (Blackman & Eastop, 2000; Capinera, 2001; Ramsey *et al.*, 2007). Crops differ in their susceptibility to the green peach aphid, but it is actively growing plants, or the youngest plant tissue, that most often harbor large aphid populations (Saljoqi, 2009). Development of the pest can be rapid, often 10 to 12 days for a complete generation resulting in over 20 generations per year reported in conducive environments (Capinera, 2001; 2005). The success of *M. persicae* in colonizing different host plants has been related to the presence of photo assimilates and phytotoxic salivary secretions (Nalam *et al.*, 2019). Phytotoxic salivary secretions cause imbalances of plant hormones and changes host metabolism that interfere with the physiological functions of the plant's (Giordanengo *et al.*, 2010).

Amaranthus (Amaranthaceae), collectively known as amaranth, is a cosmopolitan genus of annual plants, consisting of approximately 70 species, which according to the uses for human consumption can be divided into grain and vegetable amaranths (Thapa & Blair, 2018). Amaranth is a multipurpose crop supplying high nutritional quality vegetables for food and animal feed; as possessing attractive inflorescence coloration, it also may be cultivated as an ornamental plant (Mlakar *et al.*, 2009, Thapa & Blair, 2018). The genus has the ability to grow under a wide range of climatic conditions coupled with its competitive ability which permits cultivation with minimum management (Shukla & Singh, 2000; Mosyakin & Robertson, 2003; Wambugu & Muthamia, 2009). In addition, amaranth grows quickly, requires little inputs and can be harvested within a short time (4-6 weeks after planting) (Ebert *et al.*, 2011). Amaranths are important in the culture, diet, and agricultural economy in Africa, Mexico, Central and South America and northern India (Brenner, 2000; Mlakar *et al.*, 2010; Achigan - Dako *et al.*, 2014). Amaranth leaves are high in proteins, beta carotene, vitamin C, iron, calcium, phenolic compounds and antioxidants (Nana *et al.*, 2012; Kraujalis *et al.*, 2013; Rastogi & Shukla 2013; Achigan - Dako *et al.*, 2014; Nyonje *et al.*, 2014).

Despite its importance in nutritional security, amaranth is susceptible to damage by foliar insects such as leaf miners**,** leaf rolling caterpillars, cutworms, aphids, flea beetles, and mites (Aderolu *et al.*, 2013; Kagali *et al.*, 2013). Aphids are a major pest of leafy amaranth, which causes curling of leaves and they become non- marketable (World vegetable Centre, 2003; Nampeera *et al.*, 2019). Among the aphid species, the green peach aphid is one of the major pests of leafy amaranth in Kenya (Mureithi *et al.*, 2017). The pest is known to develop populations specifically adapted to certain amaranth species such as *Amaranthus retroflexus* (Magritopoulos *et al.*, 2000; Mokhtari *et al.*, 2012). Wet rot or stem rot caused by the fungus *Choanephora cucurbitarum* is the main disease of amaranth, whereas damping-off caused by *Pythium aphanidermatum* and *Rhizoctoniais* is a problem in amaranth seedbeds (Achigan - Dako *et al.*, 2014). Other diseases include leaf blight, white rust, and viral infections (Brenner *et al.*, 2000).

1.2 Problem Statement

Green peach aphids, *Myzus persicae* is a pest that can reduce amaranth yields (Nampeera *et al.*, 2020). *Myzus persicae* occurs in many amaranth producing areas in Kenya (Nampeera *et al.*, 2019) and continues to be the leading suppressor of amaranth yield among pests (Mureithi *et al.*, 2017). Yield loss from this pest can be as high as 90% when populations are not managed (World Vegetable Centre,2003, Saljoqi,2009).

The majority of amaranth growers in amaranth production areas of Kenya have relied mostly on foliar insecticides to manage *M. persicae* (Nampeera *et al.*, 2019). Some of the foliar insecticides that have been used by amaranth growers include diazinon 600g/L (Dizon 60EC™), lambda-cyhalothrin 50g/L, (Duduthrin, Karate & Pentagon 5ECTM) and, cypermethrin10% wv + chlorpyrifos 35% w/v (Cyclone505EC™) (Nampeera *et al.*, 2019). However, management of this pest has been complicated because *M. persicae* populations have developed resistance against such insecticides (Devonshire *et al.,* 1998, Fuentes-Contreras *et al.,* 2013; Bass *et al.,* 2014; de Little *et al.,* 2016; Rubio-Melendez *et al.,* 2018).

One method that can help growers to manage crop pests is host –plant resistance that functions through mechanisms of antibiosis (effect on insect biology), antixenosis (non-preference) and tolerance (same yield in the absence or presence of *Myzus persicae*) (Painter, 1951; Hill *et al.*, 2004;& Smith, 2005).

New amaranth varieties have been developed at Jomo Kenyatta University of Agriculture and Technology, JKUAT, Horticultural and Food security department. However, their resistance level to aphids which could be used in management of the aphids has not been established. Aphid-tolerant amaranth genotypes could offer a potential means of control that would complement predation of and parasitism by natural enemies while reducing the need for insecticide sprays.

Seed treatment with selected chemicals (Apron Star™ and Monceren™) could also contribute to reducing aphid damage in farmer fields. Apron Star™ and Monceren™ are registered seed treatments currently available to farmers in Kenya for protection of seeds and seedlings against early season fungal and insect pests, including aphids (PCPB, 2018). Management practices that include resistant amaranth and seed treatments with insecticides could help to prevent the buildup of *Myzus persicae* populations in amaranth production. However, there is limited information on resistance of amaranth varieties to green peach aphids and little use has also been made on seed treatment of amaranth grown in Kenya

1.3 Justification

The green peach aphid, *M. persicae* is a common pest of many vegetable crops including leafy amaranths (Ramsey *et al.,* 2007; Mureithi *et al.,* 2017). It is a highly polyphagous insect that can cause direct injury to the plants or transmit viruses (Tagu *et al.,* 2008; Mureithi *et al.,* 2017). To control aphids on amaranth, most of the leafy amaranth farmers in Kenya rely on insecticides without knowing their common names (Nyakundi *et al.,* 2010; Nampeera *et al.,* 2019). The insecticides which they used were moderately dangerous; classified as class II by World Health Organization, WHO (Mutuku *et al.,* 2014). Some of the insecticides, such as dimethoate used by leafy amaranth farmers has been banned by the government of Kenya (Mutuku *et al.,* 2014, PCPB, 2018). In addition, farmers use high insecticide application rates and frequencies for aphid control on leafy amaranth (Nampeera *et al.,* 2019).

Inappropriate usage of insecticides may increase consumers' risk of insecticide exposure and reduce farmer safety. (Park *et al.,* 2016). Up to 10 million cases of injuries and sickness, in addition to 200,000 deaths each year in developing countries, including Kenya are due to pesticide poisoning (WHO, 2017). Insecticide resistance (Bass *et al.,* 2014; Tiwari *et al.,* 2011), residues (Ministry of Environment Water and Natural Resources of Kenya, 2019) and outbreak of secondary pests (McKinney & Schoch, 2003) has also been reported when using insecticides. Due to limited information on resistance of amaranth varieties to green peach aphids and seed treatment management of this pest has been restricted to foliar spraying of insecticides (Nampeera *et al.,* 2019), whose detrimental effects on the environment and human health are well known (Macharia *et al.,* 2013; De Bon *et al.,* 2014).

African leafy vegetables including amaranth are recognized as contributors of micronutrients and bioactive compounds to the diets in Africa, including Kenya (Smith

& Eyzaguirre, 2007; Maundu *et al.,* 2009). Amaranths are among the most popular leafy vegetable in Africa (Maundu *et al.,* 2009) and is a promising food crop mainly due to its nutritional value and food security (Kimaru, 2013; Achigan - Dako *et al.,* 2014). Use of tolerant or resistant leafy amaranth varieties and insecticide seed treatment such as Apron Star™ 42 and Monceren™ (PCPB, 2018; Seed Care, 2017) available to farmers in Kenya may provide an alternative to the control green peach aphids and help reduce yield and quality losses due to aphids in amaranth. Understanding the effect of leafy amaranth varieties on *M. persicae* and the impact of amaranth treated seed on seed germination, population growth of aphids and fresh leaf yield would improve our knowledge of aphid control and use of such varieties and insecticides seed treatments in amaranth production. Knowledge gained will help in future integrated pest management, IPM interventions aimed at decreasing cost and improving the effectiveness of managing aphids in leafy amaranth to improve production, farmers livelihood and supply nutritive food for small-holder farmers and consumers in Kenya.

1.4 Objectives

1.4.1 General objective

The general objective of this study was to evaluate management options of green peach aphids (*Myzus persicae*) in new lines of amaranth.

1.4.2 Specific objectives

1. To identify farmers' management practices for the green peach aphids
2. To investigate variation in tolerance among new lines of amaranth to the green peach aphids
3. To evaluate the effect of seed treatment on green peach aphids and performance of new lines of amaranth

1.5 Hypotheses

1. There is no variation in tolerance among the amaranth lines to the green peach aphids
2. Seed treatments doesn't affect the green peach aphids and the performance of amaranth

CHAPTER TWO

LITERATURE REVIEW

2.1 Amaranth

2.1.1. Classification of *Amaranth*

Amaranthus (Amaranthaceae), collectively known as amaranth, is a cosmopolitan genus of dicotyledonous herbaceous plants that consists of approximately 70 specific species containing both cultivated and wild types, the cultivated ones are used as food grains, leafy vegetables, ornamentals and forages (Thapa & Blair, 2018). *Amaranthus blitum*, *A. dubius, A. hybridus* and *A. hypochondriacus* developed for leaf consumption are important amaranth species grown in Kenya (Das, 2012). The two principal species grown for grain include *Amaranthus cruentus* and *A. caudatus* (Mlakar, 2010; Muriuki *et al.,* 2014) while the species grown for leaves include *A. tricolor, A. dubius, A. lividus* and *A. hybridus* (Das, 2012). *Amaranthus hypochondriacus* is considered to be a dual-purpose type in which both grain and leaves are used (Muriuki, *et al.,* 2014). Whereas, *Amaranthus retroflexus* L. (redroot pigweed), *A. albus* L. (tumbleweed), *A. palmeri* S. Wats. (Palmer amaranth), *A. spinosus* L. (spiny amaranth) are weed species (Erum *et al.*, 2012).

2.1.2. Economic importance of Amaranth

Both gran and leaf amaranth are grown in Africa, Central America, Southeast Asia, and South America, while in North America the grain amaranth are grown and consumed (Brenner *et al.,* 2000, Mlakar *et al.,* 2010; Hoidal *et al.,* 2019). In Kenya there has been an increase in production, trade demand and consumption of amaranth (Onyango *et al.*, 2008; Kenya National Bureau of Statistics, KNBS, 2016). This can be attributed to amaranth's high nutritional value for both human and animals (Mlakar *et al,* 2009; Achigan-Dako *et al.,* 2014).

Amaranth leaves are high in protein, β-carotene, iron, calcium, vitamin C, minerals and phytochemicals (Kraujalis *et al.,* 2013; Rastogi & Shukla, 2013; Achigan-Dako *et al.*, 2014). In addition, antioxidants have also been detected in amaranth leaves (Khandaker *et al.,* 2008; Nana *et al.,* 2012; Kraujalis *et al.*, 2013). While most amaranth species are used as food crops, *A. retroflexus* is considered one of the world's worst weeds, because of its cytotoxic effect (cell damage or death), mostly to renal cells (Amoli *et al.,* 2009).

2.1.3 Key pests of Amaranth

Regardless of amaranth role in food and nutritional safety, amaranths suffer damage from various arthropod pests including defoliators, sucking insects, stem borers, fruit and pod borers and leaf miners (Sithanantham *et al.,* 2004; Torres *et al.,* 2011). Aphids (Hemiptera: Aphididae) are worldwide agricultural pests, which cause economic damage to many crops (Dedryver *et al.*, 2010). In Kenya, farmers ranked aphids as one of the most important pests of amaranth (Wekesa, 2010). Other studies of amaranth in Uganda also reported aphids as the most important insect pests of amaranth (Muyonga *et al.*, 2010).

2.2 Green peach aphid, *Myzus persicae*

2.2.1 Taxonomy and distribution

Green peach aphids, *Myzus persicae* is a species of aphids, belonging to the order Homoptera: and family Aphididae (Blackman & Eastop, 2007). It is a small insect (1-10 mm), soft-bodied plant-sucking insects with two color morphs, green and red, of which the former is much more common (Blackman, 1987). In addition, *M. persicae* exhibit a range of polyphenisms, such as reproduction polyphenism in which different modes change between asexual and sexual reproduction in response to photoperiod (Ogawa & Miura, 2014), primary host and temperatures (Le Trionnaire *et al.*, 2008; Davis, 2012). However, their primary mode of reproduction is parthenogenesis (clonal or asexual reproduction) (Simon *et al.,* 2002) and remain parthenogenic on secondary hosts (Miyazaki, 1987; Margaritopoulos, *et al.*, 2002). The green peach aphids reproduce all the year around in warmer climates and in warmer months. Nymphal development is completed in 6 to 11 days in warmer climates (Capinera, 2001). An individual can reproduce 12 days after birth and up to 20 generations may occur in a year in warmer climates (Capinera, 2005). The typical annual life cycle of the green peach aphids contains recurring parthenogenesis which consist of a succession of parthenogenetic generations (10–30 generations) and can even be as much as 30-40 generations per year in favorable climates followed by a single sexual one (Simon *et al.*,2002). Males are produced parthenogenetically (asexually) from unfertilized eggs (haploid), whereas female are produced sexually from fertilized eggs (diploid) (Wilson *et al.*,1997). The adult green aphids can be alate (winged) or apterous (wingless or flightless) (Brisson, 2010). The morphological differences are influenced primarily by the host plants, nutrition and temperature (Tsuchida *et al.,* 2010).

Myzus persicae, originated from Asia or Europe (Blackman & Eastop, 2007), but currently, it is found throughout the world, including all areas in North America (Capinera, 2001; Vorburger 2006; Ramsey *et al.,* 2007). Asexual species are likely to be spread in low-latitude regions while sexual reproduction is an adaptation to severe winters (Simon *et al.,* 2002; 2010). Parthenogenic reproduction is favored in many parts of the world where continuous production of crops provides suitable host plants throughout the year, or where weather allows survival on natural (non-crop) hosts (Capinera, 2001).

2.2.3 Host plants, damage and economic importance

The green peach aphids, *M. persicae* is a cosmopolitan and highly polyphagous aphid species that feeds on more than 1600 plant species in more than 60 plant families (Weber, 1985; Margaritopoulos *et al.,* 2000; Nikolakakis *et al.,* 2003; Vorburger, 2006). The adults produce nymphs on a wide range of herbaceous plants, including vegetables crops and herbaceous weeds, such as *Amaranthus retroflexus* (Capinera, 2005; Blackman & Eastop, 2000; 2007). Weeds such as field bindweed, *Convolvulus arvensis*; lambs quarters, *Chenopodium album*; and redroot pigweed, *Amaranthus retroflexus*, are also hosts of these aphids, creating pest problems in nearby crops (Annis *et al.*, 1981; Fernandez-Quintanilla *et al.,* 2002). Other ornamental plants are also suitable for green peach aphid development.

Green peach aphids can attain very high densities on actively growing plants, or young plant tissue, causing water stress, leaf curling, wilting and reduced growth rate of the plant that eventually reduces yields of several crops, including amaranth (Capinera, 2001; Saljoqi, 2009; Mureithi *et al.*, 2017; Nampeera *et al.*, 2020). Green peach aphids secrete honeydew that stimulates mold development and also attracts fungus which cause smutting of leaves and fruit (Gray & Gildow, 2003). Contamination of vegetables by the green peach aphids sometimes presents quarantine problems (Stewart *et al.*, 1980).

Green peach aphid is also capable of transmitting more than 150 plant viruses in different host plants, particularly in vegetables (Blackman & Eastop, 2000); including and not limited to beet yellows virus, lettuce mosaic virus, and cucumber mosaic virus (Kennedy *et al.*, 1962), cauliflower mosaic virus (Namba & Sylvester, 1981); Potato leaf roll virus (PLRV), (Mowry, 2005), pepper mottle virus, pepper severe mosaic virus, pepper yellow mosaic virus and Peru tomato mosaic virus (Kenyon *et al.*, 2014), potato virus Y (Bosquee *et al.*, 2018). Yield losses can be as high as 90% depending on plant species, infestation and environmental conditions (World Vegetable Centre, 2003, Saljoqi, 2009).

2.3 4 Management strategies of *Myzus persicae*

Green peach aphid is predominantly controlled using insecticides which has negative ecological consequences that include insecticide resistance (Silva *et al.*, 2012; Bass *et. al.*, 2014). In addition, beneficial arthropods, such as lady bird beetles (*Coccinella septempunctata* (L.) (Coleoptera: Coccinellidae) (Garzón *et al.*, 2015), lacewings (Neuroptera: mainly Chrysopidae), European earwig *(Forficula auricularia)* (Malagnoux *et al.*, 2015), spider communities (Gaelle *et al.*, 2016), honey bees and bumble bees (Blacquière *et. al.*,2012) are affected negatively. Other negative effects of pesticide use include environmental pollution, pesticide residues and toxic hazards to humans (Ajayi *et al.,* 2011; Ahouangninou *et al.,* 2012; De Bon *et al.,* 2014).

For biological control specialist parasitoids are not always sufficiently effective in controlling aphids, thus release of generalist predators such as *Macrolophus pygmaeus* is often needed to improve the control (Messelink *et al.*, 2008; Bompard *et al.,* 2013). This increases the cost of the biocontrol (Messelink *et al.,* 2011).

Macrolophus pygmaeus Rambur (Heteroptera: Miridae), a generalist predatory bug has proven to be a better predator in controlling *M. persicae* in sweet peppers (Messelink *et al.,* 2011; Castane *et al.,* 2013). While aphids in general are a good food source for *M. pygmaeus, M. persicae* enhances *M. pygmaeus* longevity and reproduction rate and is actively searched out as prey (Perdikis & Lykouressis, 2000). Preventive releases of a generalist predator such as *Orius majusculus* (Reuter) or *M. pygmaeus* can, in addition to specialist natural enemies such as parasitoids (mainly Aphididae), or the predatory midge *Aphidoletes aphidimyza* (Rondani) (Blumel, 2004), enhance aphid control (Messelink *et al.,* 2013; Messelink & Janssen, 2014).

The broad host range of *M. persicae* makes crop rotation a difficult tactic to implement successfully (Blackman & Eastop, 2000; Capinera, 2001). However, bands placed around the trunks of trees may harbor predators and suppress aphids and thereafter, reduce the numbers of aphids dispersing to vegetables (Tamaki & Halfhill, 1968).

Regular scouting is a very important component of management of *M. persicae,* and very useful when it is combined with economic thresholds (Harrington *et al.*, 2007; Ragsdale *et al.*, 2007) However, scouting is a compromise of accuracy and time spent looking for insects (Pedigo & Rice, 2008) and for scouting to be effective, the sampling techniques developed by the researchers for use by the growers, should be simple to use, low cost and output easily interpretable (Dent, 2000).

Weeds and insect interactions occur regularly (Norris & Kogan, 2000). Therefore, weeds within the crop field and neighboring areas, valleys, irrigation channels, fence rows and unplanted fields should be kept at low levels (Capinera, 2001). Weeding could have impacts on beneficial insects and other insect pests, mainly because living weeds are used as a food source for insect pests on which beneficial insects feed (Norris & Kogan, 2000). Weeding could also have impact with host location and increase insect damage to crops (Capinera, 2005).

Overfertilization influences performance, abundance and distribution of aphids (Douglas, 2003). High levels of nutrition, such as excess use of manures and mineral (artificial) fertilisers, particularly nitrogenous fertilisers, produces fleshy plant tissue attractive to aphids and thus increase the population of aphids' size (Karley *et al.*, 2002; Sauge *et al.*, 2010; Rousselin *et al.*, 2016).

In addition to mineral nutrients, water also affect the quality and quantity of the plant as a food resource and thus have impact on abundance of aphids (Awmack & Leather, 2002). Increased levels of water increase aphid abundance by enhancing shoot growth and henceforth the quantity of resource for aphids (Rousselin *et al.*, 2016).

2.3.4.1. Host Plant Resistance

Plant resistance is part of integrated pest management strategies and offers considerable advantage to control aphid populations in agricultural systems (Shannag & Obeidat, 2008). In relation to aphids, host plant resistance has been proven to be an important component of integrated insect pest management system in lettuce (Jian-Long & Toscano, 2006), wild relatives of cultivated potato (Pompon *et al.*, 2010), soybean (Pierson *et al.*, 2010; 2011; McCarville & O'Neal, 2013; Prochaska *et al.*, 2013). Resistance to aphids has been found in the forms of antibiosis (effect on insect survival, growth, development, and fecundity, due to morphological and chemical characteristics of the plant), antixenosis/non preference (affect insect settling and feeding) and tolerance (decrease in plant damage even in the presence of aphids) has been found in many plant species (Painter, 1951, Frei *et al.*, 2003, Smith 2005, Smith & Boyko, 2007, Smith &Clement, 2012; Zust & Agrawal, 2016). Host plant species including amaranth species have various effects on *M. persicae* (Goundoudaki *et al.*, 2003; La Rossa *et al.*, 2013, Mdellel & Kamel, 2014). The development of insect pests might be related to nutritional value, defensive compounds and morphological characteristics of host plants, including color, shape, toughness and pubescence of leaves, presence or absence of trichomes (Awmac, & Leather, 2002; Maremela *et al.,* 2013, Polat *et al.,* 2015; Atlihan *et al.,* 2017). Defensive mechanisms of plants reduce insect pest fertility rates which results in prolonged insect pest development (Bashir *et al.,* 2013). Fitness of the insect pest on host plant is indicated by a higher fertility rate and fast development (Liu *et al.*, 2004). Plant secondary metabolites, such as tannins bind to insect proteins and digestive enzymes, precipitate them through hydrogen or covalent bonds and limit their availability to insect pests and thus reduce the insect growth and development, and may also produce lesions in insect pests (Arnold & Schultz, 2002; Peters & Constabel, 2002; Barbehenn & Constabel, 2011, War *et al.,* 2012). Feeding deterrence by tannins has been reported in insect pests such as cowpea aphid, a*phis craccivora*, gypsy and winter moth, *Lymantria dispar* and *Operophtera brumata*; browntail, *Euproctis chrysorrhoea*and desert locust, *Schistocerca gregaria* (Grayer *et al.*, 1992).

Colonization and damage by aphids may be reduced by the presence of phenolic compounds such as cucurbitacins that might directly affect insect growth and development or indirectly act as oviposition deterrents (Tallamy *et al.*, 1997; Agrawal *et al.,* 1999; Balkema-Boomstra *et al.,* 2003). Glucosinolates also act as resistance factors to insect pest, such as phagostimulants for the cabbage aphid (Khattab, 2007; Costa *et al.,* 2014) Glucosinolate–myrosinase has also been reported in brassicaceous plants (Halkier & Gershenzon, 2006). Glucosinolates in plants, also lower and affect polyphagous insects such as aphids (Khattab, 2007; Costa *et al.,* 2014). Some amaranth genotypes also exhibit varying levels of resistance to pests (Steven *et al.,* 2018). However, the mechanism of how *Amaranthus blitum*, (selection one and two) *A. hybridus (*selection 3,4,5 and 6*)*, affect aphid densities is not yet understood. *Amaranthus caudatus* lectin or agglutinin from the seeds of *Amaranthus caudatus,* also confers enhanced resistance to cotton aphid (*Aphis gossypii*) and has the ability to decrease the survival rate and inhibit the development of the aphids (Rahbe *et al.,* 1995, Wu *et al.,* 2006). It is not clear whether the new lines/cultivars/varieties developed at JKUAT exibit any tolerance to *M. persicae* hnce the motivation for undertaking this study.

2.3.4.2 Seed Treatment

Seed treatment is the application of physical, biological or chemical agents to the seed before sowing to suppress, control or repel pathogens, insects and other pests that attack seeds, seedlings or plants, it ranges from a basic dressing to coating and pelleting, in addition to other methods applied to the seed (Sharma *et al.,* 2015; Pedrini *et al.,* 2017). Seed treatments may involve application of mixtures of fungicides, insecticides, rodenticides or nematicides (Munkvold *et al.,* 2014, Douglas & Tooker, 2015).

Seed treatments that possess systemic properties are appropriate and effective to protect the plants from pests and diseases during germination, emergence and early growth stages of the plant (Forsberg *et al.,* 2003; Chelsea, 2012).

Neonicotinoids are currently the most widely used group of insecticides in the world applied to the plant as seed coating (Jeschke *et al.,* 2011; Munkvold *et al.,* 2014; Douglas & Tooker, 2015). Neonicotinoids are registered in more than 120 countries and used for plant protection, veterinary products and biocides (Simon-Delso *et al.,* 2015).

Neonicotinoids allow protection of the treated plants from sucking insects (Jeschke *et al.,* 2011) including green peach aphids (*M. persicae*) (Jeschke & Nauen, 2008) and whiteflies (*Bemisia tabaci*) (Horowitz *et al.,* 2004) and plant hoppers. In addition to neonicotinoid seed treatments effects on insects, imidacloprid and thiamethoxam are the two main neonicotinoids active ingredients used as seed treatments on numerous crops such as maize, soybean, corn, cereals, cotton, oilseed rape, and other crops (Elbert *et al.,* 2008; Magalhaes, *et al.,* 2008; 2009, Zhang, *et al.,* 2011; Environmental Protection Document, EPA, 2014, Douglas & Tooker, 2015).

Neonicotinoid seed treatments could stimulate the germination and growth seedlings of crops (Duan *et al.,* 2012; Zhang *et al.,* 2015). Treatment of seeds with thiamethoxam improved germination of soybeans (Glycine max (L.) Merr.) and maize (Zea mays L.) (Cataneo *et al.,* 2010; Afifi *et al.,* 2015), improved seed vigor in green peas (Pisum sativum L.; Horii *et al.,* 2007), increased root development in wheat (Triticum aestivum L.) (Larsen & Falk, 2013) and improved tolerance of plants to biotic (such as disease and insect and disease attacks) and abiotic (such as temperature, lack of water, drought, salinity, etc.) stresses that affect plant growth and reduce yield (Gull *et al.,* 2019). Treatments with thiamethoxam incresead *Vigna mugo* to bean leaf crinkle virus (Karthikeyan *et al.,* 2009). Treated plants of rose had a greater leaf area and higher content of chlorophyll (Gupta & Krischik, 2007).

Cotton treated with imidacloprid increased places of fruiting, improved growth and yield (Gonias & Oosterhuis, 2008a). At higher temperatures cotton seed treated with imidacloprid resisted temperature stress and increased chlorophyll fluorescence yield (Gonias & Oosterhuis, 2008b). Cotton and Okra seeds treated with thiamethoxam and acetamiprid increased the plant's ability to fix carbon dioxide, promoted photosynthesis and improved chlorophyll content of okra (Preetha & Stanley, 2012). However, the mechanism of how imidacloprid and thiamethoxam affect the plants physiology, stimulate growth and protect amaranth to reduce the losses or stress is not yet understood.

Seed treatments; imidacloprid and thiamethoxam induce changes in gene expression like those changes induced by salicylic acid, defense responses (Ford *et al.,* 2010). Salicylic acid is a phenolic compound, that have a role in plant growth, including and not limited to germination and vegetative growth. Salicylic acid is also linked with plant resistance to abiotic and biotic stress (Khan *et al.,* 2015). It regulates, directly or indirectly enzyme activities of antioxidant defense system and changes responses of plant to several stresses (Khan *et al.,* 2015; Hasanuzzaman *et al.,* 2017). It increases stress tolerance of crops and could also prevent accumulation of protection products like fungicides, insecticides and herbicides in plants including amaranth (Liu *et al.,* 2021).

2.3.4.2 1 Active ingredients, mode of action and effects of seed treatments

Apron® Star 42 WS is a water dispersible powder for slurry seed treatment. It is a combination of three active ingredients namely thiamethoxam at 20g/Kg, metalaxyl –M at 20g/Kg and difenoconazole at 2g/Kg (PCPB, 2018). **Thiamethoxam** (IUPAC: (EZ)-3-(2-chloro-1,3-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene (nitro)amine) (Maienfisch *et al.,* 2001) is a second-generation neonicotinoid insecticide developed by Syngenta Crop Protection, Inc. (Greensboro, North Carolina). It works through contact, stomach and systemic activity (Wilde *et al.,* 2001). It interferes with nicotinic and acetyl choline receptors (nAchR) of the nervous system of insects (Tomizawa & Casida, 2005). The molecule impersonates acetylcholine and binds to its receptor site, thus causing irreversible harm to the nervous system (NRA,2001).

The second product Monceren® GT 390 FS Flowable Concentrate is a combination of three active ingredients namely imidacloprid at 233g/L, pencycuron at 50g/L and thiram at 107g/ L (PCPB, 2018). **Imidacloprid** ((IUPAC: (E)-1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin- 2-ylideneamine), is used as a systemic seed treatment to protect seeds and seedlings from insects' pests (Tharp *et al.,* 2000). It causes permanent blockage of the acetylcholine receptors of insect's nervous system which leads to an accumulation of neuro transmitter, resulting in paralysis and sometimes death (Kidd & James, 1991). Is effective in control of sucking insect such as aphids, mites, thrips, wire worms because of its unique plant- systemic and translaminar properties (Marrs & Ballantyne, 2004). It is used on several crops including canola, barley, cotton, sorghum, sugar beet, wheat, etc.

In addition to protecting plants, promoting growth of plants and improving yields, other benefits of seed treatments include cost effectiveness compared to broadcast and foliar applications, user friendly, no need to handle chemicals, unless if a farmer decides to do seed treatment, no reliance on favorable weather, amount of active ingredients used are also much lower compared to foliar applications (Khangura & Barbetti, 2004; Munkvold *et al.,* 2014).

2.3.4.3 Seed treatment and duration of storage

Seeds can be treated, bagged and stored till sowing at the best period to attain high yields (Conceição *et al.,* 2016; Brzezinski *et al.,* 2017). However, storage period after seed treatment can affect physiological quality of seed including low vigor (Khaliliaqdam *et al.,* 2012**)**, decrease of seedling emergency and reduction in yield (Dan *et al.,* 2010, 2013; Ferreira *et al.,* 2016). The duration of storage of treated seeds can have detrimental effects on the seeds (Dan *et al.,* 2010; Ferreira *et al.,* 2016; Santos *et al.,* 2018). Seedlings emergency reduced with soybean seeds treated with thiamethoxan during storage (Dan *et al.,* 2013). Seed treated 240 days (8 months) before sowing affected growth and yield of soybean as compared seeds treated soon before sowing (Brzezinski *et al.*, 2015).

Combinations of some insecticides affected the physiological quality of soybean treated seeds two months after of storage (Ferreira *et al.,* 2016). Pereira *et al. (*2018) found out that germination and vigor of treated soybean seeds (up to 120 days) declined over the storage period. Dan *et al. (*2010) reported decrease in seedling emergence of treated soybean seeds after storage. Seed storage after treatment for long periods may also result in phytotoxicity (Lamichhane *et al.,* 2020

Dan *et al. (*2010) observed effect on soybean seedlings when treated soybean seeds were stored (240 days). Similarly, Ludwig *et al. (*2011) reported decrease in dry matter content from soybean seeds treated with insecticides and fungicides mixtures and stored up to 120 days after seed treatment. Information on effects of storage period of either a combination of thiamethoxam, metalaxyl-M and difenoconazole) or imidacloprid (233g/l), pencycuron, and thiram) treated seeds on germination, seedling growth, insect growth and development and yield of amaranth remains inadequate, thiamethoxam reduce physiological potential decrease of seeds during storage **(**Dan *et al.,* 2013**)**. It influences the mechanisms of defense and the activities of antioxidative enzyme, it, stimulates peroxidase activity and stop oxidative stress in several crops, such as soybean (Cataneo, 2010). Thiamethoxam modify phyto hormone precursors, amino acids promote and enhance germination, vigor and plant growth (Castro *et al.,* 2008). It also removes reactive oxygen, destruct biomolecules, causing cellular death and quicken up germination under conditions of stress (Cataneo, 2010). Furthermore, as thiamethoxam moves through the cells of plants, it activates several physiological reactions, including, expression of proteins associated to mechanisms of plant defense against factors of stress, like effects of toxicity, temperatures and water scarcity (Castro & Pereira, 2008).

CHAPTER THREE

FARMERS' PRACTICES IN THE MANAGEMENT OF APHIDS OF LEAFY AMARANTH IN FOUR COUNTIES IN KENYA

3.1 Abstract

Farmer management practices of aphids on leafy amaranth were investigated during a household survey conducted in four counties of Kenya. Six hundred farmers who were growing or had grown amaranth were interviewed. Eight focus group discussions and 14 key informants were completed in Kiambu, Kisii, Kisumu and Vihiga counties. Farmers' knowledge of synthetic insecticides and non-synthetic insecticides methods in the management of aphids on amaranth was gathered in the four counties of Kenya. A majority of the farmers (86.4%) in Kiambu county used synthetic insecticides compared to farmers of Kisii (77.0%), Kisumu (33.9%) and Vihiga (79.2%) counties. In addition, 7.5% of the farmers mainly in Vihiga and Kisumu counties used non synthetic insecticides compared to farmers of Kiambu and Kisii counties. The survey showed that 58.3% of the farmers did not use any management practice, mainly in Kisii. The most widely used insecticides were lambda-cyhalothrin 50g/L, (Duduthrin, Karate and Pentagon 5ECTM), diazinon 600g/L (Dizon 60EC™), and cypermethrin10% wv + chlorpyrifos 35% w/v (Cyclone505EC™) respectively. Duduthrin was mainly used in Kiambu and diazinon in Kisii. Amaranth farmers used on weekly basis application dosages of less than 5 to more than 20 millilitres of pesticide in 15 or 20 litres of water to spray less than 0.25 to more than 2 acres of amaranth. Alternative pest management strategies such as host plant resistance and seed treatment should be exposed to farmers to improve control practices of aphids in amaranth.

3.2 Introduction

Amaranthus L. (Amaranthaceae) is a dicotyledonous herbaceous plants classified into 60 recognized species (National Research Council, 1984). Amaranth is an ancient food crop, which was cultivated over 6000 years ago in Puebla, Mexico (Brenner *et al.*, 2000). It has a worldwide distribution (Brenner *et al.,* 2000; Mlakar *et al.,* 2010; Trucco & Tranel, 2011) and is widely consumed in Africa, especially in Kenyan households (Government of Kenya, 2009). Some amaranth species are cultivated for their leaves and used as a leafy vegetable, while others are cultivated for their seeds and used as grain (Mlakar *et al.,* 2009) with high nutritional value (Srivastava, 2011).

In Kenya *Amaranthus dubius* Mart. ex Thell and *A. hybridus* L are grown as leafy vegetables, while *A. cruentus* L. is cultivated for grain and *A. hypochondriacus* is a dual-purpose species (Muriuki *et al.,* 2014). Amaranth is inexpensive to produce and grows well in numerous environments (Wambugu & Muthamia, 2009). Leafy amaranth is harvested between 20 to 45 days after sowing, depending on the variety and growing period (Ebert *et al.,* 2011). The nutritional value and environmental adaptability of leafy amaranth have created a positive effect on leafy amaranth growers in the rural, urban and peri-urban areas in Kenya (Monica *et al.*, 2011).

The most important problems of leafy amaranth are insect pests (Banjo, 2007; Aderolu *et al.,* 2013; Kagali *et al.,* 2013). Insect pests that cause losses to leafy amaranth include aphids (*Myzus persicae*), amaranth weevils (*Hypolixus nubilosus*), webworm (*Hepertogramma bipuctalis*) and leaf miner (*Liriomyza spp*.) (Palada & Chang, 2003). Pests in insect orders; such as; Coleoptera, Diptera, Hemiptera, and Lepidoptera have also affected leafy amaranth growers in Kakamega, Kiambu, Machakos, Meru, Nairobi and Narok counties in Kenya (Clarke-Harris *et al.,* 2004; Aderolu *et al.,* 2013; Kagali *et al.,* 2013; Mureithi *et al.*, 2015)

Determining of current practices used by farmers to manage aphids in leafy amaranth is needed to design and implement sustainable approaches to control aphids in amaranth. Current information is lacking regarding farmers' knowledge and control practices used for aphid pests on leafy amaranth in Kenya. A baseline survey of leafy amaranth farmers was conducted to establish the current management practices they use to manage insect pests of leafy amaranth in four counties of Kenya. The specific objectives of the baseline study were to: (1) identify the major production constraints of leafy amaranth (2) identify the major insect pests of leafy amaranth (3) determine insect pest management practices that farmers use to control major pests of leafy amaranth. counties.

3.3 Materials and Methods

3.3.1 Study area

This study was conducted in four counties of Kiambu, Kisii, Kisumu and Vihiga in central and western Kenya (Fig. 3.1). The four counties were purposely selected because their communities grow and consume African leafy vegetables, such as leafy amaranth (Abukutsa-Onyango, 2007). Kiambu County is located in Central Kenya (1°19’20” S to 0°45’49” S; 37°21’23” E to 36°29’23” E). The County has a tropical climate with temperatures between 7°C (July and August) and 34° C (January and March). It has a bi-modal type of rainfall (County Government of Kiambu, 2015). Rainfall is received between mid-March to May followed by a season with light rains between June to August. The second season is between mid-October to November (County Government of Kiambu, 2015). There three broad categories of soils which are: high level upland soils, plateau soils and volcanic footbridges soils. These soils are of varying fertility levels with soils from high-level uplands, which are from volcanic rocks, being very fertile (Kiambu County Government, 2013). High value crops such as vegetables and fresh fruits makes their growing suitable in the county (Government of Kenya, 2012). The census of 2009 in Kenya showed that Kiambu had 1,623,282 people of these 50.6% and 49.4% were female and male respectively; 57.7% of the population lived in urban centres (Kiambu County Government, 2013).

Kisii county is located in Western Kenya, Southeast of Lake Victoria (0°58’42” S to 0°30’31” S; 35°00’38” E to 34°37’10” E). The county has a tropical rainforest, equatorial climate and receives rainfall throughout the year. The average annual rainfall is 1500 mm with the first rainfall between March and June while the second season is from September to November. The months of July and January are relatively dry. The temperature ranges between 15 ^{0}C and 30 ^{0}C (Kisii County Government, 2017). Kisii has a characteristic feature of a hilly topography with several ridges and valleys (Kisii County Government 2014). Seventy five percent of the County has red volcanic soils (nitosols) which are deep in organic matter, while other parts of the county have clay soils which have poor drainage (phaezems); red loams and sandy soils. Black cotton soils (verisols) and organic peat soils (phanosols) exist in the valley bottoms (Kisii County Government, 2018). The 2009 population census in Kenya showed that, Kisii County had a population of 1,152,282, of these 52.2% and 47.8% were female and male respectively ;10.9% lived in major town centers (Kisii County Government, 2014).

Kisumu county is located in Western Kenya, along the eastern shores of Lake Victoria (0°25'07" S to 0°01'08" N; 35°20'34" E to 34°24'42" E). The county has two rainy seasons (April to May and August) of 1200 - 1300mm annual rainfall, and a dry period (January and February) with temperature between 25 ^{0}C and 35 ^{0}C (Kisumu County Government, 2018). A characteristic feature of this county is the Kano Plain, which is a flat stretch of Lake Victoria ending along the Winham Gulf (Kisumu County Government, 2018). The soils of Kano plains are black cotton soils, Seme and lower parts of Nyakach sub counties have sand and clay soils whereas the red –loamy soils predominate Kisumu West sub-county and upper – Nyakach. The lake shores are generally swampy (Kisumu County Government, 2018). In 2009 census, the population of the county was 968,909, 51% female and 49% male. Of the population listed in 2009 census, 30.6% lived in urban centers (Kisumu County Government, 2013).

Vihiga county is located in Western Kenya (0°12'28" N to 0°02'29" S; 34°55'44" E to 34°32'10" E). The county has a well-distributed rainfall all the year between 1800 - 2000 mm. The highest precipitation is in April. The average temperature is 24 ºC with the highest month in February (Institute of Economic affairs, 2011). The soils in the county are mainly sedimentary in nature (Vihiga County Government, 2013). Data from 2009 population census showed 554,622 people living in Vihiga County, of these, 52.6% and 47.4% were female and male respectively, 15.6% of the population lived in main urban centers (Vihiga County Government, 2013).

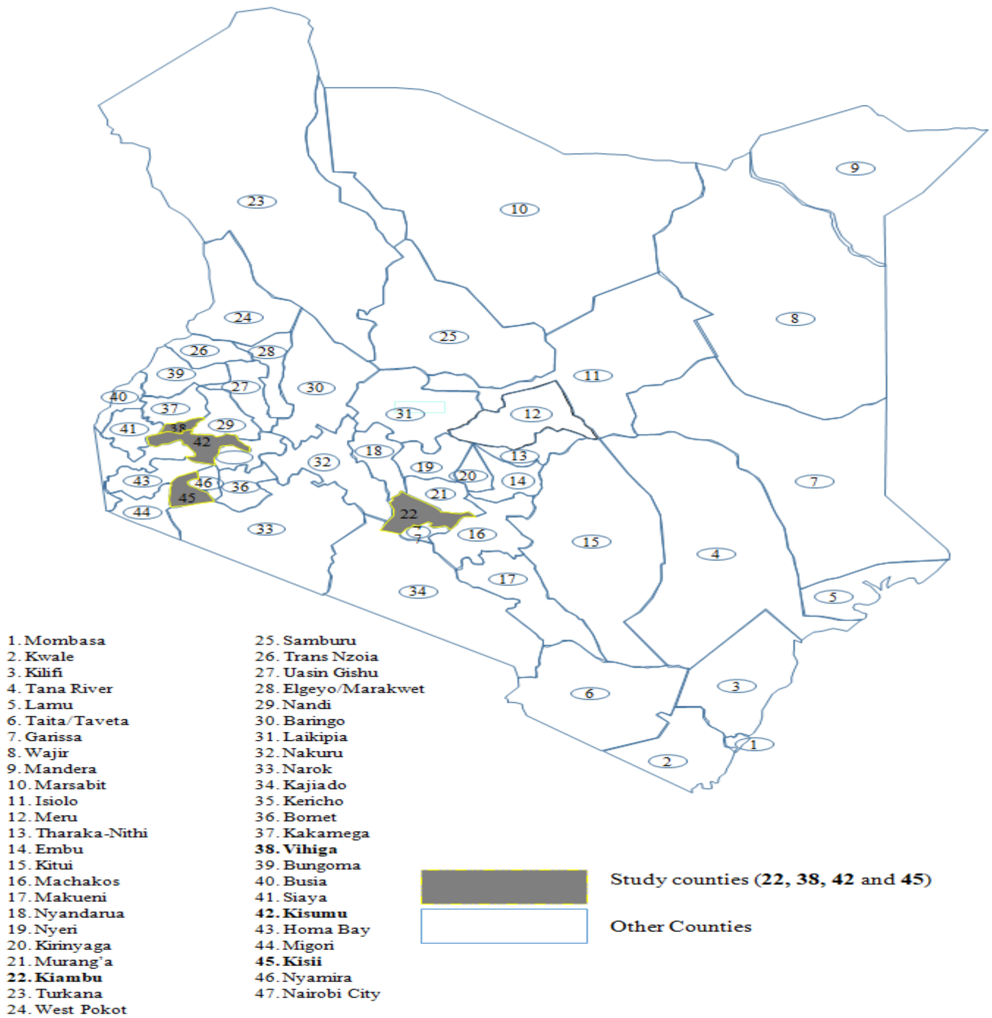

Figure 3. 1: Location of Kiambu, Kisii, Kisumu and Vihiga Counties in Kenya (Adapted from Okilwa, 2015)

3.3.2 Research methods

Research methods used in the four counties, included; farmer surveys, focus group discussions (FGD) and key informant interviews. A social scientist, from Makerere University, two agricultural extension experts of Jomo Kenyatta University (JKUAT) and the student tested surveys questionnaire, question guides and sampling procedures. The survey of the farmers and FGD questions was pretested in Juja constituency at Juja Farm, Kiambu County (1°09'12" S, 37°05'24" E) an electoral division that was not part of the research study. Five female and five male leafy amaranth growers pretested the survey questions, whereas, four female and two male participants pretested focus group discussion questions. In addition, three key informants of Juja constituency, including; an agriculture teacher at Kalimoni Senior Secondary School, agriculture student at JKUAT and a dealer in chemical pesticides and seed at Juja trading centre, Kiambu County pretested the questions for the key informant interview. The female and male survey respondents finished in almost 30 min and and 45 min respectively, whereas, the FGD of female and male farmers survey respondents, finished in 20 min and key informants' questions in 15 min. Feedback was given after pretesting, and the questions of the survey and focus group and interview refined to ease the farmers and key informants understanding. During pretesting, all the farmers questions, except those of key informants were translated into Kiswahili, a formal language spoken by most of the farmers. The survey questionnaires were administered in person. The key informants' questions were asked in English, an official language in Kenya and asked in person.

3.3.2.1 Farmer survey

A farmer survey questionnaire to determine the management practices of farmers included demographic characteristics, farm size, leafy amaranths production and management practices of pests, including aphids (Appendix I). The sample size for each constituency was computed as follows: A/B x 150, where A and B is the voter population registered of the constituency and county respectively. The sample size of each constituency within each county was determined based on data of registered voters for the 2013 general elections in Kenya (Schulz-Herzenberg *et al.*, 2015). The individual respondents were selected using, a non-probabilistic survey sampling method (snowball method), (Snijders, 1992). The agricultural of officer in each county, identified the three initial leafy amaranth participants. In each county, one hundred and fifty previous and current amaranth farmers were interviewed and a total of 600 farmers in all the four counties (150 x 4) were interviewed face-to-face. Two enumerators who knew English and Kiswahili were trained on survey methods and questionnaire administration for the survey. Farmers interviewed at the time of the survey had grown or were growing leafy amaranth.

3.3.2.2 Focus groups discussion

Two focus group discussions one for 12 men and another for 12 women were conducted in each of the four counties. The male and female FGD were separated by two age ranges of 26-35 and 36-40 years, considered youth and adults respectively. This was done to examine the different opinions and explanations for the presented results of men and women. The FGD of female and male focus groups were held simultaneous to avoid participants from interchanging ideas and influencing responses of each participant before participation (Elias, 2013). Discussion questions were translated into Kiswahili (Appendix II).

3.3.2.3 Key informants

Key informants included four people per county and a total of 16 people were interviewed in the four counties. These interviews aimed at the expert's ideas on the interpretation of the survey results in the four amaranth farming areas. The key informants were identified in each county by the agricultural officers in the county. Semi-structured interviews were used to collect experts' perceptions from the key informants (Harrel & Bradley, 2009). The student conducted the experts' interviews in English, although if the participant wanted clarification, a translator of Kiswahili assisted (Appendix III). The key informant interviews were conducted one-on-one in a location that provided privacy at the place of employment for the participant.

3. 4 Data analysis

Quantitative data recorded in the survey questionnaire were coded and entered into a Microsoft® Excel® spreadsheet. Data were then transferred to SPSS™ (release 11.0, SPSS Inc., Chicago, IL, USA) for descriptive statistics to be generated. Descriptive and correlation analysis were generated to address the objectives of this study. Descriptive statistics was used to describe the data or summarize the characteristics of the data in tables and graphs. Whereas one of the inferential statistics, Pearson chi-square (χ^2) was used to test for the differences in the variables of interest across the four counties and helped in understanding how the variables are associated and if the relationship (association) that affects among them is significant or not. Variables analyzed included production constraints, key insect pests and pest management practices used by leafy amaranth farmers for insect pest control in leafy amaranth. Cross tabulations that were used to determine relationships among counties. Associations using Fisher's exact test and correlations using Cramer's V test were also conducted. Qualitative data were summarized, interpreted and described.

3.5 Results

3.5.1 Demographic characteristics of survey respondents.

Demographic characteristics of the survey respondents is shown in Table 3.1, A majority of the leafy amaranth growers (59.2%) were female. A higher percentage of the female respondents were recorded in Kisii County (80.7%). The age range was between 22 and over 60 years, with the majority of respondents (46.2%) in the age range of 26-40 years. A higher number of respondents (48.8%) had attended primary school education. A majority of the respondents (79.3%) were married. The highest numbers of the respondents (71%) had less than 0.25 acres of land under leafy amaranth production with the majority of respondents in Vihiga (79.3%), Kiambu (77.3%) and Kisii (74.7%) counties respectively. Some farmers of Kisumu (17.3 %), Kiambu (7.3%), Kisii (2.7%) and Vihiga (0.7%) counties grew leafy amaranth on 0.5 acres.

Table 3.1:Demographic information of leafy amaranth farmers interviewed in four counties, Kenya

Demographic Information	Number of Leafy Amaranth Farmers County				Total No. of Farmers	Percentage (%) of Total Farmers
	Kiambu	Kisii	Kisumu	Vihiga		
Sex						
Male (M)	77	29	65	74	245	40.8
Female (F)	73	121	85	76	355	59.2
Age Range (years)						
Below 25	3	8	11	13	35	5.8
26-40	67	83	76	51	277	46.2
41-60	49	44	44	47	184	30.7
Over 60	25	12	19	37	93	15.5
Don't know	5	2	0	2	9	1.5
No response	1	1	0	0	2	0.3
Education level						
None	29	14	36	17	96	16.0
Primary	63	80	81	69	293	48.8
Secondary	43	51	30	48	172	28.7
Tertiary/UN	14	2	2	9	27	4.5
No response	1	3	1	7	12	2.0
Marital status						
Single	10	6	6	13	35	5.8
Married	111	128	119	118	476	79.3
Separated/divorced	8	0	2	2	12	2.0
Widowed	21	16	23	17	77	12.8
Farm size (acres)						
None	2	26	0	27	55	9.2
< 0.25	116	112	79	119	426	71
0.25	14	8	24	2	48	8
0.5	11	4	26	1	42	7
1.0	3	0	17	1	21	3.5
2.0	2	0	3	0	5	0.8
>2.0	2	0	1	0	3	0.5

3.5.2 Amaranth production

Of the 600 farmers interviewed, 92.5% of them grew leafy amaranth in the previous season, 2014 before the survey was conducted, of those 85.9% grew leafy amaranth once per season, 13.5% twice per season, and 0.5% three-times per season and 0.1% grew more than three times per season (Table 3.2). More than 87% of the 600 farmers surveyed grew leafy amaranth in the last two years before the survey (2012 and 2013) of these, 20.5%,50.2%, 22.6% and 3% grew leafy amaranth crop (s) once, twice, thrice , and four times per season respectively in 2012 and 2013. However, between 0.2% and 1.5% of farmers grew five and 12 leafy amaranth crops in two years period. A higher number of leafy amaranth farmers in the study areas grew leafy amaranth as intercrop (52.6%) with other leafy vegetables and crops such as African nightshade (*Solanum scabrum* Mill.), kale and collards (*Brassica oleracea* L.), cat's whiskers (*Cleome gynandra* L.), slender leaf (*Crotalaria brevidens* Benth), sunn hemp (*Crotalaria juncea* L.) and Swiss chard (*Beta vulgaris* L.).

Table 3.2: Frequency of growing leafy amaranth in 2012, 2013 and previous year's season

Number of times	2012 and 2013	2014
Didn't grow (0)	74	45
1	108	477
2	264	75
3	119	2
4	16	0
5	8	0
6	5	0
9	3	0
10	1	0
11	0	1
12	2	0

A majority of farmers obtained their leafy amaranth seed from previous harvest (Fig. 3.2). Whereas, 46% and 18% leafy amaranth growers of Kiambu and Kisumu respectively obtained their seed from agro- veterinary farm shops. More than 6% of the farmers in Kisii also obtained seed from Agricultural initiative (public or private sector) (Fig. 3.2). In addition, leafy amaranth growers also obtained their leafy amaranth seeds from other sources, such as; fellow farmers, agricultural extension officers, friends, churches, relatives, neighbors, rural outreach programs, seed company and universities.

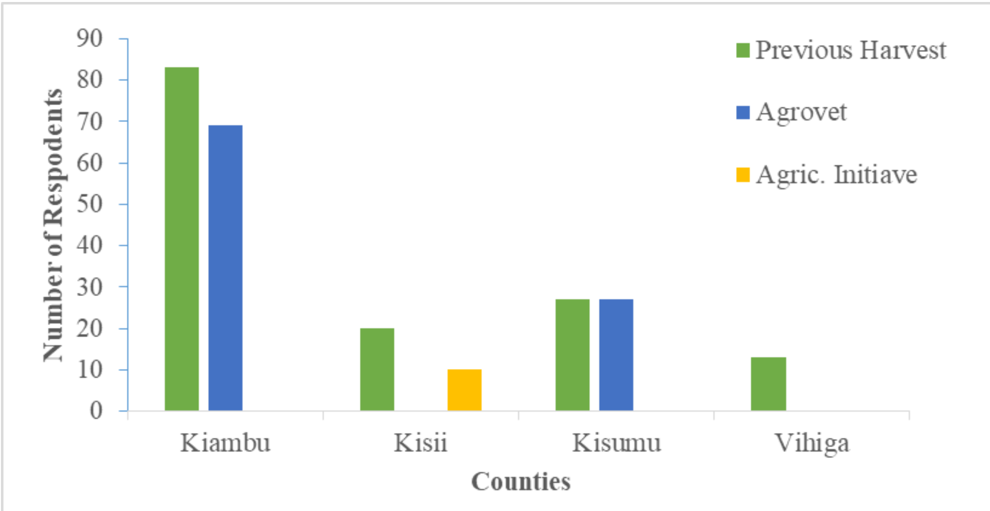

Figure: 3.2: Main sources of amaranth seeds for farmers surveyed in the four counties, Kenya

A higher number of respondents (n = 95) in Vihiga and Kisii (n = 82) grew leafy amaranth for only home consumption whereas, 67 and 66 respondents in Kisumu and Kiambu respectively grew leafy amaranth for only sale. However, 34 and 27 respondents of Kisii and Vihiga did not provide any reason for growing leafy amaranth, while four respondents of Kisumu mentioned that leafy amaranth grows naturally (Fig. 3.3).

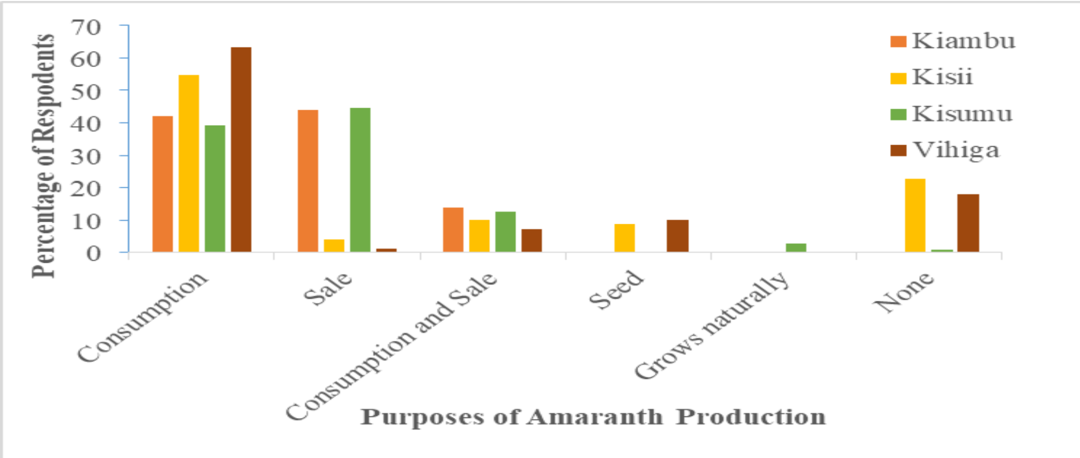

Figure 3.3: Purposes of growing leafy amaranth by farmers surveyed in the four sub counties

3.5.3 Most important constraints of leafy amaranth production

Insects (χ^2 = 126.4, df = 3, P = 0.001) were reported by a majority of respondents in Kisumu (78%) and Kiambu (71.3%) as the major constraint of *amaranthus* production, while 52% and 19.3% of the respondents in Vihiga and Kisii respectively considered insects as important. Birds (χ^2 = 361.6, df = 3, P = 0.001), were also considered by a majority of respondents in Kiambu (91.3%) and Kisumu (84.6%) respectively, whereas in Kisii (14%) and Vihiga (7.3%) birds were considered as important constraint. Lack of capital (χ^2 = 232.7, df = 3, P = 0.001), was reported by 62 % and 46.6% surveyed respondents in Vihiga and Kisii respectively. Whereas, markets (χ^2= 226.5, df = 3, P = 0.001) were reported as an important constraint by 57.3% and 53.3% of the respondents in Vihiga and Kisii respectively. Land availability (χ^2 = 147.1, df = 3, P = 0.001) for leafy amaranth production was an important constraint in Kisii (44%) and (19.3%) in Vihiga. Capital, market, land, training, moles, diseases, seed availability and weeds were not reported as major constraints in Kisumu (Table 3.3).

Table 3.3: Most important production constraints of leafy amaranth production

Major Constraints	Counties and numbers of respondents				Total	%	Statistic		
	Kiambu	Kisii	Kisumu	Vihiga			χ^2	df	P value
Insects	107	29	117	76	329	54.8	126.4	3	0.001
Birds	137	21	127	11	296	49.3	361.6	3	0.001
Capital	0	70	0	93	163	27.2	232.7	3	0.001
Market	1	80	0	86	167	27.8	226.5	3	0.001
Land	0	66	0	29	95	15.8	147.1	3	0.001
Rainfall	6	13	3	27	49	8.2			
Training	0	17	0	17	34	5.7			
Moles	0	0	0	27	27	4.5			
Diseases	5	1	0	13	19	3.2			
Seed availability	1	3	0	4	8	1.3			
Weeds	1	0	0	0	1	0.2			

The most important constraint reported by a majority of respondents in Kiambu and Kisumu counties was insect pests, while in Vihiga the number of respondents that mentioned insect pests as a constraint was higher than that of Kisii. Insect pests as the most important constraint was mentioned by 54.8% of the total number of respondents interviewed. Ninety five percent of the survey respondents who reported insect pests mentioned aphids as the major insect pest of leafy amaranthus (Table 3.4).

Aphid (χ^2 = 147.1, df = 3, P = 0.001) as the key insect pest of leafy amaranth was cited by 54.8% of all respondents across the four counties surveyed; Kisumu (72.6%), Kiambu (68.6%), Vihiga (35.3%) and Kisii (32%) (Table 3.4). Other important insect pests reported by respondents in the four counties included; cutworm (*Agrotis* spp), (χ^2=110.8, df =3, P = 0.001), leafminer (*Liriomyza* spp Diptera; Agromyzidae), (χ^2=125.1, df = 3, P = 0.001) reported by 30% and 26% respondents respectively in Kiambu County (Table 3.4) and spider mite (*Tetranychus* spp Trombidiformes;Tetranychidae) (χ^2=12.0, df = 3, P = 0.007) reported by 6.6% of respondents in Vihiga (Table 3.4).

Table 3.4: Most important field pests in leafy amaranth production

Insects	Counties and numbers of respondents				Total	%	Statistic		
	Kiambu	Kisii	Kisumu	Vihiga			χ^2	df	Pvalue
Aphids	103	48	109	53	313	95.1	83.1,	3	0.001
Cutworms	45	0	1	8	54	13.6	110.8,	3	0.001
Leaf miners	39	0	0	0	39	11.8	125.1	3	0.001
Spider mites	3	7	0	10	20	0.9	12.0	3	0.007
Worms	1	2	0	3	6	0.3			
Caterpillars	3	0	0	2	5	0.9			
Whiteflies	3	1	0	1	5	0.9			

Of the respondents who reported aphids as the most important pest of leafy amaranth, a higher percentage (96.8%) ranked it number –one and key field insect pest of leafy amaranth. Cramer's V test showed a positive correlation (0.77) between insect pests and aphids.

Aphids were also ranked as the most important pest of leafy *amaranthus* by female and male focus group discussion participants and key informants. Aphid infestation symptoms on leafy *amaranthus* included leaf curling, considered as important symptom of aphids by 49%, 43.2%,4.8 %, and 3.0% survey respondents in Kisumu, Kiambu, Vihiga and Kisii counties respectively. Similarly, leaf curling was mentioned by 72.6% and 68.6% respondents in Kisumu and Kiambu respectively, who indicated aphids was a major problem. In addition, respondents in Vihiga (35.3%) and Kisii (32%) indicated aphids were a problem. Other symptoms aphids mentioned by respondents included; mines in leaves, low yields, stunted growth and wilting.

3.5.4 Seasonal infestations of aphids on leafy amaranth

Aphid infestations were a serious problem only during the second season (October through December) as reported by 74.5 % and 68.8% of the survey respondents in Kiambu and Kisumu respectively who reported that aphids was a major insect pest. Aphids was considered a serious pest in Kisii and Vihiga by 31.2% and 28.3% respectively of leafy amaranth growers, who mentioned aphid a serious problem in the dry season during January and February. However, 29.7% and 3% of survey respondents respectively who mentioned that aphids were a major pest of leafy amaranth did not know and could not remember the season of aphid populations (Fig.3.4). Vihiga county had more respondents (64.1%) who did not know the seasons of high aphid infestations, whereas Kisumu County had more respondents (34.6 %) who could not remember the seasons of aphid infestations (Fig.3.4)

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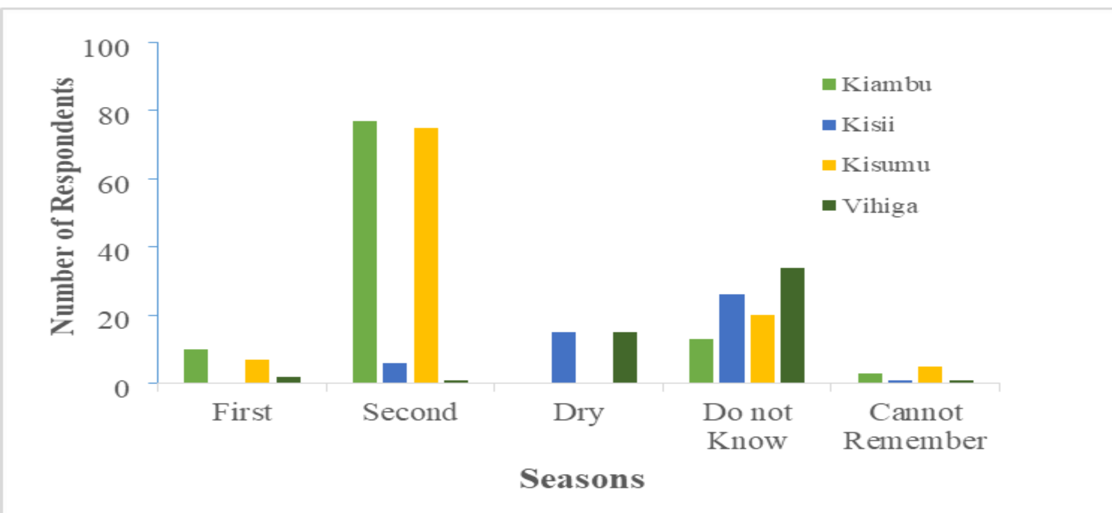

Figure 3. 4: Seasonal infestation of aphids on leafy amaranth reported by farmers

3.5.5. Management practices used by surveyed farmers to control aphids of leafy amaranth

Leafy amaranth farmers used synthetic insecticides (χ^2 = 56.8, df= 3, P = 0.001) and non-synthetic insecticides methods (χ^2 = 29.6, df = 3, P = 0.001) to control aphids of leafy amaranth (Table 3.5). A majority of respondents used synthetic insecticides (34.2%), while 7.6% used non-synthetic insecticides methods. More farmers in Kiambu (59.3%) used synthetic insecticides compared to 38% in Kisii, 36% in Kisumu and 28% in Vihiga counties. In Kisumu and Vihiga, 3.5% and 3.2% of the farmers respectively used of non-synthetic insecticides methods compared to 0.5% and 0.3% in Kiambu and Kisii respectively. Weak correlations occurred between counties and aphid control methods, r value for synthetic insecticides 0.31 and 0.22 for non-synthetic insecticides methods. Biological controls agents, host-plant resistance was not mentioned by the respondents in the farmer survey as management practices to control aphids in leafy amaranth.

3.5.5.1 non-synthetic insecticides and cultural methods used by leafy amaranth farmers to control aphids

Wood ash was the main non-synthetic insecticides (cultural) control method reported by 86.9% of the survey respondents. Of the 86.9% who used wood ash to control aphids of leafy amaranth, a majority of respondents (52.5%) and (42.5%) were from Kisumu and Vihiga respectively. None of the respondents reported using wood ash to control aphids of leafy amaranth in Kisii County (Table 3.5). Other non-synthetic insecticide control methods included use of marigold, pepper and traditional mix. Cultural practices used to control aphids in leafy amaranthus included; intercropping used by 2% of the survey respondents. Similarly, uprooting infested plants was used by 2% of the respondents (Table 3.5).

Table 3.5: Methods used by farmers to control of aphids on leafy amaranth in four counties in Kenya

Insect Pest Control Methods	Number of Farmers County Kiambu	Kisii	Kisumu	Vihiga	Farmers using control (n =251)	All farmers surveyed (%)	χ^2	df	p-value
Insecticides	89	37	37	42	205	34.17	56.8	3	0.0001
lambda-cyhalothrin	39	1	16	0					
cypermethrin + chlorpyrifos	17	0	10	0					
diazinon	1	31	0	33					
Other types	31	7	20	9					
Cannot remember	1	2	1	0					
Non-insecticides	3	2	21	19	46	7.67	29.6	3	0.0001
Wood ash	2	0	21	17	40				
Mexican marigold* (*Tagetes minuta*)	0	0	0	2	2				
Pepper or chilies (*Capsicum annum* L.)	0	1	0	0	1				
Traditional mixx	0	1	0	0	1				
Intercropping	0	0	0	1	1				
Uprooting infected plants	1	0	0	0	1				

3.5.5.2 Synthetic insecticides used by leafy amaranth farmers to control aphids

The main insecticides mentioned by survey respondents who used synthetic methods ((34.1%) to control aphids in leafy amaranthus are shown in Table 3.6. Of the survey respondents who used synthetic insecticides, 31.7%, 27.3% and 13.2% used diazinon 600g/L (Dizon 60EC™), lambda-cyhalothrin 50g/L, (Duduthrin, Karate and Pentagon 5ECTM) and, cypermethrin10% wv + chlorpyrifos 35% w/v (Cyclone505EC™) respectively. In addition, deltamethrin 25g/L (Decis 2.5EC™) and alpha-cypermethrin 10g/L (Tata Alpha 10EC™) was each used by 4.4% of the respondents who used synthetic insecticides. Similarly, thiamethoxam 250g/Kg (Actara 25WG™) and dimethoate 400g/L (Twigathoate 40 EC™) was each used by 1.9% of the respondents who used insecticides. However, only 1.5% and 1.0% of the farmers who used synthetic insecticides used permethrin 20%, 40%, and 60% (Ambush 25DC™, formerly permethrin 25WP) and propargite 21.2%+ tetradifon 7.5% (Dictator Plus 28.7EC™) (1.0%) respectively (Table 3.6). Surprisingly, 1.4% of the farmers mentioned a fungicide metalaxyl-M 40g/Kg + mancozeb 640g/Kg (Ridomil Gold MZ 68WGTM) to control aphids on leafy amaranthus. Farmers obtained insecticides from suppliers in labelled and unlabeled containers.

Table 3.6: Formulation and dosage rates of insecticides used by farmers to control aphids in leafy amaranth

Trade name and type of formulation in Kenya	**Common name and active ingredient (g/Kg)**	**Agent in Kenya**	**Dosage used by farmers (ml in 15/20 l of water)**
Dispersible Granules			
Actara 25WG™	thiamethoxam 250 g/Kg	Syngenta East Africa (E.A) Ltd.	5 and 20
Ridomil Gold MZ 68 WG™	metaxyl -M 40 g/Kg + mancozeb 640 g/Kg	Syngenta East Africa (E.A) Ltd.	Cannot estimate
Wettable Powder			
Ambush 25DC™ (formerly permethrin 25 WP)	permethrin; 20%, 40% and 60%	Syngenta E. A Ltd Formulator Twiga Chemical Industries Ltd. Nairobi.	10 and 20
Emulsifiable Concentrates			
Cyclone 505 EC™	cypermethrin 10% w/v + chlorpyrifos 35%w/v	Osho Chemical Industries Ltd.	5, 10, 15, 20 > 20
Decis 2.5 EC™	deltamethrin 25 g/L	Bayer East Africa Ltd.	5, 15, 20
Dizon 60 EC™	diazinon 600 g/L	Amiran (K) Ltd	5, 10, 15 and 20
Dictator Plus 28.7 EC™	propargite 21.2% +tetradifon 7.5%	Osho Chemical Industries Ltd.	5 and 20
Duduthrin 5 EC™	lambda-cyhalothrin 50 g/L	Twiga Chemical Industries Ltd.	<5, 5, 10, 20 >20
Karate 5 EC™	Lambda - cyhalothrin 50 g/L	Syngenta E. A Ltd	10
Pentagon 5 EC™	lambda-cyhalothrin 50g/L		5 and 20
Tata Alpha 10 EC™	alpha-cypermethrin 10 g/L	Osho Chemical Industries Ltd.	5, 10, 15 and 20
Thunder 145 O-TEQ™	imidacloprid 100 g/L+ betacyfluthrin 45 g/L	Bayer East Africa Ltd.	Cannot estimate
Twigathoate 40 EC™	dimethoate 400 g/L	Twiga Chemical Industries Ltd.	15 and 20

3.5.5.2.1 Frequency and rate of insecticide application to control aphids of leafy amaranth

In Kiambu county, farmers used higher application rates, once per week, every two weeks or more in the growing period (Table 3.7). Forty six percent and 28% of the survey respondents in Kiambu County used insecticides once a week or every two weeks respectively to control aphids of leafy amaranth. Whereas, 59.4% and 37.8% leafy amaranth farmers in Kisumu and Kisii, respectively applied insecticides every two weeks (Table 3.7). Of the leafy amaranth farmers who used insecticides in Vihiga, 23.8% of them could not estimate the frequency of the insecticides they applied.

Table 3.7: Frequency of insecticides used by respondents, Kiambu, Kisumu, Kisii and Vihiga, 2014

Frequency of application	Counties and numbers of respondents				%
	Kiambu	Kisii	Kisumu	Vihiga	
Once a week	41	13	7	4	31.7
Twice per week	6	1	1	1	4.4
Once in two wks	25	14	22	8	33.7
Once in a season	15	3	5	10	16.1
Cannot estimate	2	6	2	10	14.1

3.5.5.2.2 Dosage and rates used by farmers who use insecticides to control aphids of leafy amaranth

Farmers who used insecticides to control aphids in leafy *amaranthus,* used dosage rate of less than 5 ml or g and more than 20 ml or g of the insecticide in 15 or 20 l of water. The most used dosage rate in the four counties was 5 to 20ml of pesticide per 15 or 20 l of water. Higher dosage rates of more than 20mls of insecticides in 15 or 20 liters of water was mostly used in Kiambu compared to Kisii, Kisumu and Vihiga counties, of the farmers in Kiambu who used insecticides to control aphids in leafy *amaranthus*, 41.5% and 11.2% used 20mls and more than 20mls of insecticides respectively in 15 or 20 l of water (Table 3.8). In Vihiga 30.9% of the surveyed farmers who used insecticides to control aphids in leafy *amaranthus,* could not estimate the dosage rates they used (Table 3.8).

Table 3.8: Insecticides dosage rates used by farmers to control aphids in leafy amaranth

Dosage in 15/20liters of water	Counties and number of respondents				%
	Kiambu	**Kisii**	**Kisumu**	**Vihiga**	
Less than 5mls/gms	1	0	0	0	0.4
5mls	25	2	20	2	23.9
10mls	10	24	2	21	27.8
15mls	2	2	1	5	4.8
20mls	37	5	13	1	27.3
More than 20mls	10	0	0	0	4.8
Cannot estimate dosage	4	4	1	13	10.7

3.5.5.2.3 Insecticide effectiveness

In Kiambu, 32.5% and 49.4% of leafy amaranth farmers who used insecticides to control aphids in leafy *amaranthus* found it to be medium and highly effective respectively (Table 3.9). Whereas a majority (47.6%) of respondents in Vihiga who used insecticides rated it low in effectiveness. In Kisii and Vihiga counties 27% and 26.1% of the surveyed respondents did not know how to rate insecticides effectiveness (Table 3.9).

Table 3.9: Number of surveyed farmers who stated insecticides effectiveness to control aphids of leafy amaranth in four counties

Insecticides Effectiveness	Counties and number of respondents				%
	Kiambu	Kisii	Kisumu	Vihiga	
Low	8	4	3	20	17.0
Medium	29	12	13	9	30.7
High	44	11	16	2	35.6
Do not know	8	10	5	11	16.5

Farmers thought that, the only solution to management of aphid infestations in leafy amaranth were frequent spraying and higher application rates, which they thought caused medium and high insecticide effectiveness.

3.5.6 Gender similarities and differences.

respondentsrespondentsrespondentsrespondentsrespondentsrespondentsrespondentsresp ondentsrespondentsrespondentsrespondents. Thirty one percent and 38% of the women and men respondents respectively used insecticides to control aphids in leafy *amaranthus*, 3% of the women did not remember the insecticides they used, whereas, all men who used insecticides remembered what they used. Both women (86%) and men (90%) used ordinary wood ash as a non-synthetic alternative to control aphids in leafy *amaranthus*. Other alternative methods used by 17.3% and 9% of the women and men respondents respectively; to control aphids in leafy amaranth included Mexican marigold, hot pepper or chilies and cultural methods such as intercropping and uprooting aphid infested plants.

3.5.7 Focus Group Discussions and Key informants' interviews

Constraints of amaranth production; Major constraints mentioned by leafy amaranth focus group participants and key informants were birds, including chicken and domestic animals. Other constraints mentioned by key informants included lack of knowledge on where to get amaranth seeds and technical information on leafy amaranth production. Others included depletion of soil, small land sizes for amaranth production, diseases and competition of other leafy vegetables consumed in the study areas.

Pests of leafy amaranth; Focus group participants described pests of leafy amaranth including; beet webworm (*Spoladea recurvalis* Fabricius) caterpillars, cutworms, spider mites, stem borers, thrips and whiteflies. Whereas, key informants described moles, spider mites and whiteflies as pests of leafy amaranth.

Aphid infestation season; Key informants reported that leafy amaranth is grown during the rainy season (March through May) and harvested before the dry season, to reduce aphid infestations.

Aphid management practices; focus group discussions or key informants as management did not mention *b*iological controls agents, host-plant resistance as aphid management practices during discussions and interviews.

3.6 Discussion

3.6.1 Gender and Demographics, attributes of amaranth farmers

Vegetables, including amaranth leafy vegetables production is regarded as a woman's occupation in Kenya (Muyonga *et al.*, 2010; Onyango *et al.*, 2016). In Kisii, 121 (80.7%) respondents were females. The reason could be that women are more knowledgeable about vegetables. Maundu and Imbuni (2003) reported that the growing of vegetables in Kisii, a county in Kenya, is mainly a woman's job.

Women are more involved in leafy amaranth production as compared with men. Women farmers have access to land, but then again limited control over land. Utilization of land for cultivation of crops especially vegetables including leafy *amaranthus* is typically by females for food and personal income security (Ogunlela & Mukhtar, 2009; Nambiri 2010), evident in the study especially in Kisii and Kisumu counties. Women involved in leafy amaranth production are also more challenged by capital and markets than men (Twyman *et al.*, 2015; Cheryl, 2018). Coles and Michel (2011) also investigated gender disparities in vegetable production. Other constraints that hinder women positive development in regard to advancing of women from leafy amaranth production and results in economic disadvantage include; lack of access to land, ownership, credits and markets, in addition to higher illiteracy rates (World Bank, 2009; Laven *et al.*, 2012). The customary land laws also deny women accessibility to land because as a tradition, inheritance of land is by men and this leaves the women unsure in regards to the land for cultivation (Leavens & Anderson, 2011). Male smallholder farmers also have higher income than women because they dominate the sale processes (Osori *et al.,* 2014).

A higher percentage of the survey respondents were mainly within the economically active and productive ages (26 and 40 years) that are more likely to take up innovations, such as new production and insect pest management practices of leafy amaranth. Ayua and Omware (2013) noted that people of 35 years in another county of Kenya are enthusiastic about innovations.

More than a half of the survey respondents had either no or with at least primary school education, that may affect farmers to understand integrated pest management practices and concepts to insect pests, including aphids on leafy *amaranthus*. Women and men had accomplished primary school education. Unfortunately, the transition rate from primary to secondary has been low (Ministry of Education, MOE, 2010). Kenya demographic and health survey 2008-09, noted a decline of more male compared to female youth attending school from the age of 14 years, because of marriage and child delivery (KNBS & ICF Macro, 2010). In addition, the 2008-09 demographic and health survey also reported a higher number of women compared to men with no formal education. Likewise, the women of this study had inadequate formal or no school education that might limit their capability to read and understand the labels of insecticides for right and harmless use and to avoid exposure to dangers of insecticides (Al- Zadjali *et al.*, 2015).

3.6.2 Amaranth Production

The majority of respondents in the farmer survey grew amaranth mainly as an intercrop on less than a quarter of an acre respondents. However, some farmers in Kiambu and Kisumu counties grew leafy amaranth on 0.5 acre. This might be to their proximity to peri-urban and urban vegetable markets (Otieno *et al.,* 2009). In central Kenya, Kiambu farmer groups get high-value market for their leafy indigenous vegetables, including leafy amaranth due to the close proximity of Kiambu County and Nairobi city (Ngugi *et al.,* 2007). Most farmers used amaranth seed saved from the previous harvest as planting material. This indicates that seed systems for leafy amaranth vegetables are underdeveloped with most of the farmers producing and using seeds at farm level (Abukutsa-Onyango, 2007; Achigan-Dako *et al.*, 2014). Ayieko and Tschirley (2006) also reported that, for domestic production of indigenous leafy vegetables, a majority of farmers rely on an informal seed system for seed and planting material.

3.6.3 Major constraints of amaranth production

The highest number of respondents identified insect pests as an important production constraint. Aderolu *et al. (*2013); Yongo (2009) reported insect pest infestations as the most important constraint for amaranth production and one of the primary causes of low yields in terms of quality and quantity. Kigali *et al. (*2013) also found a diverse number of damages causing insect pests in amaranth.

Respondents in all the four counties identified aphids as the major insect pest of amaranth that damage leaf amaranth that cause leaves to curl and become non-marketable to customers (World Vegetable Centre, 2003). Aphids are also the major grain amaranth field pest in Uganda (Muyonga *et al.*, 2010). In Vihiga, the participants of the female FGD described symptoms of aphids on leafy amaranth: "Infested plants infect uninfested plants, which results in low production of vegetables for home consumption and sale, inadequate income due to money spent to buy pesticides, and lack of market for small quantity and low quality of leafy amaranth vegetables harvested.

High aphid infestation on amaranth were reported to occur in the second season especially the dry seasons. Aderolu *et al. (*2013) established that species diversity and abundance of insect pests associated with amaranth species varied from season to season.

Among aphid species, *Myzus persicae* Sulzer (Hemiptera: Aphididae), is the most damaging insect pests of leafy amaranth (Mureithi *et al.*, 2017). Some survey respondents mentioned the red spider mite (*Tetranychus urticae*) as one of the major insect pests of leafy *amaranthus.* It is not known whether predatory mites are existing on leafy amaranth in the four counties, which could be used to control red spider mite. However, Furtado *et al. (*2007) described effective predatory mite *Phytoseiulus longipes,* that could be used. Leaf holes were also mentioned by the survey respondents as one of the symptoms of aphids on leafy amaranth. Nevertheless, aphids do not cause leaf holes, but other insect pests feed on several plant parts, such as seeds, flowers, leaves, stems and roots of amaranth (Mureithi *et al.,* 2017). Foba *et al.* (2015) reported the pea leafminer *Liriomyza huidobrensis* Blanchard (Diptera: Agromyzidae) that burrows uneven white mines on leaves of wild and cultivated amaranth in Kenya and Tanzania.

Survey respondents in Kiambu and Kisumu counties mentioned birds as a major constraint of leafy amaranth production. However, lack of data on the overall relationship of farmland birds existing nearby or within the farming land of the four counties necessities further studies to establish the ecosystem of farmland birds in farming landscapes of Kiambu and Kisumu counties.

3.6.4 Farmer Management practices to control aphids in leafy amaranth

3.6.4.1 Synthetic insecticides

Synthetic insecticides were the most commonly used aphid control method. These findings differed with Muyonga *et al. (*2010) and Wekesa (2010) who found low pesticide use in controlling aphid in amaranth in Uganda and the Kisumu County of Kenya. However, the production goal of leafy amaranth is marketable fresh leaves, unlike grain amaranth. De Bon *et al. (*2014) reported that though insecticides usage in Africa is the lowest in the world, vegetable farmers frequently depend on insecticides to control insect pests because of the quick results achieved after application. Inefficient use of insecticides may have harmful effects on useful insects, development of insecticide resistance, outbreaks of secondary pest, soil and water pollution and too much pesticide residues (Macharia *et al.,* 2013). Bass *et al. (*2014), identified different mechanisms of resistance to insecticides in *M. persicae* (Sulzer) that enables it to endure various chemicals. Whereas, Garzon *et al. (*2015) and Malagnoux *et al. (*2015) reported insecticides that are lethal to earwigs, lace wings and ladybird beetles. Use of insecticides has an effect on the spider communities (Gaelle *et al.,* 2016). \

Amaranth growers used a wide variety of insecticides for controlling aphids. Respondents surveyed cited trade names of pesticides with no mention of common names. Nyakundi *et al. (*2010) also mentioned lack of knowledge about insecticides' common names in Kenya. Lack of knowledge of common names impacts insecticides use negatively since dosage rates is based on active ingredient. Some of the leafy amaranth farmers used moderately hazardous insecticides classified as class II by the World Health Organization (Mutuku *et al.,* 2014). The most commonly used insecticides by 34% of leafy amaranth farmers were Duduthrin 5EC™ (lambda cyhalothrin), Dizon 60EC™ (diazinon) and Cyclone 505EC (cypermenthrin 10% and Chlorpyriphos 35% w/v). The frequently used insecticides on vegetables in Cameroon include; cypermethrin, diazinon and dimethoate (Matthews *et al.,* 2003). Sithananham *et al.* (2004) also reported other insecticides, such as Ambush 25DC™ (permethrin), dimethoate, and Karate 5EC™ (lambda-cyhalothrin) used by vegetable farmers to control insect pests in East Africa. Key informants also stated the insecticides used by leafy amaranth farmers that included Actara (thiamethoxam), diazonal (diazinon), Duduthrin (lambda cyhalothrin) dimethoate, Karate (Lambda cyhalothrin), permethrin, and tetradifon. A county extension key informant said that, "Leafy amaranth growers used dimethoate and Karate (Lambda cyhalothrin) to control insect pests in leafy amaranth because within seven days, the vegetables could be harvested and sold without insecticide residues." Growers were commonly using dimethoate that the government of Kenya had banned (Mutuku *et al.,* 2014). McKinney and Schoch (2003) and Tiwari *et al. (*2011) noted that inefficient use of insecticides can cause insecticide resistance, pest resurgence, secondary pest infestations, residues, applicator safety and consumer health concerns.

The primary source of pesticides in the study areas was agro-veterinary shops. Epstein and Bassein (2003) had similar findings where suppliers influenced the choice of pesticides used. Sithanantham *et al. (*2004) also found that farmers purchase pesticides from agricultural and veterinary suppliers in their local area. An agricultural extension officer serving as a key informant stated, “Farmers, received guidance on aphid management mostly from agricultural and agro-veterinary shops where they bought insecticides. Other sources of information of aphid management in leafy amaranth included fellow farmers. Forms in which pesticides were purchased ranged from factory-sealed containers to cases where suppliers opened containers and sold in smaller quantities depending on growers’ demands and put into an unlabeled farmer-supplied container. “A key informant who is a community leader said that, some farmers use insecticides with no knowledge of their chemical names.”

Vegetable farmers frequently depend on insecticides to control insect pests, due to the quick control results obtained after application (De Bon *et al.,* 2014). Abang *et al. (*2013), De Bon *et al. (*2014) and Mutuku *et al. (*2014) also reported a similar pesticide application frequency. Leafy amaranth harvesting begins four weeks after sowing and is done continuously (Achigan-Dako *et al.*, 2014). Therefore, an increased frequency of insecticides may decrease farmer safety if re-entry intervals are not followed and increase vegetable consumers risk of contact with pesticide residue if preharvest intervals are not met (Park *et al.,* 2016). Midega *et al. (*2012) also noted that scheduled pesticide application is costly and could be harmful to the environment.

Farmers reported dosage rates in terms of millilitres or grams per 15 or 20 L of water without referring to the recommended insecticide labels or land area to which the insecticides were applied. It was also noted that farmers who purchased small quantities of insecticides at the time of application could not estimate the dosage rate. Among the causes of inappropriate insecticide use practices is the difficulty of calculating a correct dosage rate from a hectare basis to very small areas (Ajayi & Akinnifesi, 2007; De Bon 2014). The reported dosages rates of 5, 10, 20 and over 20 mls of insecticide per 15 or 20 L of water did not match the recommended rates for aphid control in amaranth. In addition, many farmers used a fungicide to control aphids, which indicate the necessity for insecticide training and safety education programs.

Ajayi *et al. (*2011) noted that higher risk of the negative health effects from insecticides and higher rates of exposure are mostly experienced by small-landholder farmers in low and middle-income countries including Kenya. However, the negative health effects due to inappropriate insecticide use for leafy amaranth growers could be reduced by using an integrated IPM system including appropriate product selection and dosage of application, when justified.

3.6.4.2 Non synthetic insecticides

Both male and female farmers used non-insecticides methods to control aphids in leafy amaranth including botanical extracts such as, papaya leaves, neem oil and exotic garlic and wood ash dust. (Mochian *et al.*, 2011). Muyonga *et al. (*2010) and Sithanantham *et al. (*2004) also noted that other aphid control methods including traditional pesticides, ash and a mixture of tobacco and ash.

Cultural practices to control aphids, such as intercropping and rouging infested plants were used at a lesser extent. Farmers did not mention other aphid control methods including elimination by covering the plant or host plant resistant. Karagounis *et al. (*2006) and Murray (2006) noted that use of less toxic substances such as insecticidal soap and botanicals might reduce the side effects of insecticides. Other solutions are to find alternative control methods including companion plants (Refka *et al.*, 2017), plant extracts such as from chili (*Capsicum annuum*) (Hussein & Samad, 2008), *Tagetes* species (Asteraceae) (Blagovesta *et al.*, 2005; Gayatri & Sahu 2017; Salehi *et al.*, 2018). Murray (2000), Murray (2016) and Lee (2018) found that, plant essential oils could act as feeding deterrents and repellents; these products might be included into an IPM program which offers a diversity of control methods.

3.6.5 Biological control of aphids

Biological control methods were not mentioned by respondents in the farmer survey. Farmers neither discussed natural enemies of aphids nor the negetative effects of insecticide use on natural enemies. Farmers knowledge of insect pest control in leafy amaranth by use of biological control was not directly asked as a survey question. Croft (1990), Stark and Banks (2003), and Desneux *et al. (*2007) reported that, insecticides are harmful to arthropods for biocontrol such as ladybird beetles (Coleoptera: Coccinellidae) which are common aphid predators in natural field settings (Long & Finke, 2014).

3.6.6 Host plant resistance for aphid control

Respondents did not mention any variety that was resistant to aphids, they only mentioned a susceptible variety, *A. dubius*, usually called Terere. Achigan-Dako *et al. (*2014) noted that *A. dubius* is the most important and commonly grown leafy amaranth variety in Kenya. Pierson *et al. (*2010) found out that aphids can be managed by host plant resistance. Various crops can resist aphid attack by using physical traits and allelochemicals (Growth which affect their behavior and survival (Smith & Clement, 2011).

3.6.7 IPM programs for aphid control

The production goal of grain and leafy amaranth is different. For leafy amaranth, the production goal is marketable fresh leaves. Therefore, leafy amaranth production needs effective aphid control management measures during the duration of the production season. Sustainable pest management strategies that will reduce cost of controlling aphids in amaranth, reduce insecticide use and eliminate exposure to hazardous pesticides and protect the environment need to be developed or improvement made to existing approaches.

CHAPTER FOUR

SCREENING OF AMARANTH SPECIES FOR RESISTANCE TO THE GREEN PEACH APHID (*MYZUS PERSICAE*, HEMIPTERA: APHIDIDAE)

4.1 Abstract

Myzus persicae (Sulzer) is an important pest of leafy amaranth in the tropics. Three amaranth species (*Amaranthus blitum*, *A. hybridus* and *A. hypocandracus*) containing seven amaranth varieties (Abuku 1–7) developed in Kenya for leaf consumption were screened for resistance to aphids. Five plants of each selection were artificially infested with 10 adult female *M. persicae* and the numbers of *M. persicae* were assessed over a five-week period. The weight of leaves from each of the seven varieties were measured each week for a period of five weeks. The parameters of the seven varieties evaluated at the end of this experiment included; leaf yield, numbers of aphids, leaf damage score and specific leaf area (SLA). Aphid populations assessed 49 days after sowing seeds differed significantly ($P \leq 0.001$) among the amaranth species and within varieties of the same species. *Amaranthus blitum* (Abuku 1 and 2) had significantly lower aphid populations, whereas *A. hybridus* (Abuku 5), had significantly higher aphid population and leaf damage score. Amaranth leaves without aphids are considered more marketable than those infested. Abuku 1 and 2 would be considered to produce the greatest number of marketable leaves, as they had the lowest percentage of aphid-infested leaves. However, Abuku 1 and 2 (*A. blitum)* had lower leaf yield by fresh weight, and *A. hybridus* (Abuku 3, 4 and 5) had the greatest leaf yield. Varieties that had the fewest aphids could be used by breeders to develop amaranth varieties tolerant to aphids and be used to manage *M. persicae* in amaranth production.

4.2 Introduction

Amaranthus (Amaranthaceae), known as amaranth, consists of approximately 70 species (Suresh *et al.*, 2014; Stetter & Schmid, 2017; Thapa & Blair, 2018), grown for grain, leaf, forage, and ornamental uses (Thapa & Blair, 2018). Throughout the African continent, *Amaranthus* has been regarded as a weed but also a vegetable crop for those people with limited resources. Over the last decade, the volume of production, trade, demand, and consumption of *Amaranthus* in Kenya has increased (Onyango *et al.*, 2008). Breeding programs are producing varieties from *Amaranthus* species as potential commercial cultivars for leaf production (Omondi *et al.*, 2016).

Leafy amaranth experiences damage from various arthropod pests including defoliators, leaf miners, stem borers, fruit and pod borers, leaf webbers, and sucking insects (Sithanantham *et al.*, 2004; Torres *et al.*, 2011). *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) is among the most destructive insect pests of leafy amaranth (Mureithi *et al.*, 2017). In general, *M. persicae* can cause direct injury to plants by feeding on leaves or indirectly by transmitting plant viruses (Tagu *et al.*, 2008; Mureithi *et al.*, 2017). Among the more than 100 plant viruses that *M. persicae* can vector (Blackman & Eastop, 2000; Ramsey *et. al.*, 2007) only potato virus Y has been shown experimentally to infect leafy amaranth (Mureithi *et al.*, 2017). The impact of aphid feeding, such as leaf curling, leaf discoloration, presence of honeydew, sooty mold and excuviae, as well as the insects' presence on leaves, prevents a crop from being marketable as the injury and aphid presence are unacceptable to consumers.

Currently, management of *M. persicae* on leafy amaranth in Kenya is primarily through foliar applied insecticides (Nampeera *et al.*, 2019). However, improper application of insecticides may result in insecticide resistance, have harmful effects on biological control agents, secondary pest occurrences, soil and water pollution, human health risks (Ajayi *et al.*, 2011; Ahouangninou *et al.*, 2012; Macharia *et al.*, 2013; De Bon *et al.*, 2014; Grewal *et al.*, 2017, Hanson *et al.*, 2017). Finding alternative management practices is important as the consumption of leafy amaranth could expose humans to insecticides.

Plants with resistance to aphids have significant benefit over chemical insecticides for aphid management (Pierson *et al.*, 2010; Pierson *et al.*, 2011; Prochaska *et al.*, 2013). It reduces labor required by small-scale farmers to scout for the target pest, and also reduces the risks of insecticide exposure to humans and the environment (Smith & Chuang, 2014). Resistance to aphids has been found in the germplasm of many crop species, in the forms of antibiosis (affecting insect survival, growth, development and fecundity, due to morphological and chemical features of the plant), antixenosis (or non-preference, affecting the settling and feeding of insects) and tolerance (reducing plant damage even in the presence of aphids) (Painter, 1951; Frei *et al.,* 2003; Smith, 2005; Smith & Boyko, 2007; Smith & Clement, 2011; Zust & Agrawal, 2016). Aphids' salivary components induce defense responses in several crop species (De Vos & Jander, 2009), and their feeding habit (stylet insertion, salivation and sap ingestion) also allow them to avoid allelochemicals and indigestible compounds that are abundant in plant tissues (Schoonhoven *et al.*, 2007).

There is limited evidence that *Amaranthus* spp. possess resistance to insect herbivores. Some amaranth varieties exhibit varying levels of resistance to lepidopteran pests (Steven *et al.,* 2018). *Amaranthus caudatus* produces a lectin in the seeds (Rinderle *et al.,* 1989), which has aphicidal activity when applied to various crops such as tobacco, cotton and potato (Guo *et al.,* 2004; Wu *et al.,* 2006; Xin *et al.,* 2011). The effects of *M. persicae* on the new lines of amaranth which would form a basis for their use in management of *M. persicae* is uknown. The purpose of this study was therefore to screen *Amaranthus* varieties (Abuku 1-7) for resistance to *M. persicae.* The null hypothesis was that the new amaranth lines did not differ in their interaction with *Myzus persicae*.

4.3 Materials and methods

4.3.1 Study area

Experiments were conducted in insect-proof, polyethylene high tunnels at Jomo Kenyatta University of Agriculture and Technology (JKUAT) in Juja, Kenya (latitude 1.0891°S, longitude 37.0105°E, altitude 1525 m above sea level), with an average temperature of 27±7°C and 70-80% relative humidity.

4.3.2 Treatments and Experimental layout

Treatments used in the study consisted seven Abuku varieties belonging to three *Amaranthus spp.* As follows: Abuku varieties 1 and 2 (*Amaranthus blitum*), Abuku varieties 3, 4, 5 and 6 *(A. hybridus*) and Abuku selection 8 *(A. hypochondriacus*) (Table 4.1).

Table 4.1:Details of amaranths varieties used in the study

No	Variety	Full variety name	Botanical name	Status	Attributes (Leaf Size)		
					(Young Leaf: Length)	(Young Leaf: Width)	Young Leaf Ratio Length / Width
1.	Abuku 1	Abuku amaranth 1	*Amaranthus blitum*	Advanced line	Short to medium	Short to medium	Small to Medium
2.	Abuku 2	Abuku amaranth 2	*Amaranthus blitum*	Advanced line	Narrow to medium	Narrow to medium	Small to Medium
3.	Abuku 3	Abuku amaranth 3	*Amaranthus hybridus*	Advanced line	Medium to Long	Medium to broad	Medium to Large
4.	Abuku 4	Abuku amaranth 4	*Amaranthus hybridus*	Advanced line	Medium to Long	Narrow to Medium	Medium to Large
5.	Abuku 5	Abuku amaranth 5	*Amaranthus hybridus*	Advanced line	Medium to Long	Medium to Broad	Medium to Large
6.	Abuku 6	Abuku amaranth 6	*Amaranthus hybridus*	Advanced line	Medium to Long	Narrow to Medium	Medium to Large
7.	Abuku 8	Abuku amaranth 8	*Amaranthus hypocondriacus*	Advanced line	Medium to Long	Medium to Broad	Medium to Large

All of these varieties (Table 4.1) are grown for leaves and considered leafy amaranth, except Abuku 8 which is dual purpose. All varieties were obtained from the Department of Horticulture and Food Security, JKUAT. Since *A. dubius* was used for rearing *M. persicae* used in this study, variety Abuku 7 which is *A. dubius* was therefore not evaluated to avoid telescoping association (Dixon, 1998; Ogawa & Miura, 2014).

Three trials were carried out, in January - March (trial 1), April - June 2016, (trial 2) and July - September 2016 (trial 3). In each trial, the seven amaranth varieties were arranged in a completely randomized design (CRD) with three replications. The distance between each row was 60 cm and pots within a row were separated by 30 cm.

4.3.3 Rearing of *Myzus persicae*

The population of *Myzus persica* was initiated from adults collected on *Amaranth dubius,* an aphid-susceptible amaranth cultivar (Nampeera *et al.,* 2019) in the fields of International Centre of Insect Physiology and Ecology (ICIPE). The infested amaranth originally established from the fields of ICIPE was transferred into cages to allow a large population to develop. Using a fine paint brush, *M. persicae* from the cages were placed on new clean plants of *A. dubius*. The *Myzus persica* colony was maintained at temperature of 25±1ºC, relative humidity of 60-70% and 12-h light/12-h dark photoperiod at ICIPE, Nairobi, Kenya (latitude; 1°13'17. 9"S, longitude; 36°53'48.1 "E, at 1599 m above sea level).

4.3.4 Aphid infestation and damage assessment

Seven weeks (49 days) after planting, each plant was artificially infested with adult aphids from pure progeny using methods of Hill *et al. (*2004), Mensah *et al. (*2005), and Bansal *et al. (*2013). An infested leaf with 10 apterous adult (4 to 5 days old) *M. persicae* females was placed on the adaxial or upper surface of the middle, fully-expanded leaf of each plant.

Aphid populations were assessed one week after each plant was artificially infested. Aphid numbers were estimated by destructive sampling of a randomly selected plant from each of the seven varieties and replication. The aphid numbers were estimated every 7 d for five consecutive weeks. The sampling period of all treatments ended at five weeks to limit alate production by aphids, which would have resulted in aphids moving and re-colonizing plants within the high tunnel.

4.3.5 Aphid infestation and amaranth leaf area assessment

Samples were collected by removing all leaves of a selected amaranth plant, placing the leaves in brown paper envelopes labeled with corresponding replication and treatment information. The samples were transported to the laboratory in cooler boxes packed with ice. All aphids in the envelope were counted. Prior to data analysis log transformations ($\log_{10}$) were performed on aphid populations for each trial to ensure normality and homogeneity of variance.

Leaf damage symptoms were scored using rating scale from zero to four (modified from Mensah *et al.,* [2005]), with 0 = no aphids; 1≤ 100 *M. persicae* per plant (leaf appears normal and healthy); 2 = 101 to 300 *M. persicae* per plant (yellowish leaves); 3 = 301 to 800 *M. persicae* per plant (slight curling and sooty mold on leaves); and 4 ≥ 801 *M. persicae* per plant (brown leaves with a combination of curling, sooty mold and excuviae).

Leaf area (cm^2) of each individual selection was measured with a LI-COR Li-3000 leaf area meter (Li-Cor, Lincoln, NE). Specific leaf area (SLA) $(cm^2\, g^{-1})$ was estimated as the ratio of leaf area to leaf dry mass, similar to Freschet *et al. (*2015), Liu *et al. (*2016).

4.4 Data analysis

Statistical analyses were performed separately for each trial using R, version 3.43 (R Core Team, 2017.). Data from five weeks of sampling were combined at the end of each trial period and used in these analyses. Each time we measured the same variables to make the data more reliable. Analysis of Variance (ANOVA) was used to determine if the following parameters varied by amaranth varieties trials and the interaction (amaranth varieties trial): aphid densities, leaf damage score, SLA, infested and uninfested weight and percentage of fresh leaves when evaluating effects. Tukey's HSD test at 0.05 level of significance was used to separate the means and tested the differences of amaranth varieties in each trial and of each selection across the three trials, with varieties that were less than HSD test ($P \leq 0.05$) were considered significant. All analyses were performed in R version 3.4.3 (R Core Team, 2017) from the lme4 package (Bates *et al.*, 2013).

4.5 Results

The mean number of aphids, leaf damage score and SLA recorded for each of the seven varieties in each trial are presented in Table 4.2.

4.5.1 Influence of variety on Aphid densities

A significantly greater number of aphids per plant were observed in the second trial (April - June). The mean number of aphids was similar in the first (January - March) and third (July -September) trial. The aphid density varied significantly among varieties (F=7.3; df = 6, 294; P < 0.001) and among varieties of the same species. Overall, significant greater populations of aphids were observed on *A. hybridus* selection (Abuku 5) when compared with other *A. hybridus* varieties (3,4and 6) and *A. blitum* varieties (Abuku 1 and 2). Similarly, *A. hypocandracus* selection (Abuku 8) had significantly greater aphid populations in the third trial. Overall, *A. blitum* varieties (Abuku 1 and 2) had the lowest aphid population (Table 4.2). Significant difference of varieties on number of aphids also was observed among the three trials (First: F = 8.2; df = 6, 98; P < 0.001: Second: F = 3.1; df = 6, 98; P < 0.001 and Third: F = 2.3, df = 6, 98; P = 0.039) (Table 4.2). A significant interaction of trial by selection on number of aphids was also observed. Number of aphids on *A. hybridus* varieties (Abuku 3,4,5 and 6) was significantly different. The number of aphids on Abuku 3,4 and 6 were significantly different from Abuku 5. However, the number of aphids on Abuku 5 across the three trials was similar (Table 4.2). There was no significant different observed on the number aphids nn *A. blitum* varieties (Abuku 1 and 2) and *A. hypocandracus* selection (Abuku 8) across the three trials (Table 4.2).

Table 4.2: The number of aphids of seven amaranth varieties compared across and within three trials and within a mean of the trials after aphid infestation, 2016 (Mean and standard error of the mean (±) values.

Amaranth varieties	Trial^{z}			Interaction			Mean of Trials
	First	Second	Third	df,42	Fvalue	Pvalue	
Abuku 1	1.6 ± 0.2b^{b}	13.9 ± 2.0a^{b}	6.4 ± 0.9ab^{ab}	2	4.4	0.018	7.3 ± 1.0^{c}
Abuku 2	1.5 ± 0.2a^{b}	17.0 ± 2.5a^{b}	4.2 ± 0.6a^{b}	2	3.1	0.054	7.6 ± 1.1^{c}
Abuku 3	3.2 ± 0.4b^{b}	27.2 ± 4.0a^{b}	15.4 ± 2.2ab^{ab}	2	6.5	0.003	15.2 ± 2.2^{c}
Abuku 4	3.7 ± 0.5b^{b}	50.0 ± 7.4a^{ab}	9.0 ± 1.3b^{ab}	2	12.7	0.0001	20.9 ± 3.1b^{c}
Abuku 5	87.4 ± 13.0a^{a}	139.5 ± 20.7a^{a}	34.1 ± 5.0a^{ab}	2	2.2	0.114	87.0 ± 12.9^{a}
Abuku 6	7.9 ± 1.1b^{b}	57.3 ± 8.5a^{ab}	31.8 ± 4.7ab^{ab}	2	6.4	0.003	32.3 ± 4.8b^{c}
Abuku 8	13.7. ± 2.0a^{b}	87.9 ± 13.1a^{ab}	87.9 ± 13.1a^{a}	2	1.4	0.24	63.2 ± 9.4^{b}
df, 98	6	6	6				
F	8.2	3.1	2.3				
P	0. 001	0.007	0.039				

^{z} Trial dates; First (Jan. - Mar.), Second (Apr - June) and Third (July -Sept.), 2016, Means with the same lower-case letter within a row of the three trials are not significantly different, Tukey's HSD test, alpha = 0.05; Superscript means with the same lower –case within a column of the same trial and parameter are not significantly different, Tukey's HSD test, alpha = 0.05

4.5.2 Effect of aphid infestation on leaf damage score

Leaf damage score varied significantly across varieties ($F = 6.2$; df =6, 294; $P < 0.001$). Overall, a significantly greater leaf damage score was recorded in *A. hybridus* varieties (Abuku 5) and a lower leaf damage score was recorded in *A. blitum* varieties (Abuku 2) (Table 4.3). When analyzed by trial, significant differences of the varieties were observed in the first and second trial (First: $F = 5.6$; df = 6, 98; $P < 0.001$: Second; $F = 2.5$; df = 6, 98; $P = 0.023$). However, no significant difference was observed in leaf damage score and varieties of the third trial (Table 4.3). Significant interaction of trial and selection was also noted in the leaf damage score of *A. hybridus* varieties (Abuku 4). Leaf damage score of Abuku 4 in trial one was significantly different from that of trial two and three (Table 4.3).

Table 4.3: Leaf damage score of seven amaranth varieties compared across and within three trials and within a mean of the trials after aphid infestation, 2016 (Mean and standard error of the mean (±) values.

Amaranth Varieties	Trial^{z}			Interaction			Mean of Trials
	First	Second	Third	df,42	Fvalue	Pvalue	
Abuku 1	$0.6 \pm 0.08a^{b}$	$0.8 \pm 0.1a^{b}$	$0.8 \pm 0.1a^{a}$	2	1.2	0.301	0.80 ± 0.1^{bc}
Abuku 2	$0.4 \pm 0.05b^{b}$	$0.8 \pm 0.1a^{b}$	$0.5 \pm 0.07ab^{a}$	2	4.0	0.025	0.60 ± 0.0^{bc}
Abuku 3	$0.6 \pm 0.08a^{b}$	$0.9 \pm 0.1a^{ab}$	$0.6 \pm 0.08a^{a}$	2	2.4	0.094	0.73 ± 0.1^{bc}
Abuku 4	$0.3 \pm 0.04b^{b}$	$1.1 \pm 0.1a^{ab}$	$0.6 \pm 0.08a^{a}$	2	9.7	0.0003	0.71 ± 0.1^{bc}
Abuku 5	$1.2 \pm 0.1ab^{a}$	$1.5 \pm 0.2a^{a}$	$0.8 \pm 0.1b^{a}$	2	3.2	0.050	1.20 ± 0.1^{a}
Abuku 6	$0.7 \pm 0.1a^{ab}$	$1.0 \pm 0.1a^{ab}$	$0.8 \pm 0.1a^{a}$	2	1.6	0.199	0.86 ± 0.1^{abc}
Abuku 8	$0.8 \pm 0.1a^{ab}$	$1.0 \pm 0.1a^{ab}$	$1.0 \pm 0.1a^{a}$	2	0.4	0.62	1.0 ± 0.1^{ab}
df, 98	6	6	6				
F	5.6	2.5	1.5				
P	0.001	0.023	0.172				

^{z} Trial dates; First (Jan. - Mar.), Second (Apr. - June) and Third (July - Sept.), 2016,Means with the same lower-case letter within a row of the three trials are not significantly different, Tukey's HSD test, alpha = 0.05; Superscript means with the same lower –case within a column of the same trial and parameter are not significantly different, Tukey's HSD test, alpha = 0.05, ^{x} Leaf damage score: rating scale of 0 to 4, with 0 = no aphids; 1 ≤ 100 *M. persicae* per plant (leaf appears normal and healthy); 2 = 101 to 300 *M. persicae* per plant (yellowish leaves); 3 = 301 to 800 *M. persicae* per plant (slight curling and sooty mold on leaves); and 4 ≥ 801 *M. persicae* per plant (brown leaves with a combination of curling, sooty mold and excuviae).

4.5.3 Effect of aphid infestations on varieties and specific leaf area

Specific leaf area did not differ across trials (F = 2.4; df = 2, 294; P = 0.085), although, it varied among varieties (F = 2.8; df = 6, 294; P = 0.010). Overall, a significantly greater SLA of 122.6 ± 11.9 cm^{2-g} was noted in Abuku 8 and the lowest of 29.5 ±2.8 cm^{2-g} in Abuku 2 (Table 4.4). The interaction of SLA by trial and selection was also not significantly different (F = 0.74; df = 12, 294; P = 0.707).

Table 4.4: Specific leaf area (SLA) of seven amaranth varieties compared across and within three trials and within a mean of the trials after aphid infestation, 2016 (Mean and standard error of the mean (±) values.

Amaranth Varieties	**Trial^z**			**Interaction**			**Mean of Trials**
	First	Second	Third	df,42	Fvalue	Pvalue	
Abuku 1	67.1 ± 10.0a^a	24.8 ± 3.6a^a	34.1 ± 5.0a^a	2	1.5	0.22	42.0 ± 6.2^{ab}
Abuku 2	34.6 ± 5.1a^a	25.6 ± 3.8a^a	28.3 ± 4.2a^a	2	2.0	0.14	29.5 ± 4.3^b
Abuku 3	81.9 ± 12.2a^a	27.7 ± 4.1a^a	33.7 ± 5.0a^a	2	2.0	0.13	47.7 ± 7.1^{ab}
Abuku 4	45.9 ± 6.8a^a	29.7 ± 4.4a^a	30.3 ± 4.5a^a	2	2.2	0.11	35.3 ± 5.2^b
Abuku 5	45.2 ± 6.7a^a	30.1 ± 4.4a^a	32.8 ± 4.8a^a	2	0.9	0.37	36.0 ± 5.3^b
Abuku 6	43.1 ± 6.4a^a	30.7 ± 4.5a^a	30.3 ±4.5a^a	2	0.7	0.47	34.6 ± 5.1^b
Abuku 8	181.1 ± 26.9a^a	154.1 ± 22.9a^a	32.8 ± 4.8a^a	2	0.8	0.41	122.6 ± 18.2^a
df,98	6	6	6				
F	2.1	1.05	0.69				
P	0.057	0.392	0.655				

^{z} Trial dates; First (Jan. - Mar.), Second (Apr. - June) and Third (July -Sept.), 2016, Means with the same lower-case letter within a row of the three trials are not significantly different, Tukey's HSD test, alpha = 0.05; Superscript means with the same lower –case within a column of the same trial and parameter are not significantly different, Tukey's HSD test, alpha = 0.05).

4.5.4 Total leaf weight

Total leaf weight varied significantly among varieties (*F* = 7.3; df = 6, 294; *P* < 0.001). A significant greater total leaf weight was recorded in Abuku 3 and the lowest in Abuku 1 (Table 4.5). Significant differences of the varieties were only observed in the second (*F* = 4.3; df =6, 98; *P* < 0.001) and third (*F* =3.4; df = 6, 98; *P* < 0.001) trial. No significant differences were observed in total leaf weight among the varieties in the first trial (*F* = 2.1; df = 6, 98; *P* = 0.056) (Table 4.5). A significant greater total leaf weight was observed in Abuku 5 in the second trial and Abuku 3 in the third trial. The lowest total leaf weight was observed in Abuku 1 and Abuku 6 in the second and third trial (Table 4.5). The interaction of trial by selection was also significant among species and varieties of the same species. Significant difference of total leaf weight of *A. blitum* varieties (Abuku 1 and 2) and *A. hybridus* varieties (Abuku 3,5 and 6) was noted across the three trials (Table 4.5). *A. blitum* varieties Abuku one and two was not significantly different between each other across the three trials. Whereas *A. hybridus* varieties (Abuku 5) was significantly across the three trials (Table 4.5).

4.5.5 Weight of infested leaves

Significant differences were observed in the weight of infested leaves and varieties (*F*= 11.9; df = 6, 294; *P* < 0.001). Abuku 5 had a significant greater weight of infested leaves and Abuku 1 had the lowest weight of infested leaves when averaged across the three trials (Table 4.5). There were also significant differences observed among varieties of the first (*F* = 8.7; df = 6, 98; *P* < 0.001) second (*F* = 4.9; df = 6, 98; *P* < 0.001) and third (*F* = 3.7; df = 6, 98; *P* < 0.001) trial (Table 4.5). The interaction of trial by selection was also significantly different (*F* = 2.8; df = 12, 294; *P* < 0.001). Weight of infested leaves differed significantly across trials of Significant interaction of *A. blitum* selection (Abuku 1), *A. hybridus* varieties (Abuku3, 4 5 and5). Weight of infested leaves was not significantly different across trials of *A. blitum* variety (Abuku 2), *A. hybridus* variety (Abuku 6) and *A. hypocandracus* variety (Abuku 8) (Table 4.5)

Table 4.5: Total and infested leaf weights of seven amaranth varieties compared across and within three trials and within a mean of the trials after aphid infestation, 2016 (Mean and standard error of the mean (±) values).

Amaranth Varieties	**Trial^{z}** First	Second	Third	df,42	**Interaction** Fvalue	Pvalue	**Mean of Trials**
Total leaf weight (g)							
Abuku 1	28.1 ± 4.1b^{a}	45.6 ± 6.7a^{b}	22.7 ± 3.3b^{c}	2	8.3	0.0008	32.1 ± 4.7^{c}
Abuku 2	29.7 ± 4.4b^{a}	49.4 ± 7.3a^{ab}	30.7 ± 4.5b^{abc}	2	6.3	0.0039	36.6 ± 5.4^{bc}
Abuku 3	44.5 ± 6.6a^{a}	60.2 ± 8.9a^{ab}	44.5 ± 6.6a^{a}	2	3.4	0.0422	49.7 ± 7.4^{a}
Abuku 4	47.4 ± 7.0ab^{a}	58.8 ± 8.7a^{ab}	30.9 ± 4.6b^{abc}	2	5.3	0.0085	45.7 ± 6.8^{ab}
Abuku 5	47.2 ± 7.0b^{a}	65.7 ± 9.7a^{a}	30.8 ± 4.5c^{abc}	2	15.2	0.0001	47.9 ± 7.1^{ab}
Abuku 6	30.4 ± 4.5b^{a}	45.2 ± 6.7a^{b}	24.8 ± 3.6b^{bc}	2	6.8	0.0027	33.5 ± 4.9^{c}
Abuku 8	40.5 ± 6.0b^{a}	60.2 ± 8.9a^{ab}	43.1 ± 6.4ab^{ab}	2	3.8	0.029	47.9 ± 7.1^{ab}
df, 98	6	6	6				
F	2.1	4.3	3.4				
P	0.056	0.001	0.003				
Weight of infested leaves (g)							
Abuku 1	0.3 ± 0.04b^{b}	2.6 ± 0.3a^{b}	1.5 ± 0.2ab^{b}	2	6.9	0.0024	1.4 ± 0.2^{d}
Abuku 2	0.3 ± 0.04a^{b}	3.6 ± 0.5a^{b}	1.0 ± 0.1a^{b}	2	3.1	0.0511	1.6 ± 0.2^{cd}
Abuku 3	2.3 ± 0.3b^{b}	14.6 ± 2.1a^{ab}	8.6 ± 1.2ab^{ab}	2	5.4	0.007	8.5 ± 1.2^{b}
Abuku 4	2.8 ± 0.4b^{b}	17.0 ± 2.5a^{a}	3.2 ± 0.4b^{ab}	2	14.3	0.0001	7.6 ±1.1^{bc}
Abuku 5	20.9 ± 3.1a^{a}	19.9 ± 2.9ab^{a}	6.1 ± 0.9b^{ab}	2	4.0	0.0253	15.6 ± 2.3^{a}
Abuku 6	5.2 ± 0.7a^{b}	9.7 ± 1.4a^{ab}	6.1 ± 0.9a^{ab}	2	1.7	0.189	7.0 ± 1.0^{bcd}
Abuku 8	6.9 ± 1.0a^{b}	13.5 ± 2.0a^{ab}	11.3 ± 1.6a^{a}	2	1.0	0.343	10.5 ± 1.5^{ab}
df, 98	6	6	6				
F	8.7	4.9	3.7				
P	0.001	0.001	0.002				

^{z} Trial dates; First (Jan. - Mar.), Second (Apr - June) and Third (July - Sept.), 2016 Means with the same lower-case letter within a row of the three trials are not significantly different, Tukey's HSD test, alpha = 0.05; Superscript means with the same lower –case within a column of the same trial and parameter are not significantly different, Tukey's HSD test, alpha = 0.05

4.5.6 Proportion (%) of infested leaves

The proportion of leaves, varied across varieties ($F = 9.3$; df = 6, 294; $P < 0.001$). A significant greater percentage of uninfested leaf weight was observed in Abuku 1 and 2 and the lowest in Abuku 5. Whereas, a significant greater percentage of infested leaves was observed in Abuku 5 and the lowest in Abuku 1and 2 (Table 4.6). Significant differences were also observed among varieties of the first ($F = 5.1$; df = 6, 98; $P < 0.001$), second ($F = 4.8$; df = 6, 98; $P < 0.001$) and third ($F = 3.1$; df = 6, 98; $P < 0.001$) trial (Table 4.6). A Significant interaction of uninfested and infested leaf weight across the three trials was only noted in A. hybridus varieties (Abuku 4 and 5) (Table 4.6).

Table 4.6: Percentage infested leaf weight of seven amaranth varieties compared across and within three trials and within a mean of the trials after aphid infestation, 2016 (Mean and standard error of the mean (±) values).

Amaranth Varieties	Trial[z]			Interaction			Mean of Trials
	First	Second	Third	df,42	Fvalue	Pvalue	
Abuku 1	9.0 ± 1.3a[b]	5.9 ± 0.8a[c]	6.1 ± 0.9a[ab]	2	0.1	0.832	7.0 ± 1.0[c]
Abuku 2	0.9 ± 0.1a[b]	7.3 ± 1.0aB[bc]	4.9 ± 0.7a[b]	2	2.1	0.126	4.3 ± 0.6[c]
Abuku 3	11.3 ± 1.6a[b]	24.9 ± 3.7a[ab]	25.0 ± 3.7a[ab]	2	1.5	0.223	20.4 ± 3.4[ab]
Abuku 4	6.0 ± 0.8b[b]	29.2 ± 4.3a[a]	16.3 ± 2.4ab[ab]	2	5.6	0.006	17.2 ± 2.5[bc]
Abuku 5	44.4 ± 6.6a[a]	28.4 ± 4.2ab[a]	20.4 ± 3.0b[ab]	2	3.1	0.05	31.1 ± 4.6a
Abuku 6	20.7 ± 3.0a[ab]	21.4 ± 3.1a[abc]	27.1 ± 4.0a[a]	2	0.3	0.711	23.1 ± 3.4[ab]
Abuku 8	25.3 ± 3.7a[ab]	21.7 ± 3.2a[abc]	24.6 ± 3.6a[ab]	2	0.09	0.9	23.9 ± 3.5[ab]
df, 98	6	6	6				
F	5.1	4.8	3.1				
P	0.001	0.001	0.001				

z Trial dates; First (Jan. - Mar.), Second (Apr. - June) and Third (July - Sept.), 2016

Means with the same lower-case letter within a row of the three trials are not significantly different, Tukey's HSD test, alpha = 0.05; Superscript means with the same lower –case within a column of the same trial and parameter are not significantly different, Tukey's HSD test, alpha = 0.05

4.6 Discussion

4.6.1 Response of *Myzus persicae* to *amaranthus* spp.

The results highlight the importance of considering different amaranth species for developing commercial cultivars of leafy amaranth that may be resistant to *M. persicae*. By encouraging the cultivation of leafy amaranth that is resistant to *M. persicae*, farmers may be able to produce more marketable produce without the use of insecticides. The data demonstrated the significant variation in the response of *M. persicae* to the seven varieties. For example, *A. blitum* (Abuku 1 and 2) had the lowest aphid populations and leaf damage score. Of importance for farmers, Abuku 1, 2, 3 and 4 produced the greatest percentage of uninfested leaves, which are more marketable. Conversely, it was consistently observed that Abuku 5 had the highest aphid populations, damage scores and percentage of leaves infested by aphids.

Of the potential types of host plant resistance to insect pests described by Painter (1951) (antibiosis, and tolerance, non-preference, renamed antixenosis by Kogan and Ortman (1978), the mechanism(s) of aphid resistance in *A. blitum* is unknown. Aphids secrete salivary effector proteins that induce susceptibility (increase aphid performance) and suppress defense induction and phloem sealing in the host plant (Powel *et al.,* 2006; Prado & Tjallingii; 2007; Bansal *et al.,* 2014). Additionally, aphid honeydew alters plant defense (Schwartzberg & Tumlinson, 2014). The experimental design (i.e. artificial infestation of plants and free aphid movement on and among plants) did not allow to determine the type and mechanism of resistance in tested amaranth varieties. Some amaranth genotypes also exhibit varying levels of resistance to pests (Steven *et al.*, 2018). As regards plant foliage, amaranth genotypes possess different morphological characteristics such as leaf shape, sizes and presence of trichomes that have effect on pest density (Akaneme & Ani, 2013).

4.6.2 Effect of *amaranthus* leaf weight *on Myzus persicae*

Plants with longer periods of vegetative growth have higher aphid populations (Dixon, 1998). If varieties of *Amaranthus* tested in this study varied by phenology or growth stages, it may help explain the variations observed among the species in terms of aphid abundance and confirm the variations in several trials. Leafy amaranth cultivars are grown for aspects of vegetative stage growth (i.e, large leaves). Varieties with the heaviest leaves (e.g, Abuku 5) supported the greatest aphid populations while plants with the lightest leaves (e.g., Abuku 1 and 2) supported lower aphid populations.

Differences in leaf weight of the various varieties could be due to morphological or chemical characteristics independent of an aphid infestation (Akaneme & Ani, 2013; Steven *et al.,* 2018). Traits that are independent of the aphids' impact on the plant may be more important for farmers. For example, even though selection 1 had the lowest aphid populations, their smaller size leaves coupled with the low weight of uninfested leaves might result in lower yields and potential prices received. The greater leaf mass produced by Abuku 5 might compensate for the higher amount aphid infested leaves.

4.6.3 Relationship between *Myzus persicae* and *amaranthus* spp

Improving the understanding of the relationship between *M. persicae* and *amaranthus* will require additional study to determine to what extent the morphology and physiology of amaranth leaves affects *M. persicae*. Typically, amaranth species with larger leaves had greater leaf weight which may be preferable to consumers; but these varieties were more susceptible to *M. persicae*. The increasing demand and consumption of amaranth in Kenya (Onyango *et al.,* 2008) may lead to artificial selection of cultivars with larger leaves, which may lead to more aphid-susceptible leafy amaranth cultivars being grown. Although there may be incentives for breeders to select *Amaranthus* species with larger leaves, this study highlights the importance of considering aphid resistance that allow for the production of a marketable product. Identifying traits in Abuku 1 and 2 (*A. blitum*) that contributed to the low populations of

M. persicae could aid in future breeding programs. Because a number of genes conferring resistance to aphids have been identified in crops, including cowpea (Githiri *et al.*, 1996); lettuce (Eenink *et al.*, 1982); soybean (Chiozza *et al.*, 2010; Kim *et al.*, 2010a; 2010b; McCarville & O'Neal, 2012; Hesler *et al.*, 2013) and wheat (Bokyo *et al.*, 2004), determining the genes responsible for resistance to aphids in leafy amaranth could accelerate the breeding process. If genes for resistance *to M. persicae* are identified in the *A. blitum* genome, they could be introduced into cultivars through hybridization with the various species considered for commercial release (i.e. *A. hybridus*, and *A. hypochondriacus*). The genes responsible for lower damage of *M. persicae* in Abuku 3 and 4 and higher damage in Abuku 5 of the same species also should be understood.

4.6.4 Relationship between aphid infestation and fresh leaf yield

There is some reported evidence of a positive relationship between aphid infestation and yield (Tiffin, 2000**;** Riedell & Catangui, 2006; Kucharik *et al.*, 2016), suggesting some degree of overcompensation. Such compensation could result in tolerance to the feeding by a pest. Within the data Abuku 5 that resulted in high leaf biomass despite the occurrence of an aphid infestation. Evidence for tolerance may help expand the types of resistance breeders could include in future commercial cultivars.

CHAPTER FIVE

EFFECT OF SEED TREATMENT AND STORAGE DURATION ON SEED GERMINATION AND FRESH LEAF YIELD OF FOUR SPECIES OF *AMARANTHUS*

5.1 Abstract

The use of *amaranthus* as a vegetable crop is growing, with farmers interested in improving upon production using various pesticides to manage the pests. Application of pesticides on seed can provide benefits to production with reduced effect to the environment health, but the response varies by plant and active ingredient. The impact of two commercially available seed treatments on the germination and leaf yield of several *amaranthus* species cultivated in Kenya were explored. The effect of the seed treatments was explored 24 h after application and after three months of storage, a practice often used by subsistence farmers in Kenya. Germination and fresh leaf yield were evaluated in a growth chamber and high tunnel during 2016 and 2017. Several varieties of four *amaranthus* species (*amaranthus blitum, A. hybridus, A. dubius* and *A. hypochondriacus*) were tested, including eight advanced-breeding varieties and a local cultivar commonly grown by farmers (*A. dubius*). Seeds of each selection were treated individually with either a combination of thiamethoxam (20g/kg), metalaxyl-M (20g/kg), and difenoconazole (2 g/kg) (1.25ml ai/250g seed) or imidacloprid (233g/l), pencycuron (50g/l), and thiram (3 ml ai/250 g seed), Treated seeds germinated at a higher percentage than the untreated controls for only one of the Amaranthus varieties. A subset of the *amaranthus* varieties germinated at lower percentage when treated with imidacloprid, pencycuron and thiram. The germination of seeds and overall fresh leaf yield of 24 h of seed treatment were 1.6 times more than 3 m of seed treatment. A significant interaction of selection x seed treatment ($P \leq 0.0001$) and selection x seed treatment x storage time ($P = 0.005$) was observed in fresh leaf weight, indicating seed treatments did not provide a consistent improvement in yield. The value of these seed treatments may be limited for the improvement of Amaranthus leaf production.

5.2 Introduction

Amaranth is cultivated for both leaves and seeds in Africa, Central America, Southeast Asia, and South America, and North America respectively (Brenner *et al.*, 2000, Mlakar *et al.*, 2010, Trucco & Tranel, 2011). In Sub-Saharan Africa, growing of leafy amaranth has increased household consumption and commercial production (Achigan-Dako *et al.*, 2014).

Low yields of amaranth leaves (> 1.2 tons per hectare) have been attributed to varietal selection as well as environmental stressors including pests, poor agronomic conditions, poor seed germination, and poor storage by farmers saving seed (Ayieko & Tschirley, 2006; Abukutsa-Onyango, 2007; Achigan-Dako *et al.*, 2014; Nampeera *et al.*, 2019). Pesticides applied as seed treatments have helped to increase crop yields, facilitate earlier planting, produce vigorous crop that can handle environmental stress (Sekulic, 2015). The application of pesticides to seeds has been considered by some to reduce the risk of pesticides to the environment (Monfort *et al.*, 2006). For more than 30 years seed treatments have been a widely adopted practice in crop protection worldwide (Munkvold *et al.*, 2014). Seed treatments are currently used for a wide range of crops (Jeschke *et al.*, 2011) and various seed treatments have been used for the control of a wide range of pests (Bonham *et al.*, 2009). This rapid growth of seed treatment use is associated with reduced cost of application, efficiency in delivery system, and protection of seed and seedlings during initial, critical stages of growth.

Neonicotinoids, including clothianidin, imidacloprid and thiamethoxam are now the most widely used class of insecticides in the world for a variety of crops, with the majority of applications coming from seed treatments (Simon-Delso *et al.*, 2015; Douglas &Tooker, 2015). Benefits of neonicotinoids as seed treatments have been identified, including and not limited to providing a mode of action to manage pests that are resistant to other insecticides, selectively control insect pests, and limited impacts on beneficial insects (Ohnesorg *et al.*, 2009). The yield benefit of using neonicotinoids, can exceed the cost of treatment (North *et al.*, 2016; Hurley & Mitchel, 2017), however benefits may not always be realized depending upon growing conditions and pest pressure (Johnson *et al.*, 2009, EPA, 2014).

Fungicides applied as a seed treatment are also used by farmers to manage seed-borne and soil-borne pathogens that attack seed and seedlings (Bugingo, 2018). There are several examples of fungicides applied to seeds that have produced improvements in crop production. A Fungicide seed treatment, difeconazole + metalaxyl and thiram + carbathin, protected germinating wheat seeds and seedlings from early infection and resulted in increased yield (Schaafsma & Tamburic- Ilincic, 2005). Chili (*Capsicum annuum* L.) seeds treated with difenoconazole had improved quality of fruit and increased yield (Gopinath *et al.*, 2006). Application of difenoconazole to maize seed improved emergence (Munkvold & O'Mara, 2002). The combination of thiram and carboxim improved germination and emergence of maize seed (Southwell *et al.*, 2003). Combining fludioxonil, mefenoxam, azoxystrobin and metalaxyl prevented stand and yield losses of soybean (Bradley, 2008). Snap bean seed dressed with Cruiser® or Monceren® reduced bean fly infestation and increased snap bean yields (Kaburu, 2011). *Amaranthus retroflexus* seeds treated by sub lethal herbicides; atrazine (2-Chloro-4-ethylamino-6-isopropylamino-s-triazine) or tribenuron-methyl inhibited seed germination and seedling growth of *Amaranthus retroflexus* (Yue *et al.*, 2017).

Germination can vary greatly with Amaranthus cultivation, in part because farmers often store seed for up to three months from the previous harvests to the next growing season (Nampeera *et al.*, 2019). This problem might render the left-over seed not fit for production. The relationship between seed treatment and duration of storage of amaranth for the new varieties of amaranth is unknown. The effect of seed treatments and storage duration on germination and fresh leaf yield may provide seed companies with useful information to plan for duration of storage after seed treatment. Apron Star® and Monceren® are registered seed treatments (insecticide /fungicide) currently available to farmers in Kenya for protection against sucking insects and seedling diseases pests, including aphids (PCPB, 2018). Each of these seed treatments include a mixture of a neonicotinoid along with two fungicides. The effects of seed treatments and storage duration on seed germination and fresh leaf yield of amaranth varieties and local cultivar was explored 24 h and 3 months after seed treatment.

5.3 Materials and Methods

5.3.1 Study site

Pot experiments were conducted in a laboratory and high tunnels at Jomo Kenyatta University of Agriculture and Technology (JKUAT) in Juja, Kenya (Latitude 1.0891° S and Longitude 37.0105° E) during August to November, 2016 (first trial) and January to April, 2017 (second trial).

5.3.2 Plant material

Nine amaranth varieties from four amaranth species which included multiple varieties provided by the Department of Horticulture and Food Security at Jomo Kenyatta University of Agriculture and Technology (JKUAT) were used. These varieties included species of *amaranthus blitum* (Abuku varieties 1 and 2), *A. hybridus* (Abuku varieties 3 4, 5 and 6), *A. dubius* (Abuku variety 7) and *A. hypocandracus* (Abuku variety 8). In addition to these eight Abuku varieties that are under development for future commercial use, also selected was a cultivar that is used commercially, commonly known as Terere (*A.dubius*), obtained from Agrochemical dealers, Nairobi, Kenya

5.3.3 Seed treatments

Table 5.1: Summary of treatments

Factor	Treatments	Details
Seed Treatment	Apron®	Seeds were coated with thiamethoxam, metalaxyl and difenoconazole at 1.25mls ai/250g of seed
	Monceren®	Seeds were coated with imidacloprid, pencycuron and thiram at 3mls ai/250g of seed
	Untreated Seed	Seeds were mixed with water
Post Treatment Storage Time	None	Seeds were tested 24 hours after seed coating
	3 months	Seeds were tested 3 months after seed coating

Each of the nine *amaranthus* varieties was compared with a treatment of either Apron Star®, Monceren®, or an untreated control (Table 5.1). Monceren® was provided by Bayer Crop Science (East Africa) Ltd, Nairobi, Kenya, and Apron Star® was purchased from Agro-chemical dealer in Nairobi-Kenya, varieties. The components of Apron Star® included, one insecticide (thiamethoxam) and two fungicide combinations (metalaxyl-m and difenoconazole) and the rate of application was 1.25 ml ai/250g seed. Monceren® components one insecticide (imidacloprid) and two fungicide combinations (pencycuron and thiram) and the rate of application was 3 ml ai/250g of seed. We did not test the active ingredients individually since the seed treatments we used were only available commercially in combinations.

5.3.4 Seed Storage

Seed treatments were applied to each amaranth selection and local cultivar separately either before or after a three-month storage period. The 3 months storage period was selected based on the practices assessed by surveying farmers in rural and urban Kenya (Nampeera *et al.*, 2019). Each selection of amaranth was divided into three equal parts of 250g before storing the seeds. The first part was treated and stored, the second part was stored and treated 24 h before planting, and the third part was left untreated to serve as a control. Seeds of each selection were mixed by hand at the recommended rates in a round-bottomed container, wherein the seeds and the coating agent were stirred until the agents were evenly distributed on the seeds. Hand mixing continued for 10 min before allowing the seeds to air dry on a paper towel for 24 h. Each group of 250g of seeds were sealed in brown paper envelopes and stored for three months in a store room at 25 ± 1ºC, 60-70% RH and normal storage conditions.

5.3.5 Germination test

After the three-month storage period, fifty (50) seeds from each amaranth and seed treatment combination were placed in a 15 x 100 mm individual plastic petri dishes laid with a Whatman No. 42 filter paper and sprayed with water. The individual petri dishes were closed with a lid and then placed in a growth chamber at 25 ± 1°C for nine days. Each combination of amaranth with and without a seed treatment were replicated three times with each petri dish containing 50 seeds. The germination was described based on the methods of Hossain *et al. (*2005) with adjustments to the total number of seeds used per treatment and where the seeds were grown.

The total number of seeds used per treatment were 50, whereas, Hossain used 150 seeds in each treatment. The seeds were grown in the growth chamber, while Hossain grew one seed in each polybag. Seeds were observed every day following the first signs of radicle emergence until germination ceased for all seeds at nine days. On each day, the number of seeds with emerged radicles were counted as germinated. After recording, the counted germinated seed were removed by forceps and discarded daily. The petri dishes were watered on alternate days and thereafter covered and returned to the growth chamber to keep them moist. These data were reported as a germination percentage of the seeds that germinated at the end of the test (i.e. 9 days).

5.3.6 Fresh leaf yield tests

Fresh leaf yield was measured by growing amaranth in a high tunnel. The experiment was laid out in a completely randomized design (CRD) with three replications of each treatment combination. Each combination of the nine *amaranthus* varieties and 5 treatments (combination of seed treatments and storage time) were applied to an individual plastic pot. Five plants were planted so that a treatment is harvested at five different times. Each treatment and harvest time period were replicated three times for a total of 675 plants (9 varieties X 5 treatments X 5 harvest periods X 3 replications).

Before commencement of the trials, insects such as aphids, thrips, whiteflies and leaf miners were eliminated from experimental area (high tunnel) and surrounding areas by spraying with recommended insecticides; imidacloprid 100g/L + Betacyfluthrin 45g/L (Thunder OD 145 Oil Dispersal, Bayer AG, Germany, Bayer East Africa Ltd.). For mite elimination insecticide/miticide, abamectin 18g/L (Dynamec 1.8 EC, Syngenta Crop Protection, Syngenta East Africa Ltd) was used (PCPB, 2018).

To produce these potted plants, seeds were germinated within seedling trays (54 cm length x 28 cm width) containing a mix of forest soil, cow manure and sand (4:2:1 v/v) prepared for each selection of each treatment. Three weeks after germination in the nursery trays, three plants were transplanted into a plastic pot (17 cm diameter x 21 cm height) filled with the same mixture. To ensure constant supply of water to the seedlings, pots were placed in plastic bowls (27 cm diameter x 8.5 height). Watering was consistent and thoroughly done using a watering can throughout the experimental period. Two weeks after transplanting, plants were thinned to one plant per pot. A week after thinning, 1.5 g of calcium ammonium nitrate (26% N) was applied to each plant.

Amaranth is ready for harvesting between 20 to 45 days after transplanting or sowing, depending on the variety and harvest season (Ebert *et al.*, 2011). Harvesting started (35 days to 70 days after transplanting) by randomly selecting one plant representing each selection and treatment combination per replicate once a week for five consecutive weeks. The period of harvest was selected based on the period of harvest of amaranth as described by Ebert *et al. (*2011) with modifications to the start and end of the harvest period and the harvesting method. The extended period was limited to five weeks, to avoid the occurrence of plants senescing and leaves falling from individual plants, an indication that an amaranth plant had matured past the point of producing leaves for consumption. Data of the five weeks in 2016 from the three replications was consolidated and used in analyses. Similarly, data of the five weeks in 2017 from the three replications was also aggregated and used in analyses. Since the effects of time factor (weeks) were cumulative, multiple observations which were made on each treatment were not independent of each other, therefore, they were combined with each other at the end of the experimental period to avoid unbiased estimate of experimental error. Casler *et al.* (2015) reported that, correlation of multiple observations of each treatment, provide unbiased estimate of the experimental error.

The plants were harvested, placed into labelled brown paper bags in cool boxes packed with ice and taken to the laboratory for assessment. In the laboratory the leaves per each plant were counted, removed and fresh leaf weight in grams was determined using a balance. Leaf area (cm^2) of each selection and local cultivar was measured with a LI-COR Li-3000 leaf area meter (Li-Cor, Lincoln, NE). Specific leaf area (SLA) (cm^2 g^{-1}) was estimated as the amount of leaf surface per unit to fresh weight of leaves that was modified from Freschet *et al.* (2015).

5.4 Data Analysis

ANOVA was used to determine if percent germination, fresh leaf weight (g) and SLA parameters varied by amaranth species (e.g. *Amaranthus blitum, A. hybridus*, *A. dubius* and *A. hypochondriacus*), varieties within species(e.g. variety 1 and 2), seed treatments (Apron Star™ and Menceron™) storage time (duration of treatment) and their interactions: Tukey's HSD test at 0.05 level of significance was used to separate the means and also to test the differences of amaranth species and varieties within species, treatments, storage time (duration of treatment) and of each selection across the three treatments and two storage times. Varieties treatments and storage time that were less than HSD test ($P \leq 0.05$) were considered significant. All analyses were performed in R version 3.4.3 (R Core Team, 2017) from the lme4 package.

5.5 Results

5.5.1 Effect of seed treatments on germination

The percentage of seeds germinating varied significantly among varieties (F= 82.02; df = 8, 216; P < 0.0001). A significantly greater percentage of seeds germinating was observed in *A. blitum* (Abuku one and two) and a lower in *A. hybridus* (Abuku 4), *A.dubius* (Abuku seven and Terere) and *A. hypochondriacus* (Abuku 8). The percentage of seeds germinating also varied among treatments (F = 18.4; df = 2, 216; P < 0.0001). A significantly greater percentage of germinating seed was observed in seeds treated with Apron Star® and a lower with Monceren® treated seeds. The interaction of seeds germinating among varieties and treatments (F = 6.5; df = 16, 216; P < 0.0001). A significantly greater germination seed of Apron Star® and Monceren® treated seeds was observed in *A. blitum* (Abuku 1 and 2) (Table 5.2). Whereas, a significant lower germination of seed was observed in *A. hybridus* (Abuku 4), *A. hypochondriacus* (Abuku 8) and *A.dubius* (Terere) of Apron Star® treated seeds, while in Monceren® treated seed a significant lower germination of seeds was observed in *A.dubius* (Abuku 7 and Terere) and *A. hypochondriacus* (Abuku 8) (Table 5.2). A significantly greater germination of seeds was observed in *A. blitum* (Abuku 2) and a significant lower germination of seeds were observed in *A.dubius* (Abuku 7 and Terere) of the untreated seeds (Table 5.2). A Significant interaction of germinating seed across treatments was noted in *A. blitum* varieties (Abuku 1 and 2) and *A. hypochondriacus* (Abuku 8) (Table 5.2).

Table 5.2: Percent germinating seeds of eight amaranth varieties and a local cultivar compared across and within three treatments and within a mean of treatments and varieties (Mean and standard error of the mean (±) values).

Amaranth		**Treatments**			**Interaction**		**Treatment Means**
Varieties and LC	Apron	Merceron	untreated	df,33	Fvalue	Pvalue	
Abuku 1^{z}	61.6 ± 10.2a^{a}	30.3 ± 5.0b^{a}	29.1 ± 4.8b^{a}	2	10.82	0.0002	40.3 ± 3.8^{a}
Abuku 2	51.5 ± 8.5a^{a}	24.6 ± 4.1b^{a}	34.6 ± 5.7b^{ab}	2	11.47	0.0001	36.9 ± 3.5^{a}
Abuku 3	22.0 ± 3.6a^{b}	19.9 ± 3.3a^{ab}	20.5 ± 3.4a^{bc}	2	0.07	0.933	20.8 ± 2.0^{b}
Abuku 4	2.7 ± 0.4a^{c}	4.3 ± 0.7a^{bc}	4.5 ± 0.7a^{de}	2	0.38	0.687	3.8 ± 0.3^{cd}
Abuku 5	15.8 ± 2.6a^{bc}	17.3 ± 2.8a^{ab}	22.6 ± 3.7a^{abc}	2	0.767	0.472	18.6 ± 1.7^{b}
Abuku 6	12.4 ± 2.0a^{bc}	4.0 ± 0.6a^{bc}	15.3 ± 2.5a^{cd}	2	3.049	0.0609	10.5 ± 1.1^{c}
Abuku 7	6.5 ± 1.0a^{bc}	1.1 ± 0.1a^{c}	1.5 ± 0.2a^{e}	2	1.293	0.288	3.0 ± 0.2^{d}
Abuku 8	2.1 ± 0.3ab^{c}	0 ± 0b^{c}	3.0 ± 0.5a^{de}	2	3.962	0.0287	1.7 ± 0.1^{d}
Terere^{c}	1.5 ± 0.2a^{c}	0.8 ± 0.1a^{c}	1.6 ± 0.2a^{e}	2	0.496	0.614	1.3 ± 0.1^{d}
Variety Means	19.6 ± 3.2a	11.3 ± 1.8b	14.7 ± 2.4ab				
F	37.5	10.77	19.2				
df, 99	8	8	8				
P	0.0001	0.0001	0.0001				

^{z} varieties included selection 1-8 and a local cultivar, ^{c}Terere, coated in Apron or Monceren as separate coating solutions. Means with the same lower-case letter within a row of the three treatments are not significantly different, Tukey's HSD test, alpha = 0.05; Superscript means with the same lower –case within a column of the same treatment are not significantly different, Tukey's HSD test, alpha = 0.05. L/C, Local cultivar. Data of two years was combined because it was not significant

5.5.2 Effect of storage period on germinating seed

There was significant difference in storage period of germination of seeds (F = 41.9; df = 1, 216; P < 0.0001). Seeds planted after 24h of seed treatment had a significantly greater seeds germinating than seeds of 3months of seed treatment. The interaction of seeds germinating among varieties and storage time was also significant (F= 5.3; df = 8, 216; P < 0.0001). At 24 h of seed treatment a significantly greater germination of seeds was observed in *A. blitum* (Abuku 1 and 2), whereas, a significantly lower germination of seeds was observed in *A. hybridus* (Abuku 4), *A. dubius* (Abuku 7 and Terere) and *A. hypochondriacus* (Abuku 8) (Table 5.3). At 3months of seed treatment a significantly greater germination of seeds was observed in *A. blitum* (Abuku 1 and 2), whereas, a significantly lower germination of seeds was observed in *A. hybridus* (Abuku 4), *A. hypochondriacus* (Abuku 8) and *A. dubius* (Terere). A significant interaction of germinating seed across storage periods was noted in *A. blitum* (Abuku 1) and *A. hybridus* (Abuku 3,4 and5).

Table 5.3: Percent germinating seeds of eight amaranth varieties and a local cultivar compared across and within two storage periods and within a mean of storage period and varieties (Mean and standard error of the mean (±) values).

Amaranth Variety and L/ C	Storage Periods 24 hours	3 months	Interaction df,33	Fvalue	Pvalue	Storage Period Means
Abuku 1^{z}	50.5 ± 8.4a^{a}	30.2 ± 5.0b^{a}	1	7.496	0.0097	40.3 ± 3.8^{a}
Abuku 2	41.0 ± 6.8a^{a}	32.9 ± 5.4a^{a}	1	1.937	0.173	36.9 ± 3.5^{a}
Abuku 3	27.0 ± 4.5a^{b}	14.6 ± 2.4b^{b}	1	9.152	0.0047	20.8 ± 2.0^{b}
Abuku 4	6.0 ± 1.0ac^{c}	1.7 ± 0.2b^{c}	1	6.496	0.0155	3.8 ± 0.3^{d}
Abuku 5	26.1 ± 4.3a^{b}	11.1 ± 1.8b^{bc}	1	13.93	0.0006	18.6 ± 1.7^{bc}
Abuku 6	14.3 ± 2.3a^{bc}	6.8 ± 1.1a^{bc}	1	3.569	0.0674	10.5 ± 1.1^{cd}
Abuku 7	1.6 ± 0.2a^{c}	4.5 ± 0.7a^{bc}	1	0.895	0.351	3.0 ± 0.2^{d}
Abuku 8	1.7 ± 0.2a^{c}	1.6 ± 0.2a^{c}	1	0.013	0.911	1.7 ± 0.1^{d}
Terere^{c}	1.3 ± 0.2a^{c}	1.3 ± 0.2a^{c}	1	0	1	1.3 ± 0.1^{d}
Means	18.8 ± 3.1a	11.6 ± 1.9b				
F	38.9	18.1				
df, 153	8	8				
P	0.0001	0.0001				

varieties included Abuku 1-8 and L/C-local cultivar, ^{c}Terere, planted 24h and 3m of seed treatment. Means with the same lower-case letter within a row of the two storage periods are not significantly different, Tukey's HSD test, alpha = 0.05; Superscript means with the same lower –case within a column of the same storage time are not significantly different, Tukey's HSD test, alpha = 0.05. Data of two years was combined; it was not significant.

5.5.3 Effect seed treatment and storage period on fresh leaf weight of amaranth, 2016

At 24 h of seed treatment, the fresh leaf weight differed significantly with varieties (F =18.03; df =8,377; P < 0.0001). *Amaranthus hybridus* (variety 6) and *A.dubius* (Terere) had significantly a greater fresh leaf weight and *A. hypochondriacus* (Abuku 8) a lower fresh weight. Fresh weight of leaves also different significantly among treatments (F =49.4; df = 8,377; P < 0.0001). Seeds treated with Apron® had significantly greater fresh leaf weight and a lower was observed in untreated seed (Table 5.4). The interaction of selection x treatment was on significant (F =9.97; df =16,377; P < 0.0001). (Table 5.4). Significant difference was noted in *A. hybridus* (Abuku; 3, 5, 6 and 4) when treated with Merceron®. Abuku 3,5 and 6 of *A. hybridus* were significantly different from Abuku 4 (Table 5.4). When analyzed by species, there was no significant difference of fresh weight of leaves of *A. blitum* (Abuku 1 and 2), *A. hybridus* (Abuku; 3,4,5 and 6) and *A.dubius* (Abuku 7 and Terere) when treated with Apron®. Similarly, there was no significant difference in fresh leaves of *A. blitum* (Abuku 1 and 2) and *A.dubius* (Abuku 7 and Terere) when treated with Merceron®.

At 3 months, varieties differed significantly with fresh leaf weight (F =20.3; df = 8,377; P < 0.0001), *Amaranthus hybridus* (Abuku 6) and *A. dubius* (Terere) had a significant greater leaf weight and a lower fresh leaf weight was observed in *A. hypochondriacus* (Abuku 8). There was also significant difference observed with treatments and fresh leaf weight (F =46.9; df =2,377; P < 0.0001). The interaction of varieties x treatments and fresh leaf weight was also significant (F =7.5; df =16,377; P < 0.0001). The interaction of fresh leaf weight across treatments and varieties were all significant (Table 5.4). Significant difference was observed in *A. hybridus* (Abuku 3, 5, 6, and 7) when treated with Apron®. Abuku four was significantly different from Abuku three, five and six when treated with Apron® (Table 5.4). Similarly, *A. dubius* (Abuku 7 and Terere) were significantly different from each other when treated with Apron® (Table 5.4). Fresh leaf weight of *A. hypochondriacus* (Abuku 8) was significantly different with *A. blitum*, *A. hybridus* and *A. dubius* when treated with Merceron® (Table 5.4)

Table 5.4: Fresh leaf weights of eight amaranth varieties and local cultivar compared across and within three treatments after 24 hour and three months of seed treatment and within a mean of treatment and varieties in 2016 (Mean and standards error of the mean(±) values)

Amaranth Var and L/ C and		**Treatments**				**Interaction**	
Storage Period	Apron	Merceron	untreated	df,42	Fvalue	Pvalue	Treatment Means
24hours							
Abuku 1^{z}	43.0 ± 7.1a^{a}	27.2 ± 4.5b^{bc}	19.5 ± 3.2b^{ab}	2	8.8	0.0006	30.0 ±2.8^{bc}
Abuku 2	47.8 ± 7.9a^{a}	34.0 ± 5.6ab^{b}	18.8 ± 3.1b^{ab}	2	10.24	0.0002	33.5 ± 3.2^{bc}
Abuku 3	50.3 ± 8.3a^{a}	38.9 ± 6.4ab^{ab}	24.9 ± 4.1b^{ab}	2	5.121	0.0102	38.0 ± 3.6^{ab}
Abuku 4	49.2 ± 8.2a^{a}	10.7 ± 1.7b^{cd}	21.3 ±3.5b^{ab}	2	21.28	0.0001	27.1 ± 2.6^{c}
Abuku 5	42.2 ± 7.0a^{a}	43.0 ± 7.1a^{ab}	18.7 ± 3.1b^{ab}	2	20.99	0.0001	34.6 ± 3.3^{bc}
Abuku 6	47.2 ± 7.8a^{a}	41.7 ± 6.9a^{ab}	25.2 ± 4.2b^{ab}	2	9.6	0.0003	38.0 ± 3.6^{ab}
Abuku 7	33.6± 5.6a^{a}	39.9 ± 6.6a^{ab}	13.1 ± 2.1b^{b}	2	10.22	0.0002	28.9 ± 2.7^{bc}
Abuku 8	0 ± 0b^{b}	0 ± 0b^{d}	30.8 ± 5.1a^{a}	2	123.3	0.0001	10.2 ± 0.9^{d}
Tererec	51.7 ± 8.6a^{a}	57.9 ± 9.64a^{a}	25.2 ± 4.2b^{ab}	2	28.36	0.0001	45.0 ± 4.3^{a}
Var. Means	40.5 ± 5.5a^{a}	32.6 ± 4.4b	21.9 ± 2.9c				
F	12.2	16.9	3.5				
df, 126	8	8	8				
P	0.0001	0.0001	0.0001				
3months							
Abuku 1	25.5 ±4.2ab^{bc}	36.6 ± 6.1a^{ab}	14.4 ± 2.4b^{bc}	2	9.867	0.0003	25.9 ± 2.4^{bc}
Abuku 2	32.9 ±5.4a^{abc}	34.2± 5.7ab^{ab}	16.1 ± 2.6b^{abc}	2	6.631	0.0031	27.7 ± 2.6^{bc}
Abuku 3	42.5 ± 7.0a^{ab}	40.6 ± 6.7a^{ab}	21.5 ± 3.5b^{ab}	2	4.96	0.0115	34.5 ± 3.3^{ab}
Abuku 4	13.0 ± 2.1a^{cd}	25.8 ± 4.3a^{b}	16.6 ± 2.7a^{abc}	2	3.131	0.054	18.4 ± 1.7^{c}
Abuku 5	30.2 ± 5.0a^{abc}	32.8 ± 5.4a^{ab}	12.2 ± 2.0b^{bc}	2	13.97	0.0001	25.4 ± 2.4^{bc}
Abuku 6	46.3 ± 7.7a^{a}	50.5 ± 8.4a^{a}	19.1 ± 3.1b^{ab}	2	17.81	0.0001	38.6 ± 3.7^{a}
Abuku 7	19.2 ± 3.2b^{cd}	37.3 ± 6.2a^{ab}	8.1 ± 1.3b^{c}	2	11.19	0.0001	21.5 ± 2.0^{c}
Abuku 8	0 ± 0b^{d}	0 ± 0b^{c}	26.0 ± 4.3a^{a}	2	64.92	0.0001	8.6 ± 0.8^{d}
Terere^{c}	50.2 ± 8.3a^{a}	49.9 ± 8.3a^{a}	18.9 ± 3.1b^{ab}	2	23.8	0.0001	39.7 ± 3.9^{a}
Var. Means	28.9 ± 3.9b	34.2 ± 4.6a	17.1 ± 2.3c				
F	12.9	12.8	4.7				
df, 126	8	8	8				
P	0.0001	0.0001	0.0001				

^{z}Varieties included selection 1-8 and a local cultivar, ^{c}Terere, coated in Apron Star or Monceren as separate coating solutions. Means with the same lower-case letter within a row of the three treatments of the same storage period are not significantly different, Tukey's HSD test, alpha = 0.05; Superscript means with the same lower –case within a column of the same treatment and storage time are not significantly different, Tukey's HSD test, alpha = 0.05

5.5.4 Effect seed treatment, storage period on fresh leaf weight of amaranth seed 2017

At 24 h of seed treatment, there was significancy difference of varieties and fresh leaf weight (F =27.8; df =8,378; P < 0.0001). *A. hybridus* (Abuku 3) had a significant greater fresh leaf weight and *A. hypochondriacus* (Abuku 8) a lower fresh leaf weight. There was also significant difference of treatments with fresh weight (F =40.8; df =2,378; P < 0.0001). Seeds treated with Apron® and Monceren® had a significantly greater leaf weight than untreated seeds (Table 5.5). The interaction of selection and treatment with fresh weight was also significant (F = 3.6; df =16,378; P < 0.0001). Significant difference was noted with fresh leaf weight of *A. hybridus* varieties *(*Abuku 3, 4, 5 and 6*)* when treated with Apron®, Monceren® or untreated. Fresh leaf weight of Abuku three was significantly different from Abuku five when treated with Apron® and Monceren®. Similarly, fresh leaf weight of untreated seed of Abuku three was also significantly different with that of Abuku six (Table 5.5). Significant difference of fresh leaf weight was also noted with *A. dubius* varieties (Abuku 7 and Terere) when treated with Monceren® (Table 5.5). There was no significant difference in fresh leaf weight of *A. blitum* varieties (Abuku 1 and 2) when treated with Apron®, Monceren® or untreated seed. There was also no significant difference of fresh weight of *A. dubius* (Abuku 7 and Terere) when treated with Apron® or untreated. t 3months of seed treatment, variety differed significantly with fresh leaf weight (F =31.9; df =8,378; P < 0.0001). *A. hybridus* (Abuku 4) had a significant greater fresh leaf weight and *A. hypochondriacus* (Abuku 8) a lower fresh leaf weight. Treatments also differed significantly with fresh leaf weight (F =107.9; df =2,378; P < 0.0001). Seeds treated with Monceren® had a significantly greater fresh leaf weight and a lower fresh leaf weight was observed in untreated seed (Table 5.5). There was significant difference of selection and treatment (F =6.3; df =16,378; P < 0.0001). Significant difference was only noted with fresh leaf weight of *A. hybridus* varieties *(*Abuku 3, 4, 5 and 6*)* when treated with Apron®, Monceren® or untreated. The fresh leaf weight of Abuku four was significantly different from that of Abuku three, five and six when either treated with Apron®, Monceren® or untreated seed (Table 5.5)

Table 5.5: Fresh leaf weights of eight amaranth varieties and a local cultivar compared across and within three treatments after 24 hour and 3 month of seed treatment and within a mean of treatments and varieties in 2017 (Mean and standard error of the mean (±) values).

Amaranth Var.and L/ C and Storage Period	Treatments Apron	Merceron	untreated	df,42	Interaction Fvalue
24 hours					
Abuku 1[z]	8.2 ± 1.3a^{bcd}	9.2 ± 1.5a^{cde}	2.8 ± 0.4b^{bc}	2	4.8
Abuku 2	5.2 ± 0.8ab^{cd}	5.0 ± 0.8a^{de}	2.2 ± 0.3a^{bc}	2	2.3
Abuku 3	27.4 ± 4.5a^{a}	23.8 ± 3.9ab^{a}	13.0 ± 2.1b^{a}	2	5.63
Abuku 4	18.3 ± 3.0ab^{ab}	23.1 ± 3.8a^{a}	8.5 ± 1.4b^{ab}	2	5.56
Abuku 5	14.8 ± 2.4a^{bc}	12.8 ± 2.1a^{bcd}	8.3 ± 1.3a^{abc}	2	2.95
Abuku 6	17.2 ± 2.8a^{ab}	22.2 ± 3.7a^{ab}	4.8 ± 0.8b^{bc}	2	11.04
Abuku 7	12.3 ± 2.0a^{bc}	4.3 ± 0.7b^{de}	0.8 ± 0.1b^{c}	2	9.56
Abuku 8	0 ± 0b^{d}	0 ± 0b^{e}	4.1± 0.6a^{bc}	2	12.25
Terere[c]	18.2 ± 3.0a^{ab}	17.2 ± 2.8a^{abc}	3.7 ± 0.6b^{bc}	2	22.4
Var. Means	13.5 ± 1.8a	13.0 ± 1.7a	5.3 ± 0.7b	2	
F	11.5	15.6	5		
df, 126	8	8	8		
P	0.0001	0.0001	0.0001		
3 months					
Abuku 1	6.0 ± 1.0ab^{de}	10.7 ± 1.7a^{cd}	1.7 ± 0.2b^{bc}	2	8.18
Abuku 2	5.9 ± 0.9a^{de}	8.0 ± 1.3ab^{cd}	1.3 ± 0.2b^{bc}	2	4.97
Abuku 3	18.3 ± 3.0a^{bc}	26.0 ± 4.3a^{ab}	8.2 ± 1.3b^{a}	2	14.55
Abuku 4	28.4 ± 4.7a^{a}	32.2 ± 5.3a^{a}	4.7 ± 0.7b^{ab}	2	20.84
Abuku 5	11.8 ± 1.9a^{cd}	18.3 ± 3.0b^{bc}	4.0 ± 0.6c^{bc}	2	23.03
Abuku 6	18.7 ± 3.1a^{bc}	16.4 ± 2.7a^{bc}	2.9 ± 0.4b^{bc}	2	19.26
Abuku 7	8.8 ± 1.4a^{de}	15.6 ± 2.6a^{bc}	0.4 ± 0.06b^{c}	2	11.54
Abuku 8	0 ± 0b^{e}	0 ± 0b^{d}	2.8 ± 0.4a^{bc}	2	9.85
Terere[c]	21.0 ± 3.5a^{ab}	19.3 ± 3.2a^{bc}	1.9 ± 0.3b^{bc}	2	33.07
Sel. Means	13.2 ± 1.7b	16.3 ± 2.2a	3.1 ± 0.4c	2	
F	19.4	12.8	8		
df, 126	8	8	8		
P	0.0001	0.0001	0.0001		

Varieties included selection 1-8 and a local cultivar, [c]Terere, coated in Apron Star or Monceren as separate coating solutions. Means with the same lower-case letter within a row of the three treatments of the same storage period are not significantly different, Tukey's HSD test, alpha = 0.05; Superscript means with the same lower –case within a column of the same treatment and storage time are not significantly different, Tukey's HSD test, alpha = 0.05

5.5.5 Effect seed treatment, storage period on Specific Leaf Area (SLA)

A significant greater SLA of 22.7 cm2 g^{-1} was noted in the second trial, 2017 and a least of 14.9 cm2 g^{-1} in the first trial, 2016. Significant difference was observed in the varieties (F =5.9, df = 8, 1469; P < 0.0001). A significant greater SLA that ranged from 14.8 to 26.8cm2 g^{-1} was observed in Abuku 1 through 7 and Terere. A least SLA of 3.0 cm2 g^{-1} was noted in Abuku 8. There was no significant difference that was observed among treatments (F =1.3; df =2, 1469; P = 0.25). A significant greater SLA was observed in A. hybridus (Abuku 3) for both Apron and Monceren treated seeds and a lower in *A. hypochondriacus* (Abuku 8). There was no significant difference in SLA of untreated seeds (Table 5.6). The interaction of SLA across treatments and varieties was not significant (Table 5.6)

Table 5.6: Specific Leaf Area of eight amaranth varieties and a local cultivar compared across and within three treatments and within a mean of treatments and varieties (Mean and standard error of the mean (±) values).

Amaranth Variety and LC	Treatments			Interaction			Treatment Means
	Apron	Merceron	untreated	df,174	Fvalue	Pvalue	
Abuku 1[z]	16.2 ± 2.7a[c]	17.9 ± 2.9a[a]	41.6 ± 6.9a[a]	2	1.4	0.227	25.2 ± 2.4[a]
Abuku 2	14.8 ± 2.4a[c]	21.8 ± 3.6a[a]	15.9 ±2.6a[a]	2	0.4	0.631	17.5 ± 1.6[a]
Abuku 3	29.3 ± 14.8a[a]	26.3 ± 4.3a[a]	24.8 ± 4.1a[a]	2	0.5	0.603	26.8 ± 2.5[a]
Abuku 4	18.7 ± 3.1a[bc]	15.0 ± 2.5a[ab]	19.0 ± 3.1a[a]	2	2.1	0.122	17.5 ± 1.6[a]
Abuku 5	20.5 ± 3.4a[abc]	20.4 ± 3.4a[a]	14.8 ± 2.4b[a]	2	8.3	0.0003	18.6 ± 1.7[a]
Abuku 6	17.8 ± 2.9a[bc]	18.0 ± 3.0a[a]	14.8 ± 2.4a[a]	2	3.4	0.033	16.9 ± 1.6[a]
Abuku 7	16.1 ± 2.6ab[c]	18.0 ± 3.0a[a]	9.9 ± 1.6b[a]	2	5.2	0.006	14.8 ± 1.4[ab]
Abuku 8	0 ± 0b[d]	0 ± 0b[b]	14.2 ± 2.3a[a]	2	109.9	0.0001	3.0 ± 0.2[a]
Tererec	26.4 ± 4.4a[ab]	21.5 ± 3.5a[a]	31.0 ± 5.1a[a]	2	0.9	0.403	26.3 ± 5.1[a]
Variety Means	17.8 ± 2.9a	17.7 ± 2.9a	21.2 ± 3.5a				
F	15.5	4.5	1.6				
df, 531	8	8	8				
P	0.0001	0.0001	0.11				

[v] Varieties included selection 1-8 and a local cultivar, [c]Terere, coated in Apron Star or Monceren as separate coating solutions. Means with the same lower-case letter within a row of the three treatments are not significantly different, Tukey's HSD test, alpha = 0.05; Superscript means with the same lower –case within a column of the same treatment are not significantly different, Tukey's HSD test, alpha = 0.05. Data of two years was combined because it was not significant.

5.6 Discussion

The response of amaranth to a seed treatment varied among species. This variable response was noted in the germination percentage, leaf weight and SLA. An extended time of harvest for treated versus untreated plants for some species of *amaranthus* was also observed. Seed planted 24 h and 3 m of Apron® and Monceren® seed treatments improved germination and amaranth fresh yield, more than the untreated seed. Seed treatment improved aspects of amaranth cultivation, such as higher germination percentage, leaf weight and SLA, in addition to extended time of harvest than untreated in some species of *amaranthus,* whereas in other species seed treatment did not improve the aspects of amaranth cultivation. Low germination percentage was observed with untreated seed. This might have been the seed lot used, length of storage period of the seed lot, the harvesting time of the seeds used or the processing methods used.

5.6.1 Effect of treatments and storage period on germinating seed

Seed treatments improved germination of *A. blitum* more than *A. hybridus, A. dubius, A. hypocandracus* and untreated control. This might be due to genetic variation in *amaranthus* species and varieties within species (Achigan- Dako *et al.*, 2014), morphology characteristics (Trucco & Tranel, 2011; Das, 2012). Seed planted 24 h and 3 months of seed treatment improved germination more than the untreated seed. Seeds treated with Apron® and grown after 24 hours of seed treatment improved germination above Monceren® seed treatments. The difference in percentage germination of Apron® and Monceren ® seed treatments could be attributed to the active ingredient found in Apron Star® (thiamethoxam) and Menceron® (imidacloprid) that might have affected germination.

Treatment with thiamethoxam increased germination of seeds on moistened paper in a laboratory at 13 to 25°C (Almeida *et al.*, 2013). Rice seeds also treated with thiamethoxam increased the rate of seedling emergence (Lanka *et al.*, 2017). Plant germination and seedling growth stimulation by thiamethoxam seed treatment has also been reported in carrot (Almeida *et al.*, 2012), maize (Afifi *et al.*, 2015), soybean (Stamm *et al.*, 2014), rice (Almeida *et al.*, 2013,) and wheat (Larsen & Falk 2013). However, the mechanism by which thiamethoxam and imidacloprid stimulated seed germination and seedling growth is not fully understood. Ford *et al. (*2010) characterized the effects of thiamethoxam on the physiology of Arabidopsis thaliana L., and established that transcriptional response in plants treated with imidacloprid and clothianidin (a metabolic product of thiamethoxam) was similar to that induced by salicylic acid. Maize seeds treated with thiamethoxam stimulated synthesis of gibberellic acid and inhibited synthesis of abscisic acid and thus changed germination patterns (Afifi *et al.*, 2015).

Neonicotinoid insecticides stimulated seedling growth. Ding *et al. (*2018), reported stimulatory effects of neonicotinoids on seedling growth on corn with thiamethoxam, clothianidin and imidacloprid. Neonicotinoid insecticides increase molecular seed components and their activities, such as antioxidant, phenolic contents and glucose-6-phosphate dehydrogenase and guaiacol peroxidase activity. The seed components activities strengthen the ability of crops to defend themselves against exogenous disturbances (Duan *et al.*, 2012; Tang *et al.*, 2017). Thus, imidacloprid and thiamethoxam seed treatments might have effects on plant physiology, such as plant genotype and other factors.

The germination performance of Apron® treated seeds that achieved higher germination 3 months after seed storage might be the result of an effect of the seed-coating mixtures used. Neonicotinoid seed treatments can have effects on plant health.

Across different application methods and environmental conditions, thiamethoxam declined to <10% of its initial concentration within 1 year (Hilton, 2015) would suggest the likelihood of neonicotinoid treated seed storage beyond 3 months.

5.6.2 Effect of treatments and storage period on fresh leave yield and SLA

Amaranthus hybridus and *A. dubius* produced more fresh leaves than *A. hypocandracus* less leaves. A greater SLA was obtained with Amaranthus blitum, *A. hybridus* and *A. dubius,* in contrast a less SLA was obtained in *A. hypocandracus* in all seed treatments. The reason in fresh leaf yields and SLA may be related to genetic or agronomic trait variation between species and varieties within species (Achigan- Dako *et al.*, 2014), morphology characteristics (Trucco & Tranel, 2011; Das, 2012). Comparing these varieties and advancing breeding will have a bigger impact than improving the type and deliver of a seed treatment.

Greater yields were obtained with *Amaranthus blitum*, *A. hybridus* and *A. dubius* seeds treated with either Apron ® or Monceren® and used after 24 h or 3 m of seed treatment. *Amaranthus hybridus* and *A. hypocandracus* untreated seeds showed higher yield level. On average, Apron® treated seed of 24 h of seed treatments increased yields above the untreated seed. Use of Monceren® stored seed treatment provided a similar yield increase compared with untreated seed.

The increase was also statistically significant in both Monceren® 24 h and 3 m stored treated seed, in addition to Apron Star® stored insecticide seed treatments. However, the mechanism of how yield increased as a result of the two seed treatments is not fully understood Probably, the combination of insecticides and fungicides of the insecticides seed treatments with different modes of action offered broad spectrum protection and enhanced germination and increased yield (Mathre, et al, 2001; Khangura & Barbetti, 2004). Gaspar *et al. (*2014) found yield increases were more consistent when the insecticide, thiamethoxam, was combined with mefenoxam + fludioxonil. Dual fungicide (difenoconazole and metalaxyl) and an insecticide (thiamethoxam) improved germination of wheat and increased wheat yields (Larsen & Falk, 2013). Yield increase with seed treatment might also depend on environment (Schulz & Thelen 2008). Yield increase of treated seed may depend on variety (Lueschen *et al.*, 1991). Neonicotinoid seed treatments have helped to reduce insect pest injury and increased yields (North *et al.*, 2016; Perkins *et al.*, 2018).

CHAPTER SIX

EFFECTS OF SEED TREATMENTS ON *MYZUS PERSICAE* AND FRESH LEAF YIELD OF AMARANTH

6.1 Abstract

Green peach aphid, *Myzus persicae,* are key insect pests of amaranth in Kenya. Management of this pest has been restricted to indiscriminate use of foliar application of insecticides. Seed treatments is a common method to control aphid infestations and increase leaf yield. Analysis was accomplished on seed treatment trials in 2016 and 2017 to evaluate the efficacy of two selected insecticides and storage periods on *Myzus persicae* infestation and amaranth fresh leaf yield. The analysis compared Apron® and Monceren® treated seeds, untreated control plus 24 h and 3 months of seed treatment with two *amaranthus* species, *Amaranthus blitum* (2 varieties) and *A. hybridus* (4 varieties). Each amaranth selection was treated individually with thiamethoxam 20g/Kg + metalaxyl-M 20g/Kg+ difenoconazole 2 g/Kg and imidacloprid 233g/L + pencycuron 50g/L + thiram 107g/L and untreated control. The treated and untreated seeds were planted either 24 h or 3 months of seed treatment. When analyzed, there was a significantly greater amaranth fresh leaf yield and a lower aphid infested leaves when using seed treatments compared to untreated control. A significantly greater amaranth fresh leaf weight was noted of *A. hybridus* varieties (variety 3 and 6) while *A. blitum* varieties (variety 1 and 2) had a lower fresh leaf weight. Seeds treated and planted after 24 h of seed treatment had a significantly lower aphid densities compared to seeds of 3months post seed treatment and untreated seed. A higher number of aphid densities was only recorded in *A. hybridus* (variety 5). The results showed that seed treatments included in the experiments may suppress aphids and preserve yields, but not consistently for all seed treatments. Future studies may determine the causes of differences in *M. persicae* infestations and fresh leaf weight of amaranth seeds with regards to seed treatment.

6.2 Introduction

Application of plant protection compounds such as Apron Star® and Monceren®, directly to the seed (i.e. seed treatment) before sowing may provide an alternative to the control of green peach aphids, infesting amaranths. Amaranth farmers can also use seed-applied insecticides as a means for managing green peach aphids. Seed treatments have effectively controlled a wide range of pests (Nault *et al.,* 2005; Bonham *et al.,* 2009) in many crops, including green peach aphids (McLeod, 2008) in spinach. The use of amaranth treated seeds could offer a potential means of aphid control that would reduce the frequency of insect sprays with low labor costs, limited effects on the parasitoids and predators, avoid or limit crop losses in terms of quality and yield. Consequently, vegetable productivity and market access will rise.

There is limited information on the use of Apron Star® and Monceron® insecticide/fungicide seed treatments of 24h and 3 months of seed treatment to manage *M. persicae* in amaranth production in Kenya. The objectives of this study were to determine the effects of seed treatment and storage duration on *M. persicae* and fresh leaf yield of amaranth.

Apron Star® (thiamethoxam 20g/Kg + metalaxyl –M 20g/Kg + difenoconazole 2g/Kg, Syngenta, East Africa Ltd.) and Monceron® (imidacloprid 233g/L + pencycuron 50g/L + thiram 107g/ L, Bayer, East Africa, Ltd.) seed treatments are recommended control method for the management of sucking insects such as aphids (PCPB, 2018). The active ingredient thiamethoxam and imidacloprid found in Apron Star® and Monceron® respectively can provide amaranth producers the option of green peach aphid control using seed treatments. Seed treatments would be particularly useful in situations where green peach aphids cause infestations on amaranth.

The aim of this study was to assess the potential of seed treatment (using Apron Star® and Monceron®) in management of aphids in amaranth. This entailed assessing the effects of seed treatments and storage duration on population growth and development of *M. persicae* and yield of amaranth varieties under high tunnel conditions for seeds treated and planted after 24 h of seed treatment and those subjected to three months seed storage.

6.3 Materials and Methods

6.3.1 Site locations

Two high tunnel trials were conducted under similar environmental conditions at Jomo Kenyatta University of Agriculture and Technology (JKUAT) in Juja, Kenya (Latitude 1.0891° S, Longitude 37.0105° E, Altitude 1525 m above sea level) in 2016 and 2017. High tunnels had a temperature of 25 ± 1ºC, 60-70% RH and 12:12 L: D (h) Photoperiod.

6.3.2 Test plants

The test plants that were used in both trials included two *amaranthus* species namely; *A. blitum* (2 Abuku varieties) and *A. hybridus* (4 Abuku varieties) that were obtained from the Department of Horticulture and Food Security, JKUAT. More than one selection of the same *amaranthus* spp. were used to identify the differences in aphid infestation and fresh leaf weight within varieties when using seed treatment. *Amaranthus dubius* (one Abuku amaranth 7 and the local cultivar, Terere) though grown for leaves, were not including in this study, because *M. persicae* used for this study were reared on *A. dubius* and thus eliminated them to avoid telescoping association (Dixon, 1998; Ogawa & Miura, 2014). Similarly, *A. hypochondriacus* (Abuku variety 8) which is grown for both leaves and grain was not used because the treated seeds did not germinate. This might be due to morphological features of Abuku selection 8 (morphology characteristics (Trucco & Tranel, 2011; Das, 2012). All varieties were obtained from the Department of Horticulture and Food Security, JKUAT.

6.3.3 Treatments

Seed treatments, procedures of treating seeds, storage and storage period were the same described in chapter 5. The storage period was selected based on the practices assessed by surveying farmers in rural and urban Kenya (Nampeera *et al.*, 2019). The objective was to evaluate the effect of treated seed that was planted 24 h and 3 months after seed treatment on aphid infestations and leaf yield.

6.3.4 Experimental design

Treatments and untreated check were arranged in a randomized completely block design (RCBD) with three replications. Each replication contained thirty-six (36) amaranth plants that comprised six (6) plants of each amaranth selection planted in individual plastic pots. Treatments of each selection included, 1) seeds that were not treated and planted either 24 h of water treatment, 2) or 3 months post water treatment; 3) seeds treated with Apron and planted either 24 h of seed treatment, 4) or 3 months post treatment; 5) seeds treated with Monceren and planted either 24 h after seed treatment, 6) or 3 months post treatment. All seed treatments, untreated control, 24h and 3 months of seed treatment were included in the analysis.

6.3.5 Insect pest source

The insect pest source and rearing room was the same described in chapter 4. However, *M. persicae* population colonies were rejuvenated each year from A. *dubius* fields.

6.3.6 Insect infestation procedures

The insect infestation procedures were the same described in chapter 4. However, the adults were confined to the leaves surface by placing a paper bag barrier made of khaki on the leaves of each individual plant to prevent aphid escape and allowing the aphids to infest the individual plant in a no- choice situation.

6.3.7 Data collection

Destructive sampling of individual selection and treatment was done at five (5) and 10 days after infestation (DAI). Nymphal development of the green peach aphids in warmer months of warmer climates is completed 6 to 11 days (Capinera, 2001). Reproduction of individual aphids takes place 12 days after birth (Capinera, 2005). The data of the two sampling periods was combined and these data was used in these analyses. The sampling times was limited to two to avoid yellowing, breaking and falling of amaranth leaves that might have resulted from the method that we used for aphid confinement. During each sampling, the number of live aphids were counted. To determine if the aphids were alive, they were prodded with a camel hair brush. They were considered alive if they moved upon prodding. The harvested leaves including those with live *M. persicae*, of each individual selection and treatment were packed into labelled brown paper bags and placed in cool boxes packed with ice and taken to the laboratory for assessment. In the laboratory, the number of live aphids on leaves of individual selection and treatment were counted and recorded. Data on live aphid counts were log-transformed ($\log_{10+1}$) prior to analysis to ensure normality and homogeneity of error variance. The weight of each individual selection and treatment that exhibited *M. persicae* feeding injury (infested leaves) and those that did not exhibit *M. persicae* injury (uninfested) were also recorded. The percentage weight of leaves was calculated as weight of uninfested + infested x 100. The percentage weight of uninfested leaves (uninfested /total x 100) and infested (infested /total x 100) of each individual selection and treatment was calculated and recorded. Percent infested leaves per plant was adjusted from Luiz *et al. (*2019). The adjustments included the infested leaves per plant based on the number of leaves infested with live aphids, in addition to yellowish/brown curling of leaves, sooty mods and insect cast skins. Specific leaf area (SLA) (cm^2 g^{-1}) was estimated as the ratio of leaf area to leaf dry mass, similar to Freschet *et al. (*2015), Liu *et al. (*2016).

6.4 Data Analysis

ANOVA was performed on factor effects; varieties treatments, storage time and their interactions of each trial. Variables included live aphid counts, uninfested and infested leaf weight (g), percentage weight of uninfested and infested fresh leaves and SLA. Mean were sparated using Tukey's HSD test ($P \leq 0.05$). All analyses were performed in R version 3.4.3 (R Core Team, 2017) from the lme4 package (Bates *et al.*, 2013). The. results are presented for each main effect and interactions. Only significant interactions were presented in tables.

6.5 Results

6.5.1 Effect of seed treatments and storage period on *M. persicae* numbers

A significantly greater number of live aphids was recorded in 2016 and a lower aphid count was recorded in 2017. A significant effect on live aphids was also observed in both years ($F = 9.5$; $df = 5, 1008$; $P < 0.001$). Selection 5 had a significant greater aphid density compared to other varieties when either analyzed across the two years or in each year. Furthermore, the treatments also had a significant effect on live aphids in both years ($F = 3.2$; $df = 2, 1008$; $P = 0.039$). A significantly greater number of live aphids was recoded in Apron® treated seeds and lower in Monceren® treated and untreated control. In 2016, Apron® had a significantly greater number of live aphids, whereas, Monceren® and untreated control had a lower aphid count. In 2017, a significantly greater number of live aphids was recorded in untreated control and a lower in Monceren® treated seeds. A significantly greater number of live aphids was also observed at 3months after seed treatment ($F = 6.6$; $df = 1, 504$; $P < 0.001$). A significant effect of storage time (seeds treated and planted either 24 h or 3months of seed treatment) on the population of aphids was observed also across the two years ($F = 4.1$; $df = 1, 1008$; $P = 0.041$). Seeds that were treated and planted 24 h of seed treatments had a significantly lower aphid counts and seeds that were treated and planted after 3months of seed treatment had a significantly greater number of live aphids.

6.5.2 Effect of treatments and storage period on uninfested fresh leaf yield, 2016

There was significant effect on uninfested leaves observed among varieties ($F = 10.7$; $df = 5, 504$; $P < 0.0001$), treatments ($F = 74.6$; $df = 2, 504$; $P < 0.0001$) and storage period ($F = 15.3$; $df = 1, 504$; $P < 0.0001$). Similarly, significant effect was also noted with selection x treatment and selection x treatment x storage time (Table 6.1). A significant greater uninfested leaf weight was observed in Apron® and Monceren® treated seeds and a lower in untreated control (Table 6.1). There was no significant difference in fresh leaf weight of *Amaranthus blitum* (Abuku variety 1 and 2) and *A. hybridus* (Abuku varieties 3,4,5 and 6) when treated with in Apron®. No significant difference was also noted in fresh leaf weight of untreated seeds for *Amaranthus blitum* and *A. hybridus* varieties. However, there was significant difference in fresh leaf weight observed with *A. hybridus* varieties when treated with Monceren®. Selection 4 was significantly different with varieties 3, 5 and 6 (Table 6.1). There was also significant different in fresh leaf weight of *A. blitum* and *A. hybridus* Abuku varieties that was observed across the three treatments. Treated seeds of *A. blitum* varieties were significantly different from untreated seeds. Likewise, treated seeds of varieties 3,5 and 6 of *A. hybridus* were significantly different from untreated seeds. However, fresh weight of seeds of selection 4 treated with Monceren® were not significantly different with untreated seeds (Table 6.1).

At 24 h of seed treatment, fresh weight of Apron® treated seed of *A. blitum* (Abuku selection 1 and 2) and *A. hybridus* (Abuku varieties 3,4,5 and 6) were not significantly different. Similarly, untreated seeds of *A. blitum* and *A. hybridus* varieties were not significantly different. Significant difference. Uninfested fresh leaf weight of selection 4 was significantly different from selection 3,5 and 6 when treated with Monceren®, though it wasn't significantly different with untreated seed (Table 6.1).

At 3months of seed treatment, fresh weight of Monceren® treated seed of *A. blitum* (Abuku variety 1 and 2) and *A. hybridus* (Abuku varieties 3,4,5 and 6) were not significantly different. Similarly, untreated seeds of *A. blitum* and *A. hybridus* varieties were not significantly different. Uninfested fresh leaf weight of variety 4 was significantly different from variety 3,5 and 6 when treated with Apron®, though it wasn't significantly different with untreated seed (Table 6.1).

Table 6.1: Effect of treatments, varieties and storage time on amaranth uninfested fresh leaf weight (g) after aphid infestations, 2016 (Mean and standard error of the mean (±) values)

Amaranth Varieties and Storage Time	**Treatments**			**Interaction**			**Overall mean**
	Apron	Merceron	Untreated	df,87	Fvalue	Pvalue	
Abuku 1	33.8 ± 3.5a	31.6 ± 3.3a^{b}	16.9 ± 1.7b^{ab}	2	10.62	0.0001	27.4 ± 2.0^{cd}
Abuku 2	39.8 ± 4.1a	33.8 ± 3.5a^{ab}	17.0 ± 1.7b^{ab}	2	15.27	0.0001	30.2 ± 2.2^{bc}
Abuku 3	44.0 ± 4.6a	39.1 ± 4.1a^{ab}	22.7 ± 2.3b^{a}	2	8.86	0.0003	35.1 ± 2.6^{ab}
Abuku 4	30.6 ± 3.2a	17.9 ± 1.8b^{c}	18.7 ± 1.9b^{ab}	2	4.22	0.0178	22.4 ± 1.6^{d}
Abuku 5	33.9 ± 3.2a	36.5 ± 3.8a^{ab}	14.8 ± 1.5b^{b}	2	28.94	0.0001	28.6 ± 2.1^{bcd}
Abuku 6	45.5 ± 4.7a	45.2 ± 4.7a^{a}	21.6 ± 2.2b^{ab}	2	23.98	0.0001	37.4± 2.7^{a}
df, 174	5	5	5				
F	2.5	8.3	2.4				
P	0.028	0.001	0.036				
24 hours				df, 42			
Abuku 1	43.0 ± 7.1a	27.2 ± 4.5b^{ab}	19.5 ± 3.2b	2	8.8	0.0006	29.7 ± 2.2^{ab}
Abuku 2	47.8 ± 7.9a	34.0 ± 5.6a^{a}	18.2 ± 3.1b	2	10.24	0.0002	33.1 ± 2.4^{ab}
Abuku 3	50.3 ± 8.3a	38.9 ± 6.4ab^{a}	24.9 ± 4.1b	2	5.12	0.0102	37.0 ± 2.7^{a}
Abuku 4	49.2 ± 8.2a	10.7 ± 1.7b^{b}	21.2 ± 3.4b	2	21.28	0.0001	26.6 ± 1.9^{b}
Abuku 5	42.2 ± 7.0a	43.0 ± 7.1a^{a}	18.7 ± 3.1b	2	20.99	0.0001	33.3 ± 2.4^{ab}
Abuku 6	47.2 ± 7.8a	41.7 ± 6.9a^{a}	25.2 ± 4.2b	2	9.6	0.0003	37.4 ± 2.7^{a}
df, 174	5	5	5				
F	0.5	7	1.1				
P	0.77	0.001	0.35				
3 months				df,42			
Abuku 1	25.5 ± 4.2ab^{ab}	36.6 ± 6.1a^{ab}	14.4 ± 2.1b	2	9.86	0.0003	25.1 ± 1.8^{bc}
Abuku 2	32.9 ± 5.4a^{a}	34.2 ± 5.7a^{ab}	16.1 ± 2.6b	2	6.63	0.0031	27.2 ± 2.0^{bc}
Abuku 3	42.5 ± 7.0a^{a}	40.6 ± 6.7a^{ab}	21.5 ± 3.5b	2	4.96	0.0115	33.2 ± 2.4^{ab}
Abuku 4	13.0 ± 2.1a^{b}	25.8 ± 4.3a^{b}	16.6 ± 2.7ab	2	3.13	0.054	18.2 ± 1.3^{c}
Abuku 5	30.2 ± 5.0a^{ab}	32.8 ± 5.4a^{b}	12.2 ± 2.0b	2	13.97	0.0001	23.7 ± 1.7^{c}
Abuku 6	46.3 ± 7.7a^{a}	50.5 ± 8.1a^{a}	19.1 ± 3.1b	2	17.81	0.0001	37.4 ± 2.7^{a}
df, 174	5	5	5				
F	5.6	3.6	1.8				
P	0.001	0.001	0.11				

Means with the same lower-case letter within a row of the three treatments and same storage time are not significantly different, Tukey's HSD test, alpha = 0.05; Superscript means with the same lower –case within a column of the same treatment and storage time are not significantly different, Tukey's HSD test, alpha = 0.05

6.5.3 Effect of treatments and storage period on uninfested fresh leaf yield, 2017

Significant difference in fresh leaf weight was noted in varieties (F =45.1; df = 5, 504; P < 0.0001), treatments (F =96.9; df =2, 504; P < 0.0001). The interaction of selection x treatment was significant (F =4.2; df =10, 504; P < 0.0001). A significantly greater uninfested fresh leaf weight was noted with *A. hybridus* varieties seeds treated with Apron® and Monceren®, in addition to untreated seed. Variety 3 and 4 when treated with Apron® and Monceren® had a significantly greater fresh leaf weight among *A. hybridus* varieties. In addition, untreated seed of selection 3 had also a significantly greater fresh leaf weight (Table 6.2). A lower uninfested leaf weight was noted in treated and untreated seed of *A. blitum*. No significant difference in fresh weight was observed in treated seed of *A. blitum* (Abuku variety 1 and 2).

Similarly, at 24 h of seed treatments, a significantly higher fresh leaf weight was noted with *A. hybridus* varieties and a lower with *A. blitum* varieties. Amaranthus. hybridus (variety 3) treated and untreated seeds had a significantly greater fresh leaf weight (Table 6.2). There was no significant difference noted between seed treatments and untreated seed of *A. blitum* (variety 2) and *A. hybridus* (variety 5) after 24h of seed treatment (Table 6.2).

At 3 months of seed treatment, a significantly greater fresh leaf weight was still observed with *A. hybridus* varieties and a lower with *A. blitum* varieties. Significant difference was noted in fresh leaf weight of treated and untreated seed of *A. blitum* varieties (Table 6.2). There was significant difference between selection 1 and 2 seeds treated with Monceren® and untreated seed. There was no significant difference in fresh leaf weight of selection 1 and 2 seeds treated with Apron® and untreated seed. (Table 6.2). Significant difference was also noted in fresh leaf weight of treated and untreated seeds of *A. hybridus* varieties (Abuku, 3, 4,5 and 6). Fresh leaf weight of seeds treated with Apron®, Monceren® and untreated seed of Abuku 5 were significantly different from each other (Table 6.2)

Table 6.2: Effect of treatments, varieties and storage time on uninfested amaranth fresh leaf weight after aphid infestations, 2017 (Mean and standard error of the mean (±) values)

Amaranth Varieties and Storage Time	Treatments Apron	Merceron	Untreated	Interaction df, 87	Fvalue	Pvalue	Overall mean
Abuku 1	7.1 ± 0.7a^{cd}	9.9 ± 1.0a^{de}	2.2 ± 0.2b^{bc}	2	12.35	0.0001	6.4 ± 0.4^{c}
Abuku 2	5.5 ± 0.5a^{d}	6.5 ± 0.6a^{e}	1.7 ± 0.1b^{c}	2	7.07	0.0014	4.6 ± 0.3^{c}
Abuku 3	22.8 ± 2.4a^{a}	24.9 ± 2.6a^{ab}	10.6 ± 1.1b^{a}	2	14.77	0.0001	19.4 ± 1.4^{a}
Abuku 4	23.3 ± 2.4a^{a}	27.6 ± 2.9a^{a}	6.5 ± 0.6b^{ab}	2	22.4	0.0001	19.2 ± 1.4^{a}
Abuku 5	13.2 ± 1.3a^{bc}	15.5 ± 1.6a^{cd}	6.0 ± 0.6b^{bc}	2	15.11	0.0001	11.6 ± 0.8^{b}
Abuku 6	17.9 ± 1.8a^{ab}	19.3 ± 2.0a^{bc}	3.8 ± 0.4b^{bc}	2	26.19	0.0001	13.6 ± 1.0^{b}
df, 174	5	5	5				
	18.11	18.89	8.9				
	0.001	0.001	0.001				
24 hours				df,42			
Abuku 1	8.2 ± 1.3a^{bc}	9.2 ± 1.5a^{c}	2.8 ± 0.4b^{b}	2	4.8	0.0132	6.7 ± 0.4^{cd}
Abuku 2	5.2 ± 0.8^{c}	5.0 ± 0.8^{c}	2.2 ± 0.3^{b}	2	2.3	0.112	4.1 ± 0.3^{d}
Abuku 3	27.4 ± 4.5a^{a}	23.8 ± 3.9ab^{a}	13.0 ± 2.1b^{a}	2	5.63	0.0068	21.3 ± 1.5^{a}
Abuku 4	18.3 ± 3.8ab^{ab}	23.1 ± 3.0a^{ab}	8.5 ± 1.4b^{ab}	2	5.56	0.0071	16.6 ± 1.2^{ab}
Abuku 5	14.8 ± 2.4^{bc}	12.8 ± 2.1^{bc}	8.3 ± 1.3^{ab}	2	2.95	0.0632	12.0 ± 0.8^{bc}
Abuku 6	17.2 ± 2.8a^{ab}	22.2 ± 3.7a^{ab}	4.8± 0.8b^{b}	2	11.04	0.0001	14.7 ± 1.0^{b}
df, 174	5	5	5				
F	8.6	10.36	4.2				
P	0.001	0.001	0.001				
3 months				df,42			
Abuku 1	6.0 ± 1.0ab^{c}	10.7 ± 1.7a^{c}	1.7 ± 0.2b^{b}	2	8.18	0.0009	6.1 ± 0.4^{d}
Abuku 2	5.9 ± 0.9ab^{c}	8.0 ± 1.3a^{c}	1.3 ± 0.2b^{b}	2	4.97	0.0115	5.1 ± 0.3^{d}
Abuku 3	18.3 ± 3.0a^{b}	26.0 ± 4.3a^{ab}	8.2 ± 1.3b^{a}	2	14.55	0.0001	17.5 ± 1.3^{ab}
Abuku 4	28.4 ± 4.7a^{a}	32.2 ± 5.3a^{a}	4.7 ± 0.7b^{ab}	2	20.84	0.0001	21.7 ± 1.6^{a}
Abuku 5	11.8 ± 1.9b^{bc}	18.3 ± 3.0a^{bc}	4.0 ± 0.6c^{b}	2	23.03	0.0001	11.2 ± 0.8^{c}
Abuku 6	18.7 ± 3.1a^{b}	16.4 ± 2.7a^{bc}	2.9 ± 0.4b^{b}	2	19.26	0.0001	12.6 ± 0.9^{bc}
df, 174	5	5	5				
F	14.9	10.91	8.3				
P	0.001	0.001	0.001				

Means with the same lower-case letter within a row of the three treatments and same storage time are not significantly different, Tukey's HSD test, alpha = 0.05; Superscript means with the same lower –case within a column of the same treatment and storage time are not significantly different, Tukey's HSD test, alpha = 0.05

6.5.4 Effect of treatments and storage on infested fresh leaf yield, 2016 and 2017

Data of two years was combined because it was not significant. Overall, the mean weight of infested leaves was affected by trial (F = 119.8; df 1, 1008; P < 0.001), A significantly greater weight of infested leaves was observed in 2016 and a lower in 2017. Furthermore, the weight of infested leaves was also affected by varieties (F = 9.8; df =5, 1008; P < 0.001). A significantly greater weight of infested leaves was observed in *A. hybridus* (variety 5 and 3) and a lower number in *A. blitum* (variety 1 and 2). Treatments (F =4.2, df = 2, 1008; P < 0.001) and storage time (F =4.1, df =1, 1008; P = 0.043) also had a significant effect on the weight of infested leaves.

A significantly greater weight of infested leaves was observed in seeds treated with Apron® and a lower in seeds treated with Monceren® and untreated seeds. A significantly greater weight of infested leaves was observed in seeds that were treated and planted 3months of seed storage and a lower in 24 h of seed storage. When analyzed by year, significance difference was only observed among treatments (F =13.3, df =2, 504; P < 0.001) and varieties (F = 11.8, df = 5, 504; P < 0.001) in 2016. Seeds treated with Apron® had a significant greater weight of infested leaves and a lower weight of infested leaves was observed in seeds treated with Monceren® and un treated seeds.

6.5.5 Effect treatments and storage period on percentage uninfested fresh leaf yield, 2016

The percentage weight of uninfected leaves differed significantly across varieties (F = 14.2; df = 5,504; P < 0.0001). A significant greater percentage of uninfested leaf weight was observed in *A. blitum* (selection 1 and 2) and *A. hybridus* (selection 3,5, and 6). A lower percentage of uninfested leaves was observed *A. hybridus* (selection 4). The interaction of treatment x varieties was significant (F = 8.6; df = 10, 504; P < 0.0001). Significant difference of percentage weight of uninfested leaves was noted with treated and untreated seed of *A. hybridus* varieties (Abuku 4 and 5). Percentage weight of uninfected leaves of seeds treated with Apron® and Monceren® of *A. hybridus* (selection 4) (Table 6.3). The percentage weight of uninfested leaves of treated and untreated seeds of selection 4 were significantly different. Significant difference of percentage weight of uninfested leaves of treated and untreated seeds was also noted with selection 5. The percentage weight of uninfested leaves of seeds treated with Apron® was significantly different with untreated seeds. However, the percentage weight of leaves of seeds treated with Monceren ®was similar to seeds treated with Apron® and untreated seed (Table 6.3). There was no significant different of the percentage weight of uninfested leaves of seeds treated with Apron®, Monceren® or untreated seeds of in *A. blitum* (selection 1 and 2) and *A. hybridus* (selection 3 and 6) (Table 6.3).

The interaction of storage period x treatment x varieties (F- 10.3; df =10, 504; $P< 0.0001$) was also significant. Seeds treated and planted after 24 h of seed treatment had a significant greater percentage of uninfested leaf weight and seeds treated and planted after 3months of seed treatment had a significant lower percentage of uninfested leaf weight.

At 24 h of seed treatment, there was significant difference between treated and untreated seeds (Table 6.3). Seeds treated with Apron® and Monceren® had a higher percentage of uninfested leaves than untreated seed. Percentage weight of uninfested leaves of selection 4 seeds treated with Monceren® was significantly different from selection 4 seeds treated with Apron® and untreated seed. However, no significant difference in percentage weight of seeds was observed with seeds of selection 4 treated with Apron® or untreated (Table 6.3).

There was no significant difference of the percentage weight of uninfested leaves of *A. blitum* (Abuku selection 1 and 2) and *A. hybridus* (Abuku varieties 3,5 and 6) whether treated with Monceren®, Apron® or untreated (Table 6.3).

At 3 months of seed treatment, there was significant difference of percentage weight of uninfested leaves of *A. hybridus* selection (Abuku 4), with *A. blitum* varieties (Abuku 1and 2) and other *A. hybridus* varieties (Abuku 3,5 and 6) when treated with Apron®.

The percentage weight of uninfested leaves of selection 4 treated with Apron® was significantly different from selection 4 seed treated with Monceren® and untreated seed. However, the percentage weight of uninfested leaves of selection 4 seed treated with Monceren® and untreated seeds was similar (Table 6.3). There was no significant difference of the percentage weight of uninfested leaves of *A. blitum* (Abuku selection 1 and 2) and *A. hybridus* (Abuku varieties 3, 5 and 6) treated with Apron®, Monceren® and untreated seeds (Table 6.3).

Table 6.3 Effect of treatments, varieties and storage time on percentage weight (g) of un infested amaranth fresh leaf after aphid infestations,2016 (Mean and standard error of the mean (±) values

Amaranth Varieties and Storage Time	**Treatments**			**Interaction**			**Overall Means**
	Apron	Merceron	Untreated	df, 87	Fvalue	Pvalue	
Abuku 1	92.1 ± 9.7^{a}	99.1 ± 10.4^{a}	85.7 ± 9.0^{ab}	2	2.212	0.116	92.3 ± 6.8^{a}
Abuku 2	95.1 ± 10.4^{a}	99.2 ± 10.4^{a}	97.6 ± 10.2^{ab}	2	0.964	0.386	97.3 ± 7.2^{a}
Abuku 3	94.0 ± 9.9^{a}	91.4 ± 9.6^{a}	84.2 ± 8.8^{ab}	2	1.233	0.296	89.8 ± 6.6^{a}
Abuku 4	65.3 ± 6.8b^{b}	52.5 ± 5.5b^{b}	98.5 ± 10.3a^{a}	2	10.73	0.0001	72.1 ± 5.3^{b}
Abuku 5	93.8 ± 9.8a^{a}	93.1 ± 9.8ab^{a}	79.7 ± 8.4b^{b}	2	3.612	0.0312	89.0 ± 6.3^{a}
Abuku 6	89.6 ± 9.4^{a}	98.5 ± 10.3^{a}	95.3 ± 10.0^{ab}	2	2.487	0.0891	94.5 ± 7.0^{a}
df, 174	5	5	5				
F	5.9	8.5	3				
P	0.001	0.001	0.011				
24 Hours				df,42			
Abuku 1	98.8 ± 15.2^{a}	99.2 ± 15.3^{a}	86.6 ± 13.1	2	1.867	0.167	94.9 ± 7.0^{a}
Abuku 2	99.1 ± 15.2^{a}	99.8 ± 15.3^{a}	97.5 ± 15.0	2	0.666	0.519	98.8 ± 7.3^{a}
Abuku 3	96.0 ± 14.8^{ab}	91.0 ± 14.0^{a}	84.9 ± 13.1	2	0.724	0.491	90.6 ± 6.7^{a}
Abuku 4	98.2 ± 15.2a^{ab}	19.0 ± 2.9b^{b}	99.4 ± 15.3a	2	61.09	0.0001	72.2 ± 5.3^{b}
Abuku 5	94.3 ± 14.5^{b}	97.1 ± 14.9^{a}	85.2 ± 13.1	2	1.396	0.259	92.2 ± 6.8^{a}
Abuku 6	97.5 ± 15.0^{ab}	98.4 ± 15.1^{a}	97.7 ± 15.0	2	0.109	0.897	97.9 ± 7.2^{a}
df, 174	5	5	5				
F	3.7	41.8	1.1				
P	0.001	0.001	0.34				
3 Months				df,42			
Abuku 1	85.4 ± 13.1^{a}	99.0 ± 15.2	84.9 ± 13.1	2	1.185	0.316	89.8 ± 6.6^{a}
Abuku 2	91.2 ± 14.0^{a}	98.6 ± 15.2	97.7 ± 15.0	2	1.072	0.352	95.8 ± 7.1^{a}
Abuku 3	92.0 ± 14.1^{a}	91.8 ± 14.1	83.5 ± 12.8	2	0.536	0.589	89.0 ± 6.6^{a}
Abuku 4	32.3 ± 4.9b^{b}	86.0 ± 13.2a	97.5 ± 15.0a	2	15.64	0.0001	71.9 ± 5.3^{b}
Abuku 5	93.3 ± 14.3^{a}	89.1 ± 14.9	73.9 ± 11.4	2	2.464	0.0976	85.7 ± 6.3^{ab}
Abuku 6	81.7 ± 12.6^{a}	98.5 ± 15.1	92.6 ± 14.3	2	2.599	0.0862	91.0 ± 6.7^{a}
df, 174	5	5	5				
F	7.1	1.1	2				
P	0.001	0.034	0.07				

Means with the same lower-case letter within a row of the three treatments and same storage time are not significantly different, Tukey's HSD test, alpha = 0.05; Superscript means with the same lower –case within a column of the same treatment and storage time are not significantly different, Tukey's HSD test, alpha = 0.05

6.5. 6 Effect of treatments and storage period on percentage uninfected fresh leaf yield, 2017

There was significant difference of the interaction of selection and treatment of *A. hybridus* selection (Abuku 6) The percentage weight of uninfested leaves of selection 6 seeds treated with Monceren® was significantly different with untreated seed of selection 6. However, there was no significant difference of the percentage weight of uninfested leaves of selection 6 seeds treated with Apron®, Monceren® and untreated seeds (Table 6.4). No significant difference of percentage weight of uninfested leaves was noted with *A. blitum selection (*Abuku selection 1 and 2) and *A. hybridus* (Abuku varieties 3, 5 and 6) whether treated or untreated (Table 6.4). Similarly, no significant difference of percentage weight of uninfested leaves was noted at 24 h and 3 months of seed treatment and of untreated (Table 6.4).

Table 6.4: Effect of treatments, varieties and storage time on percentage weight (g) of un infested amaranth fresh leaf after aphid infestations, 2017 (Mean and standard error of the mean (±) values).

Amaranth Variety and Storage Time	Treatments			Interaction			Overall means
	Apron	Merceron	Untreated	df,87	Fvalue	Pvalue	
Abuku 1	100 ± 10.5	99.8 ± 10.5	100 ± 10.5^{a}	2	1	0.372	99.9 ± 7.4^{a}
Abuku 2	100 ± 10.5	100 ± 10.5	98.9 ± 10.5^{a}	2	1	0.372	99.6 ± 7.4^{ab}
Abuku 3	99.4 ± 10.4	100 ± 10.5	98.1 ± 10.3^{ab}	2	0.963	0.386	99.2 ± 7.3^{ab}
Abuku 4	96.4 ± 10.1	100 ± 10.5	99.5 ± 10.4^{a}	2	0.897	0.412	98.7 ± 7.3^{ab}
Abuku 5	99.0 ± 10.4	99.9 ± 10.5	97.7 ± 10.2^{ab}	2	2.223	0.114	98.9 ± 7.3^{ab}
Abuku 6	99.1 ± 10.4ab	99.4 ± 10.4a	89.5 ± 9.4b^{b}	2	3.723	0.0281	96.0 ± 7.1^{b}
df, 174	5	5	5				
F	0.8	0.9	3				
P	0.54	0.43	0.012				
24 hours				df, 42			
Abuku 1	100 ± 15.4	100 ± 15.4	100 ± 15.4	2	1	0.376	100 ± 7.4
Abuku 2	100 ± 15.4	100 ± 15.4	100 ± 15.4	2	1	0.376	100 ± 7.4
Abuku 3	99.6 ± 15.3	100 ± 15.4	96.3 ± 14.8	2	1.158	0.324	98.6 ± 7.3
Abuku 4	99.3 ± 15.3	100 ± 15.4	99.8 ± 15.2	2	0.866	0.428	97.4 ± 7.2
Abuku 5	100 ± 15.4	99.8 ± 15.2	100 ± 15.4	2	1	0.376	99.9 ± 7.4
Abuku 6	99.0 ± 15.2	100 ± 15.4	88.8 ± 13.7	2	2.168	0.127	95.9 ± 7.1
df, 174	5	5	5				
F	0.9	1	1.8				
P	0.4	0.4	0.1				
3 months				df, 42			
Abuku 1	100 ± 15.4	99.6 ± 15.3	100 ± 15.4	2	1	0.376	99.8 ± 7.4
Abuku 2	100 ± 15.4	100 ± 1.5	100 ± 15.4	2	1	0.376	99.2 ± 7.3
Abuku 3	99.3 ± 15.3	100 ± 1.5	100 ± 1.5	2	1	0.376	99.7 ± 7.4
Abuku 4	99.8 ± 15.2	100 ± 15.4	100 ± 15.4	2	1	0.376	99.9 ± 7.4
Abuku 5	99.8 ± 15.2	100 ± 15.4	95.5 ± 14.7	2	2.694	0.793	97.8 ± 7.2
Abuku 6	99.2 ± 15.3	98.8 ± 15.2	90.3 ± 13.9	2	1.472	0.241	96.1 ± 7.1
df, 174	5	5	5				
F	1.8	1	1.4				
P	0.1	0.3	0.2				

Means with the same lower-case letter within a row of the three treatments and same storage time are not significantly different, Tukey's HSD test, alpha = 0.05; Superscript means with the same lower –case within a column of the same treatment and storage time are not significantly different, means within any lower-case letter within the row or column are not significantly different, Tukey's HSD test, alpha = 0.05. Means without any lower-case letter within the row or column are not significantly different, Tukey's HSD test, alpha = 0.05

6.5.7 Effect of treatments and storage period on percentage infested fresh leaf yield, 2016 and 2017

The percentage weight of infested leaves differed significantly across trials ($F = 31.0$; $df = 1, 1008$; $P < 0.001$), varieties ($F = 7.9$; $df = 5, 1008$; $P < 0.001$), storage time ($F = 14.5$; $df = 1, 1008$; $P < 0.001$) and treatments ($F = 9.8$; $df = 2, 1008$; $P < 0.001$). A significantly greater percentage of infested leaves was observed in 2016 and a lower in 2017. *A. hybridus* variety (Abuku 5) had a significant greater percentage weight of infested leaves and *A. blitum* variety (Abuku 1 and 2) had a lower percentage weight of uninfested leaves. A greater percentage weight of infested leaves was observed with seeds treated and planted 3months of seed treatment and a lower with seeds of 24 h of seed treatment. Untreated seeds had a significantly greater weight of infested leaves, whereas Apron® and Monceren® treated seeds had a lower percentage weight of infested leaves. When analyzed by year, there was significant difference of percentage weight of infested leaves observed among varieties ($F = 12.0$; $df = 5,504$; $P < 0.001$), treatments ($F = 5.9$; $df = 2,504$; $P = 0.002$) and storage time ($F = 19.8$; $df = 1,504$; $P < 0.001$) in 2016. Whereas in 2017, significant difference of percentage weight of infested leaves was only observed with treatments ($F = 6.2$; $df = 2,504$; $P = 0.002$).

6.5.8 Effect of treatments and storage period on SLA

In 2016, SLA differed significantly with variety ($F = 5.5$; $df = 5,504$; $P < 0.001$). A significant greater SLA was noted with treated seed and a lower SLA was observed in untreated seed. There was a significant difference in SLA of *A. hybridus* varieties (Abuku varieties3,4,5 and 6) treated with Monceren® and Apron®. *Amaranthus. hybridus* varieties (Abuku 3 and 4) seeds treated with Apron® had a greater SLA. Similarly, *A. hybridus* variety (Abuku 4) seeds treated with Monceren® had also a greater SLA. Abuku 3 of untreated seed had a greater SLA (Table 6.5)

In 2017, there was a significant different of SLA of *A. hybridus* varieties treated and untreated seeds (Table 6.5). Significant difference in SLA across the three treatments was only noted in *A. hybridus* selection (Abuku 5 and 6). SLA of treated seed of Abuku 5 was significantly different from untreated seed. Whereas SLA of selection 6 seeds treated with Monceren® was significantly different from untreated seed. There was no significant difference in SLA of selection 6 seeds treated with Apron®, Monceren® and untreated seed) (Table 6.5).

Table 6.5: Specific Leaf Area (SLA) ($cm^2 g^{-1}$) comparisons among varieties and treatments 2016 and 2017 (Mean and standard error of the mean (±) values

Year	Amaranth Varieties	Treatments				Interaction		Overall mean
		Apron	Merceron	Untreated	df, 87	Fvalue	Pvalue	
2016	Abuku 1	7.1 ± 0.7a^{cd}	9.9 ± 1.0a^{de}	2.2 ± 0.2b^{bc}	2	12.35	0.001	13.4 ± 0.9b
	Abuku 2	5.5 ± 0.5a^{d}	6.5 ± 0.6a^{e}	1.7 ± 0.1b^{c}	2	7.0	0.001	13.2 ± 0.9b
	Abuku 3	22.8 ± 2.4a^{a}	24.9 ± 2.6a^{ab}	10.6 ± 1.1b^{a}	2	14.77	0.001	20.7 ± 1.5a
	Abuku 4	23.3 ± 2.4a^{a}	27.6 ± 2.9a^{a}	6.5 ± 0.6b^{ab}	2	22.4	0.001	12.3 ± 0.9b
	Abuku 5	13.2 ± 1.3a^{bc}	15.5 ± 1.6a^{cd}	6.0 ± 0.6b^{bc}	2	15.11	0.001	14.8 ± 1.1b
	Abuku 6	17.9 ± 1.8a^{ab}	19.3 ± 2.0a^{bc}	3.8 ± 0.4b^{bc}	2	26.1	0.001	14.9 ± 1.1b
	df, 174	5	5	5				
	F	18.11	18.89	8.9				
	P	0.001	0.001	0.001				
2017	Abuku 1	17.9 ± 1.8^{b}	20.2 ± 2.1	17.3 ± 1.8	2	1.8	0.164	37.1 ± 2.7
	Abuku 2	17.8 ± 1.8^{b}	33.0 ± 3.4	14.5 ± 1.5	2	0.8	0.421	21.8 ± 1.6
	Abuku 3	36.9 ± 3.8^{a}	31.4 ± 3.3	30.4 ± 3.3	2	0.3	0.72	32.9 ± 2.4
	Abuku 4	26.1 ± 2.7^{ab}	21.5 ± 2.2	18.7 ± 1.9	2	2.6	0.077	22.1 ± 1.6
	Abuku 5	24.7 ± 2.6a^{ab}	24.8 ± 2.6a	17.4 ± 1.8b	2	6.4	0.001	22.3 ± 1.6
	Abuku 6	19.6 ± 2.0ab^{b}	21.6 ± 2.2a	15.2 ± 1.6b	2	4.1	0.01	18.8 ± 1.4
	df, 174	5	5	5				
	F	4.0	0.4	1.9				
	P	0.001	0.78	0.09				

Means with the same lower-case letter within a row of the three treatments are not significantly different, Tukey's HSD test, alpha = 0.05; Superscript means with the same lower –case within a column of the same treatment are not significantly different, Tukey's HSD test, alpha = 0.05. Means without any lower-case letter within the row or column are not significantly different, Tukey's HSD test, alpha = 0.05. Ama. Var. – Amaranth varieties.

6.6 Discussion

6.6.1 Effect of treatments and storage period on *M. persicae* densities

The data indicated that, seeds treated and planted after 24h of seed treatment were effective and managed aphid infestations in amaranth and increased amaranth fresh leaf weight than seeds of 3months of seed treatment. The use of Monceren® insecticide seed treatments reduced aphid densities. The mechanism by which imidacloprid found in Monceren® reduced aphid densities is not fully understood.

Evidence indicates that neonicotinoid seed treatments, possess systemic properties effective to protect the plants from pests, such as aphids (Forsberg *et al.,* 2003; Chelsea 2012). However, it might be related to differences in toxicity effectiveness of different neonicotinoid insecticides to insect pests (Byrne *et al.,* 2007, Shan *et al.,* 2012). Imidacloprid and thiamethoxam found in Monceren® and Apron® respectively, exhibit high insecticidal activities in the control of insect pests which cause damage immediately after sowing to early seedling stages (early-season pests), including aphids (Hemiptera: Aphididae), leafhoppers (Hemiptera: Ciccadellidae); leaf beetles Coleoptera: Chrysomelidae); corn borers (Lepidoptera: Crambidae) seed corn maggot, Diptera:Anthomyiidae); thrips (Thysanoptera: Thripidae); white grubs Coleoptera:Scarabaeidae); wireworms (Coleoptera: Elateridae) and cut worms (Lepidoptera:Noctuidae) (Wilde *et al.,* 2004; Davis *et al.,* 2009, 2010; Ding *et al.,* 2018, Hesler *et al.,* 2018).

The active ingredient imidacroprid and thiamethoxam both have been found to have lethal and sub lethal effects on aphids in crops such as soybean (McCornack & Ragsdale, 2006; Magalhaes *et al.,* 2008; 2009; Frewin, *et al.,* 2014). Both *A. blitum* (varieties one and two) and *A. hybridus* (variety three, four and six) had lower aphid densities, however, *A. hybridus (*variety five) had greater aphid densities with or without seed treatments. *Amaranthus blitum* (variety one and two) had smaller leaves while *A. hybridus* variety three leaves were tough/ hard with trichomes, whereas variety four and six had shorter leaves. *Amaranthus hybridus* (variety five) had bigger (broader) leaves. The hardness of selection three leaves could have prevented penetration of the aphid stylet.

Morphological characteristics, including presence or absence of trichomes, toughness or hardness of leaves, etc, defensive compounds and nutritional value, can affect insect development including aphids on host plants (Awmack & Leather 2002; Maremela *et al.,* 2013, Polat *et al.,* 2015; Atlihan *et al.,* 2017). Amaranth accession resistant to leaf Webber, *Spoladea recurvalis* had smaller leaves compared to the susceptible accession that had also broader and longer leaves (Steven *et al.,* 2018). The mechanism of how *A. blitum,* (variety one and two) *A. hybridus (*variety 3,4,5 and 6*)*, affect aphid densities is not yet understood, however, synthesis of salicylic acid by isochorismate pathway and the phenylalanine ammonium lyase pathways when plants are infested by aphids have been reported by Dempsey *et al. (*2011). Salicylic acid and Jasmonic acid mediated signaling pathways against chewing insects and phloem-feeding insects have been also reported by Howe and Jander (2008), Pieterse *et al. (*2012). Resistance to leaffolder *Cnaphalocrocis medinalis* in rice plants mediated by Salicylic acid and ethylene signalling pathways have been reported by Wang *et al. (*2011). Salicylic acid signaling pathway are also activated by insect eggs (Reymond, 2013).

The results also demonstrate that 24h of seed treatment is significantly more efficient in controlling aphids compared to 3m of seed treatment. Seed treatments by pesticides have been reported to reduce damage of pathogens, soil born pests and early season pests (Hitaj *et al.* 2020). However, storing seed for long periods after treatment could result in phytotoxicity (Lamichhane *et al.,* 2020). The mechanism of which of which 24h of seed treatment efficiently controlled aphids of amaranth is not understood. It might be that at 24 h of seed treatment there was enough insecticide seed treatment on seeds that were taken up by plants and protected the new leaves from aphids. Movement of insecticides in plant is mostly via xylem to the leaves (Maienfisch *et al.,* 2001; Weichel & Nauen 2003). Hence, if there is enough insecticide to be taken up by plants, then new leaves are protected from insect pests including aphids (Weichel and Nauen 2003).

6.6.2 Effect of treatments and storage period on uninfested fresh leaf yield

The use of insecticide seed treatments increased the weight of uninfested amaranth fresh leaves more than the untreated control. On average where direct comparisons were made, the weight of uninfested leaves were increased more where insecticide treatments were used more than untreated control. Seeds treated and planted after 24h of seed treatment increased amaranth fresh leaf weight than seeds of 3m of seed treatment. Storage period after seed treatment could affect vigor, decrease seedling emergency and reduce yield (Dan *et al.,* 2010, 2013; Khaliliaqdam *et al.,* 2012; Ferreira *et al.,* 2016**).** Brzezinski *et al.* (2015) reported that seed treatment of 240 days before planting affected crop development and ultimately yield as compared to seed treatments applied at planting. *Amaranthus hybridus* (variety three and six) had greater uninfested leaf weight compared to *A. blitum* (selection one and two). Seeds treated with insecticides and fungicides and stored two months of seed treatment reduced physiological quality and yield of soybean seeds **(**Ferreira, *et al.,* 2016; Sandini, *et al.,* 2019**).**

Insecticides might have reduced aphid densities and protected amaranth leaves from aphid infestation. Thus, insecticide seed treatments may directly manage aphids by killing them as they feed on leaves or indirectly by increasing fresh leaf yield. Neonicotinoids, thiamethoxam and imidacloprid act as neurotoxins and are introduced into the insect body (Tomizawa & Casida 2005). When they reach the nAChR, activation occurs and causes an increase in sodium ion conductance and depolarization of the post-synaptic membrane which triggers an action potential to occur and an insect is left in a constantly excitable state which leads to hyperexcitation, convulsion, paralysis and death of the insect (Yu, 2008).

6.6.3 Effect of treatments and storage period on infested fresh leaf yield

A greater infested leaf weight was also observed in *A. hybridus* (selection three and five), while *A blitum* (selection one and two) had lower infested leaf weight. It may be attributed to differences in morphological characteristics of the spp., varieties and within varieties of the same spp., such as shape, toughness and presence or absence of trichomes (Awmack & Leather 2002; Maremela *et al.*, 2013, Polat *et al.*, 2015). that are independent of aphid infestations. *Amaranthus hybridus* (selection three and five), selection three had bigger, longer and tough leaves with trichomes whereas selection five had broader leaves. *Amaranthus blitum* (selection one and two) had smaller leaves.

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 General discussion

7.1.1 Farmers aphid management practices in leafy amaranth production

Farmer aphid management practices in leafy amaranth differed across the four counties surveyed. Of all the respondents who controlled aphids in amaranth, 34 percent used synthetic (insecticides) and 8% used non-synthetic methods. However, some of the insecticides' they used, such as dimethoate is banned to be used on fruits and vegetables in Kenya (PCPB, 2018). Furthermore, farmers used insecticides' inappropriately that could lend to risks associated with use of pesticides, (Ajayi *et. al. (*2011), Tiwani *et al. (*2011); Bass *et al.* (2014) and Park *et al. (*2016). Farmers do not practice biological control practices, exploit host plant resistance and seed treatments to control aphids in amaranth. Farmer management practices identified during the survey, focused group discussions and key informants' interviews were not adequate in management of aphids in amaranth production. Enhance capacity, provide wider management options, encourage use of safer approaches, such as biological control, host plant resistance and use of seed treatment could be used by leafy amaranth growers for management of aphids in amaranth production. This will lend sustainability, reduction in the costs of production, improvement of farmers' livelihoods, supply of safety quality food and consumption. and reduce or eliminate the risks associated with the use of synthetic and incorrect use of synthetic insecticides.

7.1.2 Host plant resistance

This study showed that *A. blitum (*selection one and two*)* had the lowest population of aphids and weight of fresh leaves, whereas *A. hybridus (*selection five*)* had the highest population of aphids and greatest leaf weight. Different mechanisms of aphid resistance in amaranth between species and among varieties such as antibiosis, Painter (1951), Kogan and Ortman (1978) may help to explain the differential response to *M. persicae* in amaranth production. However, the response of aphids does not only depend on the resistance mechanisms, but also on aphid salivary effector proteins that increase aphid performance, Prado and Tjallingii (2007), Bansal *et al. (*2014). The period of vegetative growth is also important for growth and development of aphids (Dixon, 1998). The present study showed that leaves of variety 1 and 2 significantly reduced the population increase of *M. persicae*, whereas the leaves of selection 5 significantly increased population of *M. persicae*. This result suggests that genes conferring resistance to aphids may influence the effect of *M. persicae* on *A. blitum* (variety 1 and 2) and A. *hybridus* (variety 3, 4 and 5). The present study also showed that selection 5 produced higher biomass despite of aphid infestation. This result suggests evidence of a compensatory mechanisms, such as tolerance that considerably increased biomass production in selection 5 despite aphid infestation. Tiffin (2000) reported mechanisms of tolerance to herbivore damage. The results of this study could help breeders to select amaranth varieties in relation to aphid management and also devise an integrated pest management strategy for *M. persicae* in amaranth production.

7.1.3 Seed treatment

In this study, seed treatment increased germination, in addition, it reduced the infestation of *M. persicae*, increased fresh leaf yield and SLA in amaranth species such as *A. blitum* and *A. hybridus*. Seeds also planted 24 h and 3 months after seed treatment reduced infestation and increased yield more than the untreated seed. It is hypothesized that seed treatment affected germination, aphid population growth and leafy amaranth fresh leaf yield. This study has been conducted on seed treatments of Apron® and Monceren® and their effects on germination, aphid growth and infestation and fresh leaf yield of amaranth. This study showed that seeds treated with Apron® and grown after 24 hours of seed treatment were effective in improving germination, whereas seeds treated with Monceren® insecticide seed treatments reduced aphid densities. Kaburu, (2011) found Gaucho®, Monceren® and Cruiser ®seed dressings moderately effective while Apron Star® was least effective in controlling bean fly infestation in snap bean.

The weight of uninfested leaves were increased more where insecticide treatments were used than untreated control. The effectiveness of Apron® and Monceren® on germination, population growth of aphids and fresh yield may be related to the active ingredients in Apron® (thiamethoxam - 20g/kg, metalaxyl-M- 20g/kg, difenoconazole-2g/kg) and Monceren ® (imidacloprid - 233g/l, pencycuron-50g/l, and thiram-107 g/l). It has been shown in earlier studies that a dual fungicide (difenoconazole and metalaxyl) and an insecticide (thiamethoxam) improved germination of wheat and increased wheat yields (Larsen and Falk, 2013).

7.2 Conclusions

Based on objective one which is to identify farmers' management practices for the green peach aphids. Amaranth farmers used both synthetic insecticides and non-synthetic methods to control aphids in leafy amaranth with the majority of them using synthetic insecticides which they thought were more effective in aphid management. Farmers did not mention use of host plant resistance, seed treatment or biological control to manage aphids in *amaranthus*.

For objective 2 which was to investigate variation in tolerance among new lines of amaranth to the green peach aphids. Certain leafy amaranth varieties have variations in the levels of tolerance to *M. persicae*; for example, *A. blitum* (variety 1 and 2) was more tolerant whereas *A. hybridus* (variety 5) was less tolerant to *M. persicae*.

For objective 3 which was to evaluate the effect of seed treatment on green peach aphids and performance of new lines of amaranth. The Use of Apron® and Monceren® amaranth treated seed also reduced infestation of *M. persicae* and increased fresh leaf yield of *A. hybridus* amaranth species. Seeds treated and used after 24 h of seed treatment effectively reduced aphid densities and increased fresh leaf yield. Use of Apron Star® and Monceren® treated seeds of 24 h and 3 months of seed treatment also improved germination and fresh leaf yield.

Overall, amaranth growers depend on insecticides to control aphids in amaranth production because of the quick control results after application. Farmers also use non-synthetic and cultural methods such as wood ash dust, botanical extracts, exotic garlic, papaya leaves, intercropping, and rouging to control aphids in amaranth. However, aphid- tolerant amaranth varieties could offer a potential means of control that would reduce aphid infestation in amaranth production. The use of tolerant amaranth varieties which are not preferred to aphids will reduce cost, insecticide use, exposure to harmful pesticides, impact on the environment and natural enemies. Seed treatment with Apron® and Monceren® could also contribute to reducing aphid damage in farmer fields.

7.3 Recommendations

1. Future studies using both no-choice and choice-based assays are needed to determine what type and mechanism of resistance these *amaranthus* species may possess.
2. Aspects of seed treatment in respect to morphology features in *A. blitum* (varieties one and two) and *A. hybridus* (varieties two, three, four, five and six) in addition to the factors that influenced response of aphids to Apron® may lend to future studies.
3. Explore interaction of Apron® and Monceren® seed treatments and storage time period on outbreaks of *M. persicae* and fresh leaf yield of amaranth.

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APPENDICES

Appendix I: Survey questions of 600 leafy amaranth farmers in four counties about their amaranth production and pest management practices.

No.	Questions			
Q1.1	Demographic Information of farmers**;** gender, age, education level ,and size of the farm			
Q1.2	How many times have you grown leafy amaranth in the last two years?			
Q1.3	Have you grown leafy amaranths in the first season of 2014?			
Q1.4	Which varieties of amaranth do you grow?			
Q1.5	Do you make your own seed from previous harvest?	Yes	No	
Q1.6	Do you get seeds from other sources	Yes	No	
Q1.7	If Yes above, please indicate the other sources			
Q1.8	What was the mode of acquisition?			
Q1.9	Do you grow amaranth as a pure stand or as an intercrop	Pure stand	Intercrop	Both
Q1.10	If intercropped, what are the intercrops grown			
Q1.11	Did you use seeds or transplants?	Seeds	Transplants	
Q1.12	Were plants thinned and thinned leaves eaten as vegetables	Yes	No	
Q2	Which production constraints do you face during production, rank them from 1 being the most important?			

Q3	If pest are one of the constraints, can you rank the pests in order of importance? (1 most important and damaging)	Pests	Rank	
Q3.1	Can you rank the control methods of the most damaging pest?	Pests	Control Method	Rank
Q3.2	In case of aphids what are the damaging symptoms they cause?			
Q.4	In which season of the year do you get a high level of infestation of aphids? Name the season and month			
Q.5.	Which control methods have you been using for aphids?			
Q.5.1	If you have been using insecticides for aphid control, name the types of insecticides, dosage and frequency	Insecticide	Dosage	Frequency
Q5.2	What type of other pest control methods have you been using	Other pest control	Dosage	Frequency
Q.5.3	When did you apply pest control on aphids			
Q.5.4	How did you apply it?			
Q.5.5	How would you assess the effectiveness of the pest control methods of aphids on a scale of 1(low) to 5 (high)	1,2,3,4,5		
Q.6	Do you have birds eating your seeds	Yes	No	

Appendix II: Questions used to guide eight focus-group discussions in four counties

No.	Questions			
1.	What vegetables do you grow?			
2.	What do you know about amaranth?			
3.	Is amaranth one of the most economically important vegetable grown?	Yes	No	
4.	Is amaranth grown for sale or only home consumption?	Sale	Home	
5.	Do you know of any pest attacking amaranth?			
6.	What are the key pests attacking amaranth?			
7.	In case of aphids what are the losses incurred by aphids?			
8.	What are management options of aphids in amaranth?			
9.	How did you get knowledge about the management of aphids in amaranth?			
10.	How do you assess the effectiveness of the management practices of aphids in amaranth?	Low	Medium	High
11.	Have you ever received assistance in the management of aphids in amaranth?	Yes	No	
12.	Whom did you receive assistance from?			
13.	Do you have any other questions on amaranth?			

Appendix III: Questions to guide interviews 16 key informants four counties in Kenya.

No.	Questions
1.	To what extend is amaranth grown in this county?
2.	In which season do they most grow and harvest amaranth?
3.	Which production constraints do the farmers in this county face during the production of amaranth?
4.	How can you rate the insect problem on amaranth in this county?
5.	Pests in the order of importance/with the Pests control method used Control method used
6.	In case of aphids what are symptoms of aphid attack?
7.	Have the farmers been using insecticides for aphid control in amaranth? If yes which insecticides?
8.	Do you think these farmers have been using non chemical methods for aphid control in amaranth? If yes, what are they?
9.	Have you given advice to anyone about management of aphids in amaranth in the past 12 months?
10.	Do you have farmer groups involved in amaranth in this county?
11.	Is there anything else about amaranth production that you would like me to know?