ANALYSIS OF PYRETHRUM PLANT EXTRACT AND ITS ANTI-APHID EFFECT ON GROWTH, YIELDS, CHLOROPHYLL AND PHYTONUTRIENT CONTENTS OF AFRICAN NIGHTSHADES

BY

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DECLARATION

I certify that this thesis is my own work and effort and has not been submitted for a degree in Maseno University or any other University. All sources of information have been acknowledged by means of references.

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ABSTRACT

African nightshades (*Solanum scabrum)* is one of indigenous vegetables widely consumed in Kenya. It is used for food, income and for medicinal purposes, but faces low yields due to severe damage by aphids. Yield losses due to aphids is estimated to about 84-96% in Kenya. Aphids have developed resistance to synthetic pesticides. There is need to search for ecofriendly alternatives such as the use of botanicals like pyrethrum which are cheaper. Pyrethrum extracts are known to control insect pests of most crops. Past studies have concentrated on the insecticidal effects of pyrethrum flower extracts on pests like *Tribolium confusum* and *Costelytra zealandica* but there is little information on anti-aphid effects of pyrethrum extracts on African nightshades. The objectives of this study were to determine the phytochemical constituents in pyrethrum flower, leaf and root parts, to determine the effect of different concentration levels of pyrethrum extracts on aphids affecting *S. scabrum,* to determine the anti-aphid effect of pyrethrum extracts on growth parameters and yields of *S. scabrum* and to determine the anti-aphid effect of pyrethrum extracts on chlorophyll and phytonutrient contents of *S. scabrum*. The study was conducted at Maseno University farm. A field experiment was laid in Randomized Complete Block Design with three replications. Five treatments consisting of 0%, 33% ,67% and 100% pyrethrum flower, root and leaf extracts and duduthrin were applied. Phytochemical screening of extracts was carried out. *Solanum scabrum* seeds were locally sourced from the Botanic Garden, Maseno University. Seeds were germinated and raised in 3m by 1m plots caged with mosquito net. After four weeks the seedlings were thinned to 9 seedlings per plot. Aphids (*Aphis fabae*) were obtained from International Center of Insect Physiology and Ecology- Nairobi were reared then introduced to every seedling (10 aphids) in all plots. Aphid population was determined on leaves and by use of yellow water pan traps and data collected weekly after treatment. Growth parameters including plant height, number of curled leaves, leaf area, fresh and dry weights, yield/ha, chlorophyll content and phytonutrients content was recorded. The data was subjected to analysis of variance and means were separated using Least Significant Difference at $P = 0.05$. The study revealed that pyrethrum extracts contained phenols, saponins, alkaloids, flavonoids, tannins, triterpenoids and phytosterols. Triterpenoids were absent in the leaf extracts. Phenols, flavonoids and triterpenoids were more in flowers than in root and leaf extract. The root exhibited more amounts of saponins, alkaloids and phytosterols. Aphid population was significantly ($p \leq 0.05$) reduced in all plant treated with pyrethrum extracts. Outstanding concentration were 100% and 67% pyrethrum flower and root extracts which recorded zero number of aphids on day 69^{th}, 76^{th} and 83^{rd} day but leaf extracts showed a reduced effect. The highest concentration (100%) of pyrethrum extracts reduced aphid population significantly, leading to a high plant growth, yield, chlorophyll and phytonutrient content of *S. scabrum* as compared to control. This observation may have been attributed to the death of aphids. The use of 100% pyrethrum flower or root extracts is recommended to farmers in aphid control so as to realize improved yield and production of *S. scabrum* in order to reduce the overdependence on synthetic pesticides.

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CHAPTER ONE

INTRODUCTION

1.1 Background Information

African nightshades (*Solanum scabrum* Mill). is one of the commonly consumed African indigenous vegetable in Kenya (Edmond and Chweya, 1997) and its role in balanced diet is indisputable. *Solanum scabrum* have been gaining attention in recent years and have become more economically competitive compared to other vegetables (Kamga *et al*., 2016). This is attributed to higher levels of nutrients than those in exotic vegetables such as kales and collards (Edmonds and Chweya, 1997). Most of rural communities in Kenya depend on these nutrient-dense vegetables as food, important income source and for medicinal purposes (Onyango *et al*., 2000). In contrast, this vegetable has received little research attention compared to other food crops despite their short growing cycles, economic benefits and role in ensuring food security (Kamga *et al.*, 2016), this could be attributed to its low leaf yields and frequent pest attack. Insect pests remain one of the most significant constraints to nightshade production (Omondi, 2015). Farmers need information on cheap, effective and safe ways particularly botanicals to protect this potential vegetable specifically against insect pests which includes aphids, black-ants and white flies that severely reduce the yields (Carnot *et al*., 2017).

African nightshades in Kenya are severely damaged by aphids (Carnot *et al*., 2017). *Aphis fabae* reduces both weight and caloric content of young African nightshades by as much as 64 and 113%, respectively, depending on the number of feeding aphids and yield losses due to aphids are estimated to be about 84-96% in Kenya (Ngurwe *et al*., 2018). Aphids are tiny, 1-3 mm long, soft-bodied, pear-shaped insects. They can be winged or wingless and vary tremendously in colour and shape, and feed on almost every part of the plant (Capinera, 2001). Aphids have been known to cause the greatest manace on *Solanum spp*, kales, wheat and several crops because they are serious phloem feeders and persistent in a farm (Ashilenje *et al.*, 2011). Aphids feed by sucking plant sap, feeding on the growing tips and undersides of leaves, where they congregate in large numbers (Capinera, 2001). Aphids can attain very high densities on young plant, and direct feeding of aphids results into reduced growth rates, yields of green leaves, chlorosis and reduced stem reserves and storage organs (Golawska *et al*., 2010). Aphids also have very short life cycle, high

reproductive rate and are resistant to different insecticides which make their management and control difficult (Qureshi *et al*., 2002), hence the need to come up with a better and proper control method in order to realize improved Solanum productivity.

Majority of smallholder vegetable farmers use synthetic chemicals to reduce this crisis yet their use has not substantially reduced the pest losses (Mamun and Ahmed, 2011). Additionally, overzealous and indiscriminate use of many synthetic pesticides has resulted in a number of environmental and toxicological problems. A potential means to break the reliance on pesticides is by using alternative sources like plant extracts. These plant based products possess bioactive compounds called secondary metabolites which are preferred because they are environmentally safe, locally available and cheap and effective with no side effects or resistance (Qureshi, 2002). or can be integrated with other practices for effective pest management.

Plants secondary metabolites constitute a major source of bioactive substances. One widely proven organic insecticidal plant extracts are those from pyrethrum (Qureshi *et al.,* 2002). Pyrethrum belongs to the family Compositae; the largest family among the angiosperms with a world-wide distribution (Ghafoor, 2002). There are three species of pyrethrum namely, the *Chrysanthemum cinerariaefolium*, *Chrysanthemum roseum*, and *Chrysanthemum coccineum;* these plants are economically important as a natural source of insecticide (Wandahwa *et al.,* 1996). Pyrethrum have bioactive substances which are insecticidal (Wandahwa *et al.,* 1996). Information on secondary metabolites in pyrethrum flower, root and leaf extracts is lacking.

Pyrethrum extracts contain active ingredients which block the voltage gated sodium channels in nerve axon insects, resulting in a knockdown effect, hyperactivity, and convulsions hence death of insect pests. This occurs when the insects ingest or are exposed to these botanicals. Pyrethrum flower extracts are sprayed on broccoli plants for protection from several common insect pest including aphids, leafhoppers and spider mites. Haouas *et al*. (2008) investigated insecticidal activity of flower and leaf extracts from *Chrysanthemum* species against flour beetle (*Tribolium confusum).* They reported that high toxicities were noted in flower methanolic extracts as compared to leaf methanolic extracts. Little has been documented on the anti-aphid effect of pyrethrum leaf, flower and root extracts on aphids. Additionally, Qureshi *et al*. (2002) used garlic and pyrethrum flower to evaluate insecticidal effect against grass grub beetles (*Costelytra zealandica),* a rapid knockdown was observed from extracts compared to synthetic insecticide

used. However, these studies of Haouas *et al*. (2008) and Qureshi *et al*. (2002) disregarded the use of pyrethrum root extracts which perhaps might have a distinct toxicity and has not been done hence the need to investigate the anti-aphid effects of different concentrations of pyrethrum flower, root and leaf extracts on aphids affecting *Solanum scabrum*.

Lawal (2015) found that aphids can attain very high densities on young plant tissue, causing water stress, wilting, chlorosis, distortion of the leaves, abscission of blooms and plant stunting. In addition, they lead to reduced growth rate and appreciable reduction in yield of the plant. La Rossa *et al*. (2013) reported that aphid populations can grow to extremely high levels under favorable environmental conditions in a short time, covering sprouts, leaves, flowers, and fruits which reduces availability of photosynthetic active radiation to the plant. Lo *et al*. (1999) reported that large populations of *Myzus persicae* developing on leaves of peach and nectarine plants stunted young shoots if left unchecked. Aphids infest the underside of leaves of this vegetable causing them to curl hence young apices of affected plants fail to develop which reduces crop yield (Ashilenje *et al*., 2011). These studies depicted the effects and extent of damage caused by aphids on plant growth and yields, However, the anti-aphid effects of pyrethrum extracts on growth and yields of *Solanum scabrum* is still lacking hence needs investigation.

The leaves and shoots of African nightshade consist of important phytonutrients which include: 1.0 mg iron, 4.3 g protein, 38 kcalories, 5.7 g carbohydrates, 1.4 g fiber, 442 mg calcium, 20 mg ascorbic acid, 3660 µg ß-Carotene, 75 mg phosphorus, and 0.59 mg riboflavin per 100 g fresh weight (Muthomi and Musyimi, 2009). According to Chander *et al*. (2006) aphid infestations contribute to germination reduction, stand reduction, assimilation rate reduction, assimilate sucking and tissue consumption. In an experiment to assess assimilation rates of wheat, Chander *et al.* (2006) found that aphids are assimilate sappers which greatly reduced plant assimilation rate and together with the direct feeding resulted into reduced stem reserves and storage organs. In addition, aphids cause chlorosis as reported by Lawal (2015). Aphids are known to greatly reduce food manufactured by the plant hence less phytonutrients including proteins, carbohydrates, fatty acids and minerals stored in storage organs. However, limited information is available on the anti-aphid effects of pyrethrum extracts on the chlorophyll and phytonutrient contents of *Solanum scabrum* because no studies has been done on the same. Therefore, the present investigations on effect of controlling aphids using different pyrethrum part extracts was carried out.

1.2 Statement of the Problem

Insect pests mainly aphids remain one of the most significant constraints to African nightshade production (Omondi, 2015). *Aphis fabae* reduces both weight and caloric content of young African nightshades by as much as 64 and 113%, respectively, depending on the number of feeding aphids (Ngurwe *et al*., 2018). Yield losses due to aphids is estimated to about 84-96% in Kenya. Aphids are difficult to control due to their short life cycle, high rate of reproduction and resistance to different insecticides used by farmers which necessitates the need to come up with a better and proper control method in order to realize improved *S. scabrum* productivity. In order to support sustainable vegetable production, alternatives like plant extracts need to be identified. Pyrethrum extracts have bioactive substances which are insecticidal (Wandahwa *et al.,* 1996). There is little information on secondary metabolites in pyrethrum flower, root and leaf extracts. Aphids can attain very high densities on young plant tissue, covering sprouts, leaves, flowers and fruits causing water stress, wilting, and reduced growth rate of the plant or kill young shoots if left unchecked. There is scanty information on the control of aphids using different parts of pyrethrum extracts. Botanicals like pyrethrum extracts can be used as safer management strategies for aphid control. The menace associated with aphids and impacts on plant growth and yields is well known but arguably there is little information on the anti-aphid effects of pyrethrum extracts on growth and yields of *Solanum scabrum.* The direct feeding of aphids results into reduced growth rates of green leaves causing chlorosis which reduces availability of photosynthetic pigments of the plant. Aphids suck assimilates which results into reduced stem reserves and storage organs. Stem reserves and storage organs store important phytonutrients including proteins, carbohydrates, fatty acids and minerals. There is need to investigate the anti-aphid effects of pyrethrum extracts on chlorophyll and phytonutrient contents of *Solanum scabrum* which is unknown.

1.3 Justification

Solanum scabrum is a popular traditional vegetable with high nutritional and medicinal benefits, though most farmers consider it uneconomical because of low leaf yields compared to other high-yielding crops. *Solanum scabrum* not only thrives everywhere but has won a place in heart and plates of local farmers which is increasingly their food security and source of income. They have shorter growing cycles than staple crops, vegetables can be less affected by environmental threats such as drought. Farmers rely on chemicals in aphid control which has resulted in a number of

environmental and toxicological problems yet their use has not substantially reduced the pest losses. In addition, development of resistance in aphids have made its chemical control unattainable. To realize increased *Solanum scabrum* production, one of the sustainable ecological friendly approaches is the use of botanicals which may reduce aphid infestation. Extracts from various parts of pyrethrum can serve as a viable alternative for the management of many sucking insects. Pyrethrum extracts have been effective against most pests affecting agricultural crops including aphids, spider mites, thrips and several others. Farmers can utilize 100% pyrethrum flower and root extracts to control aphids in order to realize Solanum scabrum productivity. Pyrethrum extracts are cheap, available, user and environmentally friendly and compatible with organic farming in which most small scale farmers practice.

1.4 Broad Objective

To determine phytochemical constituents of pyrethrum plant part extracts and its anti-aphid effect on growth, yields, chlorophyll and phytonutrient contents of African nightshades

1.5 Specific Objectives

i. To determine the phytochemical constituents in pyrethrum flower, leaf and root parts.
ii. To determine the effect of different concentrations of pyrethrum extracts on aphid population on *Solanum scabrum*
iii. To determine the anti-aphid effect of pyrethrum extracts on growth parameters and yields of *Solanum scabrum*
iv. To determine the anti-aphid effect of pyrethrum extracts on chlorophyll and phytonutrient contents of *Solanum scabrum*

1.6 Hypotheses

i. Pyrethrum flower, leaf and root parts have phytochemical constituents
ii. Different concentrations of pyrethrum extracts have effect on aphids affecting *Solanum scabrum*
iii. Pyrethrum extracts have anti-aphid effect on growth parameters and yields of *Solanum scabrum*
iv. Pyrethrum extracts have anti-aphid effect on chlorophyll and phytonutrient content of *Solanum scabrum*

CHAPTER TWO

LITERATURE REVIEW

2.1 African nightshades (*Solanum scabrum*)

African nightshades are vegetable crops belonging to the genus Solanum and night shade family Solanaceae. Nine African nightshades species have been identified and at least five black nightshade species reported by Kimiywe *et al*. (2007) to be common in Kenyan including *Solanum physalifolium*, *Solanum scabrum*, *Solanum american*, *Solanum nigrum* and *Solanum villosum*. In Kenya different ethnic communities have given black nightshade various names: Managu (Kikuyu), kitulu (Kamba), ndunda (Taita), osoga (Luo), isoiyot (Kipsigis), tmomoi (Maasai) (Ondieki *et al*., 2011).

Solanum scabrum is prostrate or erect annual herb, up to 1m tall, the leaves are alternate, dark green, soft and thin (Edmonds and Chweya, 1997). The size of the leaves is quite variable, while the shape is ranging from ovate and lanceolate to diamond-shaped, ovate to heart-shaped, with wavy or large-toothed edge. Petioles are 1 to 3 cm long with a winged upper portion (Edmonds and Chweya, 1997). The flowers are 0.7cm across with prominent bright yellow colour and have many anthers surrounded by greenish to nearly white petals, which recurve when aged. The berries have in most cases a diameter of 6 to 8 mm and dull black, purple-black, red or orange color with many flat seeds (Edmonds and Chweya, 1997).

2.1.1 Origin and Cultivation of African nightshades

The Solanaceae family is made up of approximately 90 genera and between 2000 and 3000 species and is well distributed throughout the tropical and temperate regions of the world (Edmonds and Chweya, 1997). African nightshade is largely grown in Kenya as well as in Nigeria. *Solanum scabrum* occurs as a cultivated vegetable from Liberia to Ethiopia, and south to Mozambique and South Africa. The wide range of diversity of *Solanum scabrum* found especially in Nigeria and Cameroon suggests that its origin is likely to be in the warm humid forest belt of West and Central Africa. Outside Africa, *Solanum scabrum* can be found in Europe, Asia, Australia, New Zealand, North America and the Caribbean (Muthomi and Musyimi, 2009).

Table 1. Taxonomic position of *Solanum scabrum* Mill.

Kingdom	**Plantae**
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Solanales
Family	Solanaceae
Genus	*Solanum* L.
Species	*Solanum scabrum* Mill

Retrieved from Muthomi and Musyimi, (2009)

Solanum scabrum is propagated from seeds and does well in varying degree of climatic conditions. Mostly traditional vegetables are adapted to specific marginal soil and climatic conditions, and often can be grown with minimal external inputs (Hughes and Ebert, 2013). These vegetables do well in soils with high nitrogen and phosphorus. African nightshades prefer sandy loams and friable clay soil types. Its optimum soil pH ranges between 6.0-6.5 and it is sensitive to frost. According to Muthomi and Musyimi, (2009), *Solanum scabrum* should be watered at least once a week for good growth.

Annual rainfall of 500-1200mm is required for optimal growth of the plant. In addition, full sunlight is also recommended as shady conditions reduces growth leading to reduced leaf yield and total plant weight. They have shorter growing cycles than staple crops, vegetables can be less affected by environmental threats such as drought. The production of traditional African nightshades is still very low, farmers can employ botanicals, an effective means to control pests especially during rainy season when aphid population is high (Chweya, 1997). This will perhaps reduce food shortages. The production, sale and consumption of traditional African nightshades

vegetables will strengthen income, food and nutrition security in locations with erratic rainfall. African nightshades production mainly is rain fed (Onyango *et al*., 2000).

2.1.2 Economic and Nutritional Importance

Traditional vegetables like African nightshades are valuable sources of nutrients (Yang and Keding, 2009), with some having important medicinal properties. Vegetables contribute substantially to food security (Kipkosgei *et al*., 2003). Species of the African nightshades are known to have a long tradition as alternative food or fodder plants. Some plants of Solanaceae family are known as weeds in horticulture and agriculture, competing with the main crops for moisture, nutrients and light. In Kenya, fruits of both wild plants and cultivated varieties of black nightshade are used for nutrition (Edmonds and Chweya, 1997).

Solanum scabrum is widely used as medicinal plant. Leaf extracts are used to treat diarrhoea in children and certain eye infections and jaundice (Muthomi and Musyimi, 2009). African nightshades varieties are believed to have a high nutritional value. Young shoots and leaves are blanched, boiled or stir-fried, cooked with other vegetables or added to soups (Kipkosgei *et al*., 2003). Infusions of leaves and seeds are rubbed onto the gums of children who have developed crooked teeth. *Solanum scabrum* is used as fodder for cattle and goats. Both the leaves and fruits are a source of dyes (Muthomi and Musyimi, 2009). The Keiyo's of Rift Valley feed pregnant and lactating mothers as well as circumcision initiates with the belief of improved production of breast milk and quick recovery, respectively (Kipkosgei *et al*.,2003).

In East Africa the raw fruit is chewed and swallowed to treat stomach ulcers or stomach-ache. For instance, nightshade leaves are consumed to manage diabetes, high blood pressure, anaemia, peptic ulcers, colds, coughs and sight problems (Kimiywe *et al*., 2007). The leaves of African nightshades consist of 87.2 g water, 1.0 mg iron, 4.3 g protein, 38 kcalories, 5.7 g carbohydrates, 1.4 g fibre, 442 mg calcium, 20 mg ascorbic acid, 3660 µg ß-Carotene, 75 mg phosphorus, and 0.59 mg riboflavin per 100 g fresh weight (Muthomi and Musyimi, 2009). These vegetables often provide higher amounts of provitamin A, vitamin C and several important minerals than common, intensively bred vegetables like kales and collards (Kamga *et al*., 2016). Despite a number of studies to determine the phytonutrient content of *solanum scabrum*, there is no similar study that have been conducted to assess the anti-aphid effect of pyrethrum extracts on phytonutrients of *Solanum scabrum*.

2.1.3 Challenges of African nightshades (*Solanum scabrum*) production

African nightshades production in Kenya is faced with many constraints which have been attributed to a number of biotic and abiotic production constraints. Among such constraints are pest and diseases which affect the quality and quantity of yields others include poor farming practices, poor weed management, low soil fertility and post-harvest losses (Qureshi *et al*., 2002). Carnot *et al*. (2017) stated that yield of African nightshades like most vegetables are seriously affected by a complex of pests and diseases. Vegetables in tropical countries are the crops, which are often attacked most seriously by arthropod pests. Vegetables are grown mostly as intensive crops with considerable input, such as fertilizers, irrigation water, insecticides and often cultivated continuously in a limited area with narrow crop rotations. Thus insects find optimum conditions to develop high populations.

2.1.4 Insect Pests of African nightshades (*Solanum scabrum*)

Insect pests are one of the most significant constraints to nightshade production. A study carried out by Carnot *et al*. (2017) showed that the most economically important pests to African nightshades are: locusts (*Zonocerus variegatus*), beetles (*Podagrica spp*), aphids (*Aphis fabae* and *Myzas persicae*), nematodes (*Meloidogyn spp*), flea beetles (*Epilachna hirta*), leaf hoppers, grasshoppers, leaf miners, white flies (*Bemisia tabaci*) and thrips (*Frankliniella spp*). In addition to damages caused to the plants by direct feeding, some pests such as nematodes, whiteflies, aphids and thrips are vectors of viruses (Lawal, 2015).

2.2 Overview of Synthetic Pesticide use in Kenya

In Kenya the use of pesticides is an indispensable tool in combating damage from pests and ensuring sustainable food production with improved yield and greater availability of food. Agricultural activities have been faced with destructive activities of numerous insects, pests, fungi and weeds which lead to immense decrease in yields (Wondafrash, 2007). Majority of smallholder vegetable farmers use synthetic chemicals to reduce this crisis. Overzealous and indiscriminate use of many synthetic pesticides has resulted in a number of environmental and toxicological problems (Mamun and Ahmed, 2011). Insect resistance, pest resurgence, chemical residues and high cost have all acted to reduce the use of chemical insecticides which now have only limited use against pest control (Qureshi *et al*., 2002).

Human exposure to pesticides is an important health and social issue as it usually results in serious health problems such as epilepsy, stroke, respiratory disorders, stomach and intestinal upset, cancer and convulsions (Tijani, 2006). Cancer is becoming a major concern in Kenya today and is partially linked to overzealous use of synthetic pesticide which can be reduced by using alternatives like plant extracts. Lawal (2015) reported that in parts of the developing world, pesticide poisoning causes more deaths than infectious diseases. The intense use of pesticides to kill resistant pests induces more resistance until further increases in pesticide use actually reduce agricultural yield (Qureshi *et al*., 2002).

Aphids are economically important pests that are difficult to control because of their mobility, tremendous reproductive ability, and resistance to many synthetic pesticides (Capinera, 2001). Inspite of aphid resistance to chemicals, use of synthetic pesticide still remains the most common practice. Lawal (2015) reported that, over time, aphid populations in pepper develop resistance against compounds of synthetic insecticides including organochlorines, organophosphates, carbamates, and synthetic pyrethroids. There is need to find an alternative to be used for aphid control perhaps using pyrethrum extracts may yield great success without aphid resistance therefore the need to find out the effect of pyrethrum flower leaf and root extracts against aphids affecting *Solanum scabrum*.

Due to repeated usage of insecticides aphids have evolved several insecticide resistance mechanisms, including the detoxification of insecticides by elevated esterases (Khan *et al*., 2011). Anstead *et al*. (2005) reported development of resistance in aphids to more insecticides than any other insect. In view of the several disadvantage of synthetic pesticide use, it is therefore incumbent to promote the use of botanicals which are safer, cheaper and environmentally friendly means of controlling agricultural pest and diseases. This is a potential means to breaking the reliance on pesticides as using alternative sources like botanicals which will play a big role in reducing pollution, health risks and crop losses to pests (Mamun and Ahmed, 2011). Anti-aphid effect of pyrethrum extracts on growth, yields, chlorophyll and phytonutrients of African nightshades (*Solanum scabrum*) remain unknown hence the need this study.

2.3 Pyrethrum (*Chrysanthemum cinerariaefolium*) and Potential Use in Pest Control

Pyrethrin insecticides block the volt-gated sodium channels in nerve axon insects, resulting in a knockdown effect, hyperactivity, and convulsions (Isman, 2006). Wang *et al*. (1997) reported that the insect powder known as pyrethrin is produced from the dried flowers of *Chrysanthemum cinerariaefolium*. Pyrethrum extracts are used as insecticides to control insect pests of crops and ornamental plants (Ashilenje, 2011). They destroy aphids, bed bugs (*Cimex lectularius*), leafhoppers, spider mites, harlequin bugs, ticks, pickleworms, and imported cabbage worms, among others, in gardens and farms (Qureshi *et al*., 2002). For instance, they are sprayed on broccoli plants for protection from several common insect pests. However, its use in aphid management is unknown hence there is need to investigate.

2.3.1 Origin and Distribution of Pyrethrum

Pyrethrum belongs to the family Compositae; the largest family among the angiosperms with a world-wide distribution and comprises of about 1535 genera and 2300 species distributed in 3 sub-families and 17 tribes (Ghafoor, 2002). They are perennial plants with a daisy-like appearance and white petals. There are three species of pyrethrum namely, the *Chrysanthemum cinerariaefolium*, *Chrysanthemum roseum*, and *Chrysanthemum coccineum. Chrysanthemum cinerariaefolium* looks more like the common daisy than other pyrethrums do. Its flowers, typically white with yellow centers, grow from numerous fairly rigid stems. The plant is economically important as a natural source of insecticide (Wandahwa *et al.,* 1996).

Chrysanthemum cinerariaefolium occurs in the wild on the Dalmatian coast of former Yugoslavia. It was introduced into Japan in 1881, which became the principal producer between the first and second world war (Wandahwa *et al.,* 1996). In the early 1920's, it was planted in Switzerland and France. It includes perennial, annual or biennial herbs. *Chrysanthemum cinerariaefolium* is small and perennial, with a daisy-like appearance and white petals. Wandahwa *et al.* (1996) reported that pyrethrum has been tried in many countries including Rwanda, India, Zaire, New Guinea, Nepal, China, Kenya and Brazil.

Chrysanthemum coccineum is the Persian chrysanthemum, it is a perennial plant native to Caucasus and looks somewhat like a daisy. It produces large white, pink or red flowers. The leaves resemble those of ferns and looks more like the common daisy than other pyrethrums do. Its

flowers, typically white with yellow centers, grow from numerous fairly rigid stems. *C. coccineum* also contains insecticidal pyrethrins, but it is a poor source compared to *C. cinerariifolium* (Wandahwa *et al.*, 1996).

Other species, such as *Chrysanthemum balsamita* and *Chrysanthemum marshalli*, also contain insecticidal substances, but are less effective than the two species *C. coccineum* and *C. cinerariaefolium*. *Chrysanthemum coccineum* was the first species to be used. It was introduced into Europe in the 19th century and into the United States about 1860. Later, *C. cinerariaefolium* was found to be most effective and became the main source of pyrethrum. Pyrethrum plants are now found in countries such as Kenya, India, Tanzania, Ecuador, Brazil, Russia, Japan and Australia (Michuki,1994).

2.3.2 Chemical Compounds of Pyrethrum (*Chrysanthemum cinerariaefolium)*

The active ingredients from the pyrethrum flower, which are known as pyrethrins, do not usually exceed 2% of the dry mass (Roncevic *et al*., 2014). However, when a pyrethrum flower is stored for prolonged periods at high temperatures, after harvesting, the pyrethrins may degrade. The pyrethrum plant parts like leaf and root are disregarded as source of essential ingredient perhaps they might show significant toxicity and needs investigation. Furthermore, numerous biological properties mainly insecticidal, larvicidal, pesticidal, etc have been reported for pyrethrum flower extracts. However, few studies have reported on aphicidal effect of flower extracts of pyrethrum but the anti-aphid effects of different concentrations of pyrethrum flower, leaf and root on growth, yields, chlorophyll and phytonutrient content is still lacking hence need investigation

The oil is made up of pyrethrum esters of chrysanthemic acid and pyrethric acid which is made of three alcohols, namely: pyrethrolone, cinerolone, and jasmololone. Pyrethrin I, comprises three esters of chrysanthemic acid: pyrethrin I, cinerin I, and jasmolin I. Pyrethrin II comprises three esters of pyrethric acid: pyrethrin II, cinerin II, and jasmoline II (Wang *et al*., 1997). Organic solvents are used to extract the active insecticidal constituents to give a concentrate with six types of pyrethrins (Wang *et al*., 1997) pyrethrin I and II, cinerin I and II, jasmolin I and II. Pyrethrins are insecticidal antagonist acts by delaying the closure of voltage-gated sodium ion channels in the nerve cells of insects this leads to hyper excitation hence death of the insect. The insect usually loss motor coordination therefore paralysis.

Techniques that have been used to extract pyrethrins include ultrasonic extraction, Soxhlet extraction, and supercritical fluid extraction. The most common is Soxhlet extraction which involves placing the sample in an extractor, and subsequently distilling, while introducing fresh portions of solvent at intervals (Nagar *et al*., 2015). The amount of pyrethrins extracted using this technique also depends on the solvent used. For instance, acetonitrile gave the best results in a study carried out by Nagar *et al*. (2015). For the case of extracts, ethanol solvent will be used this is in accordance to Nowrid (2017) who reported that the use of ethanol in extract preparation will result in to collection of highest amount of extracts compared to methanol. The basic parameters influencing the quality of an extract are: Plant part used as starting material, solvent used for extraction and extraction procedure:

2.3.2.1 Plant Material

Plant based natural constituents can be derived from parts of the plant like bark, leaves, flowers, roots, fruits and seeds. This indicates that any part of the plant may contain active components. Fresh or dried plant materials can be used as a source for the extraction of secondary plant components. In most of the reported works, underground parts (roots, tuber, rhizome, bulb etc.) of a plant were used extensively compared with other above ground parts in search for bioactive compounds possessing antimicrobial properties (Subasri and John, 2016).

2.3.2.2 Choice of solvents

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The choice depends on the targeted compounds to be extracted (Subasri and John, 2016). According to Subasri and John (2016), the various solvents that are used in the extraction procedures are: **Water:** Water is universal solvent, used to extract plant products with pesticidal activity. Scientist use primarily water but plant extracts from organic solvents have been found to give more consistent pesticidal activity compared to water extract. **Acetone:** Acetone dissolves many hydrophilic and lipophilic components from the two plants used, is miscible with water, is volatile and has a low toxicity to the bioassay used, it is a very useful extractant, especially where more phenolic compounds are required to be extracted.

2.3.3 Phytochemicals of *Chrysanthemum spp*

Phytochemicals are biologically active chemical compounds which are derived from plants. They play a key role to protect plants from pathogenic infections and damage. Plants have different types of phytochemicals such as phenolic acids, flavonoids and lignans which contribute to the plant's color, aroma and flavor. Phytochemicals accumulation takes place in different parts of the plants, such as roots, stems, leaves, flowers, fruits and seeds (Costa *et al*, 1999). There are more than 4,000 phytochemicals which have been classified by protective function, physical characteristics and chemical (Velavan, 2015). Phytochemicals play a significant influence as insecticidal, antifeedants, repellants, larvicidal, etc.

Chrysanthemum flowers are known as a unique class of material which possess rich contents of flavonoid, chlorophyll, carotenoids, soluble sugar, amino acid, vitamin C and chlorogenic acid at the time of bud stage and young flower stage (Ma *et al*., 2016). Chrysanthemum flowers at the early flower opening stage contain higher flavonoids and volatile oil and in the middle of the flowers the content of chlorogenic acid, luteolin, 3,5-O-dicaffeoyl quinic acid are higher (Wu *et al*., 2016). Chrysanthemum essential oils have typical aroma compounds covered with characteristic aroma and include: α-pinene, β-thujene, α-terpinolen, β-cubebene, caryophyllene, (Z)β-farnesene, (-)-spathulenol, linalool, camphor, camphene, 4-terpineol, Z-citral and 4-isopropyltoluene (Xiao *et al*., 2016).

A study done by Kim *et al*. (2015), found that flowers of twenty-three cultivars of Chrysanthemum contained the anthocyanins, cyanidin 3-glucoside and cyanidin 3-(3″-malonoyl) glucoside and the following carotenoids: lutein, zeaxanthin, β-cryptoxanthin, 13-cis-β-carotene, α-carotene, trans-β-carotene, and 9-cis-β-carotene. Using a microwave-assisted extraction approach ten flavonoid glycosides were extracted from the flowering heads of Chrysanthemum *spp* (Zhou *et al*., 2015). The main components identified were flavonoid glycosides, including three luteolin glycosides, three apigenin glycosides, three kaempferide glycosides, and one acacetin glycoside (Zhou *et al*., 2015). Dai *et al*. (2013) identified chlorogenic acid, caffeic acid, 1,3-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, luteolin-7-O-beta-D-glucoside, 3,4-dicaffeoylquinic acid, linarin and luteolin in *Chrysanthemum indicum* using a high-performance liquid chromatography (HPLC).

Leaves of Chrysanthemum *spp* main bioactive components are flavonoids, galuteolin, quercitrin, chlorogenic acid and 3 ,5-O-caffeoylquinic acid (Wang *et al*., 2015). These leaves contain octa-cosyl alcohol, β-sitosterol, lupeol, α-amyrin, daucosterol, ineupatorolide B, syringin, chlorogenic acid, petasiphenol, physcion, acacetin, eupatilin, quercetin, diosmetin, luteolin, apigenin, apigenin-7-O-β-D-glucopyranoside, quercetin-3-O-β-D-glucopyranoside, luteolin-7-O-β-D-gluco pyranoside, apigenin-7-O-β-D- neospheroside, and acacetin-7-O-β-D-glucoside (Wang *et al*., 2015). Roots of Chrysanthemum spp. contains 2-hexenal, tricyclene, sabinene, limonene, α-thujone, γ-terpinene, 1-octan-3yi-acetate, α- terpineol, myrtenol, α-cubebene, Longifolene, α-copaene, α-gurjunene, β-farnesene, δ-cadinene and α-cedren (Yao *et al*., 2015).

2.3.4 Success of Pyrethrum (*Chrysanthemum cinerariaefolium*) in Pest Control

Effective alternatives to hazardous synthetic pesticides do exist for small holder farmers in Africa including biological control with fungi and viruses, harnessing natural enemies as well as use of botanicals (Grzywacz *et al*., 2014). Because chrysanthemums contain pyrethrins which are pest specific, biodegradable, inexpensive, less prone to pest resistance and nontoxic to man and beneficial organisms, the pyrethrum extracts are used as insecticides to control insect pests of crops and ornamental plants. Pyrethrum flower extracts are sprayed on broccoli plants for protection from several common insect pest including aphids, leafhoppers and spider mites (Ashilenje, 2011).

Garlic and pyrethrum have been used to evaluate insecticidal effect against grass grub beetles which provided rapid knockdown as compared to synthetic insecticide used (Qureshi *et al*., 2002). This indicated that pyrethrum extracts could be effective insecticide rather than expensive organophosphates and organochlorides hence could be used in controlling aphids on *Solanum scabrum*. This is because the compounds in synthetic pesticides have significant and persistent toxic effects to non-target and beneficial organisms (Qureshi *et al*., 2002). A study carried out to determine the relative toxicities of the pyrethrins to female houseflies found that at 20°C, pyrethrin I was more effective than pyrethrum II (Sawicki *et al*., 1962). Therefore, pyrethrum extracts can be a success in aphid control. In another study, to compare the antifeedent activities of pyrethrins against white flies (*Bemisia tabaci*) and aphids (M*yzus persicae*), the latter were more sensitive to pyrethrins (Prota and Bouwmeester, 2014). Therefore, the sensitivity of aphids to pyrethrins provides a platform to investigating the anti-aphid effect of pyrethrum extracts on African nightshades (*Solanum scabrum)* hence the reason for using pyrethrum in this study.

Farmers need information on cheap, effective and safe ways particularly botanicals against aphids. Pyrethrum root extracts whose potential has not been fully utilized can be exploited. Haouas *et al.* (2008) investigated insecticidal activity of flower and leaf extracts from *Chrysanthemum* species against *Tribolium confusum* indicated that high toxicities were noted in flower methanolic extracts as compared to leaf methanolic extracts. Root extracts was not investigated in the study perhaps it might have shown distinct toxicity owing to the fact that it also contains secondary metabolites. The anti-aphid effects of pyrethrum extracts on growth, yields and phytonutrient content of *Solanum scabrum* is unknown hence the need to carry this study. These studies (Prota and Bouwmeester, 2014 and Haouas *et al*., 2008) have entirely concentrated on using the flowers as source pyrethrins disregarding other pyrethrum plant parts like roots and leaves therefore the need to investigate the anti-aphid effect of pyrethrum root and leaf extracts as well. This may be attributed to the less amount of bioactive compounds in root and leaf.

Pyrethrins were also observed to be degraded in the presence of ultraviolet (UV) light regardless of humidity or the presence of oxygen (Cox, 2002) hence environmentally safe. The use of crude plant based materials that are home harvested and prepared using only basic technology is where plants may have most to offer small holders, and in Africa this approach is currently and has been historically widespread (Isman, 2015). In a field experiment, the fate of pyrethrins in peaches have shown that they degrade quickly (Angioni *et al*., 2005). These studies point out the safety in using pyrethrins. There is scanty information on anti-aphid effect of pyrethrum extracts on growth, yields, chlorophyll and phytonutrient contents of African nightshades (*Solanum scabrum*) hence this study will investigate this.

2.4 Aphid (*Aphis fabae*) as an Agricultural Pest

Aphids are small and soft-bodied insects often called plant lice, they belong to the family Aphididae and the order Homoptera. They are a major pest of African nightshades (Ashilenje *et al*., 2011) which cause leaves to curl and become unattractive to customers. Aphids are tiny, 1-3 mm long and pear-shaped insects with piercing-sucking mouthparts that enable them to feed on plant sap. There are thousands species of aphids within numerous genera (Lawal, 2015). *Aphis fabae* is a polyphagous and economically important pest on agricultural crops with a worldwide distribution (Lawal, 2015). *Aphis fabae* has an extremely wide host range of over 100 plants including a wide variety of vegetables and ornamental crops. Aphids can reach pest proportions

rapidly through asexual reproduction all year round on a wide variety of secondary hosts including potatoes, tomatoes, brassicas, beets, cereals, pasture clovers, peas and peppers (Lawal, 2015).

2.4.1 Biology and Life Cycle of Aphids

Aphids are highly variable in both form and life cycle and within the same species, there are winged and non-winged forms and there are often both asexual and sexual life cycles within which there are distinct phenotypes (Lawal, 2015). They vary in colour from pale green, yellow or pink, white, dark brown to black. *Aphis fabae* spend most of the growing season as parthenogenetic viviparous females with a generation time of about one week. Aphids reproduce without mating and they give birth to live aphids instead of laying eggs. All parthenogenetic aphids are born as wingless nymphs, but can develop into either alate (winged) or apterous (wingless) morphs at maturity (Blackman and Eastop, 2000). Usually the formation of alate forms within a population is triggered by overcrowding, limited food, need for migration or the presence of genetic plant resistance (Lawal, 2015).

Females reproduce live offspring that already have aphids developing inside them (Capinera, 2001). These characteristics give aphids an extremely high rate of reproduction and this is why they are difficult to control with sprays hence need to develop botanicals which can curb the crisis caused by these aphids therefore this study investigated the effects of pyrethrum extracts on aphids affecting *Solanum scabrum.* An entire colony can be generated from one surviving individual (Ashilenje *et al*., 2011). Life cycle of aphids consist of egg which are very thin and shiny black hatches to first instar, this hatches into second instar, then third instar and the fourth instar matures to adult which can give birth to live aphids. Immature stages are called nymphs, they are young aphids and look like wingless adults but they are smaller. They become adults within 7 to 10 days. This occurs under favourable conditions. Unfavourable conditions enhances sexual reproduction where male and female mate hence laying of eggs (Lawal, 2015).

2.4.2 Effects of Aphids on growth and yields of crops

Aphis fabae has an extremely wide host range of over 100 plants including a wide variety of vegetables and ornamental crops. It is an important insect pest of the Solanaceae family and can attain very high densities on young plant tissue, causing water stress, wilting, and reduced growth rate of the plant (Lawal, 2015). *Aphis fabae* reduces both weight and caloric content of young

African nightshades by as much as 64 and 113%, respectively, depending on the number of feeding aphids. Yield losses due to aphids is estimated to about 84-96% in Kenya. La Rossa *et al*. (2013) reported that aphid populations can grow to extremely high levels under favorable environmental conditions in a short time, covering sprouts, leaves, flowers, and fruits which reduces the availability of photosynthetic active radiation to the plants. Lo *et al*. (1999) reported that large populations of *Aphis fabae* developing on leaves of peach and nectarine plants will stunt or kill young shoots if left unchecked. These studies depicted the menace associated with aphids and impacts on plant growth and yields, arguably, there is still little information on the anti-aphid effects of pyrethrum extracts on growth and yields of African nightshades (*Solanum scabrum*).

High densities of these aphids can also cause actively growing leaves to curl, thereby forming pockets and folds which is known to provide shelter to the aphids but consequently unknown to give protection against insecticide treatments (Iguchi *et al*., 2012). Among piercing-sucking insects, aphids are especially important pests of agriculture; they cause plant damage both by the direct removal of nutrients and by transmitting the majority of insect-vectored plant viruses. Chander *et al*. (2006) reported that the direct feeding of aphids results into reduced growth rates of green leaves hence low leaf yields. Aphids infest the underside of leaves of this vegetable causing them to curl hence young apices of affected plants fail to develop which reduces crop yield (Ashilenje *et al*., 2011). However, information on the anti-aphid effects of pyrethrum extracts on yields of African nightshades (*Solanum scabrum*) is still lacking hence the reason this study was carried out.

2.4.3 Effects of Aphids on Chlorophyll and Phytonutrient contents of crops

African nightshade leaves consist of important phytonutrients which consist of 87.2 g water, 1.0 mg iron, 4.3 g protein, 38 kcalories, 5.7 g carbohydrates, 1.4 g fiber, 442 mg calcium, 20 mg ascorbic acid, 3660 µg ß-Carotene, 75 mg phosphorus, and 0.59 mg riboflavin per 100 g fresh weight (Muthomi and Musyimi, 2009). According to Chander *et al*. (2006) aphid infestations results into impacts which can be elaborated with different pest damage mechanisms. The pest damage mechanisms can be defined as plant physiological processes affected by pests. They include germination reduction, stand reduction, light stealing, assimilation rate reduction, assimilate sucking and tissue consumption (Chander *et al*., 2006).

Assessing assimilation rates of wheat, Chander *et al.* (2006) found that aphids are assimilate sappers which greatly reduce plant assimilation rate and together with the direct feeding results into reduced stem reserves and storage organs. Stem reserves and storage organs stores important phytonutrients including proteins, carbohydrates, fatty acids and minerals (Lawal, 2015). The anti-aphid effects of pyrethrum extracts on phytonutrient content of *Solanum scabrum* is unknown hence the reason for undertaking this study. Aphids can be serious and persistent in vegetable farm (Onyango *et al*., 2000) due to very short life cycle and high reproductive rate which make their control with chemicals difficult. Lawal (2015) reported prolonged aphid infestation can cause appreciable reduction in plant yield in pepper because they consume plant nutrients and their sucking behavior can cause chlorosis and distortion of the leaves, abscission of blooms, and plant stunting and wilting. Chlorosis results into reduced concentrations of chlorophyll leading to reduction in food manufactured by the plant (Lawal, 2015). Aphids exude honeydew, a sticky substance that encourages growth of sooty molds, this damages the plant by shading the leaf surface thus reducing the availability of the photosynthetic active radiation to the plants (Chander *et al.,* 2006). There is a dearth of information on the anti-aphid effect of pyrethrum extracts on chlorophyll of *Solanum scabrum*.

2.4.4 Flame photometry or flame atomic emission spectrometry

Photoelectric flame photometry, a branch of atomic spectroscopy is used for inorganic chemical analysis for determining the concentration of certain metal ions such as sodium, potassium, lithium, calcium, Cesium, the species is examined in the form of atoms. In flame photometry the species (metal ions) used in the spectrum are in the form of atoms. The basis of flame photometric working is that, the species of alkali metals (Group 1) and alkaline earth metals (Group II) metals are dissociated due to the thermal energy provided by the flame source in flame photometer. A schematic representation of flame photometer is shown in Figure 1. Due to this thermal excitation, some of the atoms are excited to a higher energy level where they are not stable. The absorbance of light due to the electrons excitation can be measured by using the direct absorption techniques. The subsequent loss of energy will result in the movement of excited atoms to the low energy ground state with emission of some radiations, which can be visualized in the visible region of the spectrum. The absorbance of light due to the electrons excitation can be measured by using the direct absorption techniques while the emitting radiation intensity is measured using the emission

techniques. The wavelength of emitted light is specific for specific elements (https://vlab.amrita.edu/?sub=2&brch=294&sim=1351&cnt=1). Sodium for instance, relies on the fact that the sodium ion emits light at a wavelength of 589nm when excited in a gas flame (Figure 2). Intensity of light produced is proportional to the concentration of the element therefore sodium concentration is read from a calibration curve (Appendix 5).

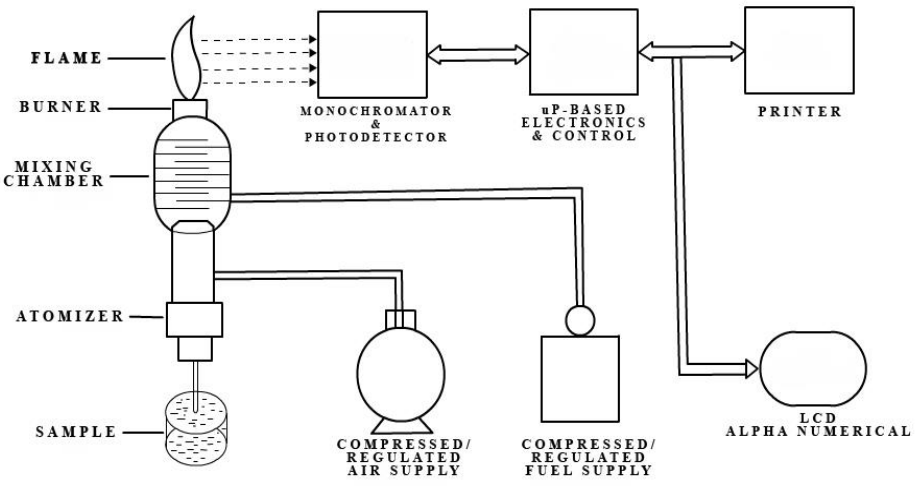

Fig 1: A schematic representation of flame photometer

The energy level diagram of the sodium atom is shown in figure 2

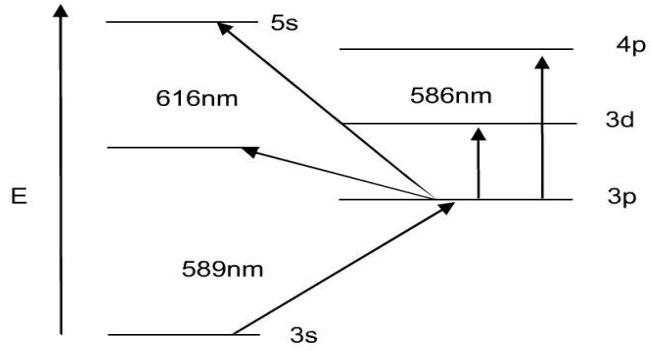

Fig 2: Energy level diagram for atomic sodium

The intensity of emitted light is directly related to the concentration of the sample.

The comparison of emission intensities of unknown samples to either that of standard solutions (plotting calibration curve), or to those of an internal standard (standard addition method), helps

in the quantitative analysis of the analyte metal in the sample solution. The flame emissions of the alkali and alkaline earth metals in terms of the emission wavelength and the characteristic color produced by each element is shown in table 2.

Table 2: Flame emissions of elements

Name of the element	Emitted wavelength range (nm)	Observed colour of the flame
Potassium (K)	766	Violet 
Lithium (Li)	670	Red 
Calcium (Ca)	622	Orange 
Sodium (Na)	589	Yellow 
Barium (Ba)	554	Lime green 

Retrived from: https://vlab.amrita.edu/?sub=2&brch=294&sim=1351&cnt=1

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Site

The study was carried out at Maseno University farm between March- June, 2018. Confirmatory study of the same was repeated in September to December, 2018. The experimental area lies at latitude 0º1'N0º12'S and longitude 34º25'E-34º47'E. The soils are classified as acrisol, deep reddish brown friable clay with pH ranging from 4.5 to 5.5. Soil organic carbon and phosphorus contents are 1.8% and 4.5 mg kg-1, respectively (Netondo, 1999). It is approximately 1,500 m above the sea level and receives an annual mean precipitation of 1,750 mm with bimodal pattern of distribution and the mean air temperature is 28.7ºC with a 40% relative humidity (Ambede *et al*., 2012).

3.2 Collection of Pyrethrum

Pyrethrum flowers, leaves and roots were collected from Kenya Agriculture and Livestock Research Organization (KALRO) - Kitale branch. The flowers, leaves and roots were packed in separate bags and transported to Maseno. The plant parts were sundried in shade until completely dry for ten days according to the procedure of Cox (2002), who reported that the process of drying under sun light helps in increasing pyrethrin content. They were ground separately using heavy duty laboratory mill into fine powder then stored in airtight plastic containers at room temperature (25 ºC) for extractions. This kind of storage prevented moisture absorption from the atmosphere.

3.3 Preparation of Pyrethrum Extracts

Pyrethrum plant extracts were prepared weekly during the study period by quantifying 100 grams of dry flowers, 100 grams of dry leaves and 100 grams of dry roots separately. Each part of the pyrethrum was soaked in 250 ml of 70% ethanol under fume hood at room temperature for 3 days to extract most bioactive compounds (Haouas *et al*., 2008). For the case of extract preparation, ethanol solvent was used this is in accordance to Nowrid (2017) who reported that the use of ethanol in extract preparation will result in to collection of highest amount of extracts compared to methanol. Ethanol dissolves more bioactive compounds in pyrethrum. The solvents and different parts of pyrethrum (flower, leaves and roots) were filtered using filter paper (Whatman

no. 1). The extracts were taken to dryness under vacuum at 35°C and then dissolved in acetone to obtain a final concentration of 100%. Different concentrations that is 67% and 33% was prepared from the final extract (Haouas *et al*., 2008). The choice of the different fore mentioned concentrations was based on the procedure of Jangam *et al*., (2014) where they stated that for highest % repellency to be found in plant extracts, 100% followed by 67% concentration level should be employed and minimum % repellency is seen in 33% in some selected herbal plants for pest management (among them is pyrethrum). As the dose increases, the repellant effect also increased. The use of such plant extracts can control the population of serious pests like aphids and mealybugs in an environmental friendly way (Jangam *et al*., (2014).

3.4 Phytochemical Screening

The solvent extracts of pyrethrum plant parts (flower, leaf and root) were subjected to routine qualitative chemical analysis to identify the nature of phytochemical constituents present in sample (Subasri and John, 2016). The varying amount was distinguished from the intensity of their colours. The choice of phytochemicals screened was linked to their cytotoxic, deleterious and defensive mechanisms against phytophagous insects like aphids

3.4.1 Determination of Phytosterols

To 3 ml of flower extracts, chloroform was added then filtered. The filtrates were treated with 3 drops of acetic anhydride, boiled and cooled. Concentrated Sulphuric acid was added. Formation of brown ring at the junction indicated the presence of phytosterols. The procedure was repeated for leaf and root extracts (Subasri and John, 2016).

3.4.2 Determination of Alkaloids

To 3 ml of flower extracts, 1 ml of dilute Hydrochloric acid was added then filtered. Two drops of Mayer's reagent was then added to the filtrate. Formation of yellow coloured precipitate or turbidity indicated the presence of alkaloids. The procedure was repeated for leaf and root extracts (Subasri and John, 2016).

3.4.3 Test for Phenols

To 3 ml of flower extracts, 3 drops of ferric chloride (5%) solution was added. Formation of bluish black colour indicated the presence of phenols. The procedure was repeated for leaf and root extracts (Subasri and John, 2016).

3.4.4 Test for Flavonoids

To 3 ml of flower extracts, two drops of sodium hydroxide solution was added. Formation of intense yellow colour, which became colourless on addition of dilute acid, indicated the presence of flavonoids. The procedure was repeated for leaf and root extracts (Subasri and John, 2016).

3.4.5 Test for Saponins

To 3 ml of flower extracts, 2 ml of water was added then shaken. Formation of foamy lather indicated the presence of saponins. The procedure was repeated for leaf and root extracts (Subasri and John, 2016).

3.4.6 Triterpenoids

To 3 ml of flower extracts, a piece of tin and 2 drops of thionyl chloride was added. Formation of violet or purple colour indicated the presence of triterpenoids. The procedure was repeated for leaf and root extracts (Subasri and John, 2016).

3.4.7 Detection of tannins

To 3 ml of flower extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicated the presence of tannins. The procedure was repeated for leaf and root extracts (Subasri and John, 2016).

3.5 Preparation of Plot and Planting of African nightshades (*Solanum scabrum*)

The land measuring 18.5m by 13m was cleared first and primary cultivation carried out. Secondary cultivation followed after one week (Kipkosgei *et al*., 2003). Forty-five (45) experimental plots were prepared following the procedure of Ahmed (2000), Kora and Teshemo (2016) with some modifications. The total number of plots (45) were used following the treatments used (5) for every pyrethrum plant part (3) and replications (3). A plot of 3m by 1m was prepared by raising the soil about 15 cm above ground (Masinde *et al*., 2009). A path of 50cm was left between plots and 100cm path between blocks. African nightshades (*Solanum scabrum*) seeds were locally sourced from School of Agriculture, Maseno University. According to Masinde *et al*. (2009), african nightshades are best adjusted to soils with high nitrogen and phosphorus. Therefore, Di-Ammonium Phosphate (DAP) fertilizer rich in nitrogen and phosphorus was applied to the soil and mixed thoroughly before sowing. Six sowing lines (rows) at a spacing of 40 cm was made in

every plot and 3 seeds were sown. Watering was done twice daily, in the morning and evening (Kipkosgei *et al*., 2003; Muthomi and Musyimi (2009) for good growth. After four weeks, seedlings were thinned to 9 seedlings per plot according to Carnot *et al*. (2017). Each seedling had a spacing of 40 cm by 40cm on slightly raised beds (Kipkosgei *et al*., 2003). The plots were caged using mosquito net placed vertically above and to a depth of 50cm in order to minimize migration and monitor the aphid population (Araka *et al*., 2016). The plots were manually maintained weed-free. African nightshades (*Solanum scabrum*) took 12 weeks to mature.

3.6 Rearing of Aphids and Infestation of Vegetables

Aphids (*Aphis fabae*) free from viruses were obtained from International Center of Insect Physiology and Ecology (ICIPE)-Nairobi. To obtain enough aphids for the study, the aphids were reared on three potted disease-free *Solanum scabrum* seedlings in Maseno University greenhouse for two weeks prior to their introduction to vegetables in caged experimental plots in the farm. Enough aphids were collected from the spreader plants and 10 aphids (constant number) were introduced to each 5 weeks-old African nightshades seedling (Ashilenje *et al*., 2011). Aphid population was monitored for 10 days to ensure equal infestation, also the movement of aphids on the plant following introduction was observed (Ashilenje *et al*., 2011).

3.7 Experimental Design and Treatment

A field experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. Experimental treatments (5) were pyrethrum flower, leaf and root extracts (active ingredient 12g/litre pyrethrum) according to Qureshi *et al*. (2002). Three levels were: 0%, 33%, 67% and 100% of pyrethrum flower, leaf and root extracts and a synthetic chemical Duduthrin® (Lambda-cyhalothrin 17.5g/litre) was used as standard check and sterile water as untreated control. In summary treatments are as follows:

Treatment 1: No plant extract/sterile water (P1=0%)

Treatment 2: Plants treated with 33% of pyrethrum extracts (P2=33%)

Treatment 3: Plants treated with 67% of pyrethrum extracts (P3=67%)

Treatment 4: Plants treated with 100% of pyrethrum extracts (P4=100%)

Treatment 5: Plants treated with Duduthrin (Standard check)

3.8 Determination of Aphid Population

Aphid population was assessed following the procedure used by Muthomi *et al*. (2009). Aphid population was monitored on leaves and by use of yellow water pan traps. Leaves were used to monitor wingless (apteral) aphids while the yellow water pan traps were used to monitor winged (alate) aphids. Five plants were randomly selected from each plot with 9 plants (Ahmed, 2000) and counting of aphids from each plant was done every 24 hours after treatment application to the vegetable till the end of experiment. Treatments used were: 0%, 33%, 67% and 100% of pyrethrum flower, leaf and root extracts and a synthetic chemical Duduthrin® (Lambda-cyhalothrin 17.5g/litre) was used as standard check and sterile water as untreated control. The rate 25ml/litre of water was used since it is the rate recommended for aphids, thrips, leafhoppers and caterpillers (Qureshi *et al*., 2002). In the untreated plot (negative control), the seedlings were sprayed with sterile water and the standard check plot (positive control) was sprayed with synthetic chemical Duduthrin® at a rate of 35ml/20litre of water. The spraying was done in the evening using knapsack sprayer at seven-day interval according to Ahmed, (2000) throughout the season to get full control of the target (aphids) pests, keeping in mind good coverage of the plant. Treatments were therefore applied when the aphid population reached economic threshold Level *viz*. 35% of infested irrespective of the stage of growth (Kamal, 1997). The threshold was achieved when the tallied number of aphids per plant reached 30. The average number of aphids detected in each count was computed for each treatment. The yellow water pan traps consisted of yellow basins ¾ filled with water and a few drops of liquid detergent added to break the surface tension to make the trapped insects sink to the bottom and most of them died hence were disposed. Two water pan traps were placed in each plot. The traps were replaced weekly and aphid counts taken (Muthomi *et al*., 2009).

3.9 Determination of Growth Parameters

Plant growth parameters were determined after six weeks of planting at seven day intervals from the day the treatments were initiated according to Ambede *et al*. (2012). These included plant height, number of curled leaves, leaf length, leaf width, leaf area, number of fruits and number of damaged fruits. These parameters were determined to study the effects (anti-aphid) of the pyrethrum treatments on the intended pest.

3.9.1 Plant Height

After six weeks of planting, the plant height was taken weekly till the end of experiment. Five plants were randomly selected from each plot with nine plants then measured (cm) from soil level up to the upper most leaf of the plant by a meter rule (Said *et al*., 2015).

3.9.2 Number of Curled Leaves

The number of curled leaves curled leaves from six-week old plants were counted weekly and recorded by selecting five plants randomly in each plot with nine plants and means were calculated.

3.9.3 Leaf Length and Width

Five plants were randomly selected from each plot. Leaf length (L) and width (W) were measured (cm) weekly from six-week old plants using a meter rule till the end of experiment.

3.9.4 Leaf Area

After six weeks of planting, five plants were randomly selected from each plot then leaf area was determined weekly (cm^2) till the end of experiment using the formula of Otusunya *et al*. (2007) as indicated below:

$LA=0.5(L*W)$

Where L=length of leaf

W=maximum width.

3.9.5 Number of Fruits

Five plants were randomly selected from each plot and the number of fruits were counted weekly till the end of experiment. This was done after 8 weeks after planting during which the fruits had started to form.

3.9.6 Number of Damaged Fruits

At the 8^{th} week after planting, five plants were randomly selected from each plot and the number of damaged fruits were noted weekly till the end of experiment

3.9.7 Shoot and Root Fresh Weights

Shoot and root fresh weights were determined (g) at the end of experiment on 12th week after planting. Five plants were randomly selected from each plot. The plants parts were carefully uprooted from the soil, cleared off debris, separated into shoot and root and measured separately using electronic weighing balance (Ondieki *et al*., 2011).

3.9.8 Shoot and Root Dry Weights

Shoot and root dry weights were determined (g) at the end of experiment (12th week) after planting. Five plants were randomly selected from each plot with nine plants. The plants (roots and shoots) were packaged separately in envelopes and dried to constant weight at 80◦C in an oven. Root and shoot dry weights were then obtained using an electronic weighing balance (Ondieki *et al*., 2011).

3.9.9 Determination of Yield

After 12 weeks of planting, the total weight (kg) of cured leaves of all plants from each plot (3m by 1m) after each picking was summed then the yield per hectare for each treatment was obtained using the formula of Said *et al*. (2009):

Yield/ha=Cured leaves(kg/ha)

Therefore:

Cured leaf (kg/ha) = (Total cured weight (g) ÷ Net area harvested) ×10,000.

3.10 Determination of Chlorophyll and Phytonutrient Content

3.10.1 Chlorophyll Content

Chlorophyll content was determined from 8 week-old plants during the vegetative stage (Ondieki *et al.*, 2011). Determination of chlorophyll followed the formula of Adelusi *et al*. (2006). It involved selecting a third fully expanded leaf from shoot apex sampled from five plants in each plot containing nine plants. About 3 grams of leaf was grounded in 10ml of 80% (V/V) acetone using mortar and pestle. They were then left overnight for 24 hours to allow maximum extraction of chlorophyll. The absorbance of the resulting extract was measured using a spectrophotometer

(Model: Novaspec II, Pharmacia Biotech, Cambridge, England) at 645 and 663 nm in order to determine the content of chlorophylls *a* and *b* respectively. Total chlorophyll was calculated by adding chlorophylls *a* and *b*. The resulting Chlorophyll *a*, *b* and total concentration was calculated as follows:

Chlorophyll a =13.19 A664-2.57 A645 (mgg-1 fresh weight)

Chlorophyll b =22.1 A664-5.26 A664 (mgg-1 fresh weight)

Total Chlorophyll =7.93 A664+19.53 A645 (mgg-1 fresh weight)

Where A664 is the absorbance at 664nm and A645 is the absorbance at 645nm.

3.10.2 Phytonutrient content Analysis

3.10.2.1 Standard stock solution preparation

Stock solution was prepared from analytical reagent grade NaCl, KCl and $CaCO_3$ dried in oven at 105^0C for 1hour. Part of the dried standard chemicals weighed; that is 0.2543g of NaCl, 0.1910g of KCl and 1.4g of [(NH4) SO4.FeSO4]. The amount of potassium, sodium and calcium was dissolved in deionized water. The dissolved salts were transferred into 1000ml volumetric flask and diluted to the mark to prepare10ppm for sodium and potassium and 100ppm for calcium ions.

Five plants (*Solanum scabrum*) were sampled from each of the 45 plots. Ten grams of leaves were picked from the same position in all plants then washed with deionized water and dried at 65°C for 12 hours (Bertin *et al*., 2014). The samples were ground sufficiently using grinder and stored well for sodium, calcium and potassium analysis.

3.10.2.2 Determination of Sodium, Calcium and Potassium

The concentration of mineral elements was determined using flame photometry. Prior to the determination of the total element concentrations a complete destruction of the organic matrix of homogenized sample was done and during mineralization process all organic compounds were converted in to inorganic elements (Bertin *et al*., 2014). The samples 0.5 g of the whole *Solanum scabrum* powder was microwave digested, with 6 ml nitric acid (60%v/v) and 1ml of sodium peroxide (30%) at 250-600 W for 30 minutes in closed vessels. Digested samples were diluted appropriately with pure water. Determination of absorbance of sodium, calcium and potassium

was performed by photometry (AOAC, 2005). The choice of these nutrients was based on the imbalances caused by aphids caused when they such these minerals.

3.11 Data Analysis

Data collected were subjected to statistical analysis using SAS Procedure (SAS Institute, 1998). A one-way analysis of variance was used to compare the means of various growth parameters among the treatments and a two-way analysis of variance (ANOVA) was used to determine the interaction between aphid population and growth parameters and treatment. Means were separated and compared using the Least Significant Difference (LSD) at p=0.05 (Steel and Torrie, 1992).

CHAPTER FOUR

RESULTS

4.1 Determination of Phytochemicals in Pyrethrum Flower, Root and Leaf Parts

The phytochemical screening of plant parts of pyrethrum (flower, leaf and roots) indicated the presence of phenols, saponins, alkaloids, flavonoids, triterpenoids, tannins and phytosterols as shown in Table 3. Findings from this study showed that different pyrethrum parts contain varying amounts of secondary metabolites and was distinguished by the intensity of the colours. Pyrethrum flower extracts recorded the highest amount of phenols, flavonoids and triterpenoids, average amount of saponins, alkaloids, tannins and little amount of phytosterols. Pyrethrum leaf extracts showed average amount of phytosterols, alkaloids, phenols, flavonoids, tannins, little amount of saponins but triterpenoids were absent. Pyrethrum root extracts indicated a higher amount of saponins, alkaloids and phytosterols but showed little amount of phenols, flavonoids and tannins.

Table 3: Secondary metabolites in pyrethrum (*Chrysanthemum cinerariaefolium*) flower, leaf and root extracts.

Secondary Metabolite	**Presence or Absence in Plant Parts**		
	Flowers	Leaves	Roots
Phytosterols	+	++	+++
Alkaloids	++	++	+++
Phenols	+++	++	+
Flavanoids	+++	++	+
Saponins	++	+	+++
Triterpenoids	+++	—	++
Tannins	++	++	+

Key

+++: Present in high amount

++: Present in average amount

+: Present in little amount

—: Absent

4.2 Determination of Effect of pyrethrum extracts on aphid population on *Solanum scabrum*

The effect of different concentrations of pyrethrum extracts on aphid population on *Solanum scabrum* are presented in Tables 4a and 4b. From results on Table 4a different concentrations (33%, 67% and 100%) of pyrethrum flower, root and leaf extracts showed no significant difference ($p \geq 0.05$) in aphid number in day 60 but the fore mentioned concentrations in subsequent days (Day 62,69,76 and 83) were significantly different. *Solanum scabrum* treated with 100% pyrethrum flower extracts recorded zero (0.00 aphids) number of aphids on 69th and 83rd days while 67% recorded zero count (0.00 aphids) on 76th and 83rd days. The effect of duduthrin on aphid population was not significantly different ($p \geq 0.05$) from the effect of 100%, 67% and 33% pyrethrum flower treatments. On 76th day, plants treated with duduthrin recorded zero aphid count but the number increased to 11 aphids on 83rd day. From the results (Table 4a), different concentrations of pyrethrum root extracts showed no significant effect on aphid population on 60th day. On 62nd day, significant effect on aphid population was noted from 33%, 67% and 100% pyrethrum root extracts as compared to control (0%) which maintained a higher aphid count of 126 aphids. Though, aphids were sensitive to 33% pyrethrum flower, root and leaf extracts, their number was not greatly reduced. A similar fore mentioned observation was noted on 69th, 76th and 83rd days. On 76th and 83rd days plants treated with 67% pyrethrum root extracts significantly reduced aphid population to zero, also on 69th day plants treated with 100% pyrethrum root extracts caused 100% kill of aphids. *Solanum scabrum* treated with varying concentrations of pyrethrum leaf extracts on 60th day showed no significant difference in the aphid population (Table 4a). On 62nd, 76th and 83rd days, significant effect on aphid population were noted from 33%, 67% and 100% pyrethrum leaf extracts as compared to control (0%) where the number of aphids continuously increased. Aphid number varied significantly in treatments 33%, 67% and 100% pyrethrum leaf extracts as compared to control but aphid population on *Solanum scabrum* treated with duduthrin did not differ significantly with 33% on 76th day. *Solanum scabrum* treated with control showed no significant effect ($p \geq 0.05$) on aphid number therefore, a higher aphid number observed all through. The pyrethrum extracts used in the study were found to be more or as effective as the standard check Duduthrin® (Lambda-cyhalothrin 17.5g/litre). There were significant interactions between pyrethrum extract concentration and days on aphid population ($p=0.0001$) (Appendix 3.1).

Overally, results from Table 4b indicated that pyrethrum root extracts caused significant reduction in aphid population (61.27 aphids) as compared to flower extracts and leaf extracts which recorded 68.91 and 69.23 aphids respectively. In addition, all the varying concentrations of pyrethrum extracts (33%, 67 % and 100%) showed significant effect on aphid population as compared to control. Generally, aphid population reduced significantly from 60th day to 83rd day.

Table 4a: Effect of different concentrations (33%, 67% and 100%) of pyrethrum flower, leaf and root extracts on aphid population on *Solanum scabrum* from 60th to 83rd days

Plant parts	**Treatments Extract concentrations**	***Days***				
		Day 60	***Day 62***	***Day 69***	***Day 76***	***Day 83***
Flower	0%	100.67a	160.33a	195.00a	266.00a	323.00a
	33%	107.00a	77.00b	6.00b	2.67b	1.67b
	67%	104.00a	53.00bc	3.33b	0.00b	0.00b
	100%	106.67a	39.00c	0.00b	7.67b	0.00b
	Duduthrin	104.67a	40.00c	15.00b	0.00b	11.00b
	LSD	46.45	31.46	29.95	29.07	24.63
Root	0%	108.00a	126.00a	168.00a	203.00a	251.00a
	33%	104.00a	45.00b	8.67b	1.33b	3.00b
	67%	108.67a	47.33b	5.33b	0.00b	0.00b
	100%	112.33a	58.00b	0.00b	5.33b	2.00b
	Duduthrin	110.33a	48.33b	18.00b	2.33b	3.00b
	LSD	66.68	50.55	21.92	31.89	35.34
Leaf	0%	103.00a	142.00a	180.00a	207.67a	275.33a
	33%	107.00a	86.00b	19.33bc	8.33b	4.33b
	67%	105.33a	71.67b	27.33b	11.00b	2.67b
	100%	104.00a	67.33b	2.33c	2.33b	5.00b
	Duduthrin	102.00a	67.67b	18.00bc	3.67b	6.67b
	LSD	47.77	48.67	24.85	30.55	26.96

Means with the same letter down the column are not significantly different at $p \leq 0.05$.

Table 4b: Overall Effect of different concentrations (33%, 67% and 100%) of pyrethrum flower, leaf and root extracts on aphid population on *Solanum scabrum* from 60th to 83rd days

Variables	Aphid population
Pyrethrum plant parts	
Flower Extracts	68.91a
Leaf Extracts	69.23a
Root Extracts	61.27b
LSD	5.38
Extract concentration	
100%	34.13b
67%	35.98b
33%	38.76b
0%	186.71a
Duduthrin	36.76b
LSD	6.95
Day	
Day 60	105.89a
Day 62	75.24b
Day 69	44.36d
Day 76	48.09d
Day 83	58.76c
LSD	6.95

Means with the same letter down the column are not significantly different at $p \leq 0.05$.

4.3 Determination of Anti-aphid effect of pyrethrum extracts on growth parameters and yields of *Solanum scabrum*

4.3.1 Plant Height

Plant height responses of *Solanum scabrum* as a result of aphid control by use of varying concentrations of pyrethrum flower, root and leaf extracts are presented in Table 5a and 5b. The control of aphids by application of the different concentrations (33%, 67% and 100%) of pyrethrum flower extracts had no significant effect ($p \geq 0.05$) on plant heights of *Solanum scabrum* on 60th, 62nd, 69th, 76th and 83rd days as compared to control (0%) (Table 5a). In addition, plant

height of *Solanum scabrum* treated with duduthrin did not differ significantly with those from pyrethrum flower treatments. The control of aphids by application of the various concentrations (33%, 67% and 100%) of pyrethrum root extracts had no significant effect ($p \geq 0.05$) on plant heights of *Solanum scabrum* on 60^{th} ,62^{nd} ,69^{th}, 76^{th} and 83^{rd} days as compared to the control (0%) (Table 5a). The results from Table 5a indicated that the plant heights of *Solanum scabrum* treated with 33%, 67% and 100% pyrethrum leaf extracts did not differ significantly with those from duduthrin and control (0%) on 60^{th}, 62^{nd}, 69^{th},76^{th} and 83^{rd} days. Results presented in Table 5b showed an overall performance of different concentration of pyrethrum flower, leaf and root extracts on plant height of *Solanum scabrum*. Overally, there was no significant difference ($p \geq 0.05$) on plant height of plants treated with both pyrethrum flower extracts and pyrethrum root extracts. The two fore mentioned plant part extracts were significantly different ($p \leq 0.05$) from pyrethrum leaf extracts. In addition, 100% extract concentration recorded significantly higher plant height (68.37cm) as compared to control. Plant height increased significantly from 60^{th} day to 83^{rd} day.

Table 5a: Anti-aphid effects of different concentration (33%, 67% and 100%) of pyrethrum flower, leaf and root extracts on plant height of *Solanum scabrum* from 60^{th} to 83^{rd} day

Parameter	Plant Part	Treatment Extract concentrations	Days				
			Day 60	Day 62	Day 69	Day 76	Day 83
Plant Height (cm)	Flower	0%	39.33a	40.33a	48.33a	50.00a	55.00a
		33%	45.67a	48.67a	56.00a	61.27a	64.67a
		67%	48.00a	50.00a	58.33a	63.67a	71.67a
		100%	59.33a	63.00a	74.67a	77.67a	82.67a
		Duduthrin	58.67a	60.00a	71.67a	76.67a	80.67a
		LSD	30.30	29.76	33.35	38.88	41.79
	Root	0%	34.33a	35.67a	40.33a	43.67a	47.80b
		33%	39.67a	42.00a	52.67a	59.00a	63.33ab
		67%	43.33a	45.33a	55.33a	68.33a	73.33ab
		100%	67.33a	69.67a	74.33a	82.90a	88.00a
		Duduthrin	52.33a	54.33a	65.67a	69.33a	78.03ab

		LSD	35.21	35.28	38.22	39.94	39.37
	Leaf	0%	30.00a	39.33a	42.00a	47.33a	51.00a
		33%	45.33a	48.67a	49.00a	52.00a	52.67a
		67%	49.00a	50.00a	50.33a	53.67a	59.00a
		100%	53.66a	54.67a	56.33a	59.67a	61.67a
		Duduthrin	49.67a	51.67a	59.67a	64.60a	68.00a
		LSD	15.46	26.58	26.04	26.28	26.22

Means with the same letter down the column are not significantly different at $p \leq 0.05$.

Table 5b: Overall anti-aphid effects of different concentration (33%, 67% and 100%) of pyrethrum flower, leaf and root extracts on plant height of *Solanum scabrum* from 60th to 83rd day

Variables	Plant Height (cm)
Pyrethrum plant parts	
Flower Extracts	60.24a
Leaf Extracts	52.32b
Root Extracts	57.76a
LSD	2.48
Extract concentration	
100%	68.37a
67%	55.96c
33%	52.04d
0%	43.42e
Duduthrin	64.06b
LSD	3.20
Day	
60	48.31d
62	50.22d
69	56.98c
76	61.98b
83	66.36a
LSD	3.20

Means with the same letter down the column are not significantly different at $p \leq 0.05$.

4.3.2 Curled Leaves

Results presented in Tables 6a and 6b shows the effect of controlling aphids using different concentration 33%, 67% and 100% of pyrethrum flower, leaf and root extracts on leaf curling of *Solanum scabrum*. The control of aphids by application of the 33%, 67% and 100% pyrethrum flower extracts showed no significant effect ($p \geq 0.05$) on curled leaves of *Solanum scabrum* as compared to control (0%) on 60^{th} ,62^{nd} ,69^{th} ,76^{th} and 83^{rd} days (Table 6a). The control of aphids by application of 33%, 67% and 100% pyrethrum root extracts had no significant effect ($p \geq 0.05$) on curled leaves of *Solanum scabrum* as compared to control (0%) on 60^{th} ,62^{nd}, and 69^{th} (Table 6a). On day 76^{th} a significant effect on curled leaves of *Solanum scabrum* was noted from plants treated with 33%, 67% and 100% pyrethrum root extracts as compared to 0%. Also, 83^{rd} day showed a significantly different number of curled leaves on plants treated with 33%, 67% and 100% as compared to control. From Table 6a, the control of aphids by application of the various concentrations: 33%, 67% and 100% pyrethrum leaf extracts had no significant effect ($p \geq 0.05$) on curled leaves of *Solanum scabrum* on 60^{th} ,69^{th},76^{th} and 83^{rd} days except 62^{nd} day (Table 6a). In addition, Duduthrin showed no significant difference on leaf curling of *Solanum scabrum* as compared to the different concentrations of pyrethrum extracts but a higher number of curled leaves was observed from plants under control (0%) (Table 6a).

Overally, the control of aphids using pyrethrum flower extract showed no significant difference on number of curled leaves as compared to pyrethrum root extracts, the effect on number of curled leaves of plants treated with pyrethrum leaf extracts showed significance compared to fore mentioned pyrethrum extracts. The use of different concentrations (33%, 67% and 100%) of pyrethrum extracts had significant effect on number of curled leaves of *Solanum scabrum* as compared to control. The least number of curled leaves (4.25) was recorded from plants sprayed with 100% pyrethrum extracts More curled leaves (11.89) were recorded from plants treated with control (0%) (Table 6b). The effect of controlling aphids using pyrethrum extracts significantly lead to reduction in number of curled leaves from 62^{nd} to 83^{rd} day. The least number of curled leaves (3.31) was recorded from 83^{rd} day as compared to number of curled leaves on 62^{nd} day as shown in Table 6b.

Table 6a: Anti-aphid effects of different concentration of pyrethrum flower, leaf and root extracts on curled leaves of *Solanum scabrum* from 60th to 83rd day

Parameter	Plant Part	Treatment Extract concentrations	Days				
			Day 60	Day 62	Day 69	Day 76	Day 83
Curled Leaves	Flower	0%	19.00a	27.67a	10.00a	10.00a	5.33a
		33%	8.00a	21.00a	9.33a	2.67a	5.00a
		67%	10.33a	16.33a	6.33a	2.33a	4.00a
		100%	8.00a	6.00a	4.33a	2.33a	2.67a
		Duduthrin	9.67a	12.33a	5.33a	4.33a	3.33a
		LSD	20.08	24.77	6.92	8.66	5.02
	Root	0%	12.67a	27.33a	10.00a	5.00a	7.33a
		33%	10.67a	23.33a	4.67a	3.67ab	3.00b
		67%	10.66a	14.00a	4.67a	2.33ab	1.33b
		100%	8.00a	8.00a	4.33a	1.67b	1.33b
		Duduthrin	8.67a	11.00a	8.33a	2.33ab	3.00b
		LSD	16.42	20.09	9.46	2.74	2.70
	Leaf	0%	10.00a	13.00a	10.67a	6.67a	3.00a
		33%	7.33a	11.67a	5.33ab	4.67a	2.67a
		67%	3.33a	9.67a	3.00b	2.67a	6.67a
		100%	2.67a	8.67a	2.33b	1.67a	2.33a
		Duduthrin	1.67a	6.00a	2.00b	6.27a	2.00a
		LSD	10.49	9.70	7.41	5.08	2.70

Means with the same letter down the column are not significantly different at $p \leq 0.05$.

Table 6b: Overall anti-aphid effects of different concentration (33%, 67% and 100%) of pyrethrum flower, leaf and root extracts on curled leaves of *Solanum scabrum* from 60th to 83rd day

Variables	Curled leaves
Pyrethrum plant parts	
Flower Extracts	8.65a
Leaf Extracts	5.28b
Root Extracts	7.92a
LSD	1.71
Extract concentration	
100%	4.25c
67%	6.24bc
33%	8.24b
0%	11.89a
Duduthrin	5.75c
LSD	2.21
Day	
60	8.76b
62	14.40a
69	6.04c
76	3.91cd
83	3.31d
LSD	2.21

Means with the same letter down the column are not significantly different at $p \leq 0.05$.

4.3.3 Leaf Length and width

Leaf length and width responses of *Solanum scabrum* as a result of aphid control by use of 0%, 33%, 67% and 100% of pyrethrum flower, root and leaf extracts and duduthrin are presented in Table 7a and 7b. Aphid control using 33%, 67%, 100% pyrethrum flower extracts did not show significant effect ($p \geq 0.05$) on leaf length of *Solanum scabrum* compared to control on 60th day. The control of aphids by application of 33%, 67%, 100% pyrethrum flower extracts caused significant differences in leaf length of *Solanum scabrum* compared to control on 62nd, 69th, 76th and 83rd day with 100% recording highest leaf length. The effect of controlling aphids using 0%,

33%, 67% and 100% pyrethrum root extracts showed no significant effect on leaf length on 60th, 62nd and 76th day. Plants treated with 100% pyrethrum root extracts resulted into a significantly higher leaf length on 76th and 83rd day (9.57cm and 10.17cm respectively). On these days' (76th and 83rd days) plants treated with 100% recorded a higher leaf length as compared to those treated with duduthrin. Control of aphids by application of 33%, 67%, 100% pyrethrum leaf extracts did not show significant effects on leaf length of *Solanum scabrum* compared to control in all days except day 62nd (Table 7a).

The effect of controlling aphids using 33%, 67% and 100% pyrethrum flower extracts showed no significant effect ($p \geq 0.05$) on leaf width of *Solanum scabrum* on 60th and 62nd days compared to control. On 69th, 76th and 83rd days, significant differences on leaf width was noted with 100% recording the highest leaf width of 6.60cm, 7.17cm and 7.77cm respectively compared to control. Additionally, plants treated with duduthrin on 69, 76th and 83rd days did not differ statistically with 67% and 33% pyrethrum flower extracts but a significant difference on leaf width from plants treated with 100% was noted (Table 7a). The use of 33%, 67%, 100% pyrethrum root extracts to control aphids did not show significant effects on leaf width of *Solanum scabrum* compared to control on 60th, 62nd and 76th days. On 69th and 83rd days, significant differences ($p \leq 0.05$) on leaf width was noted from plants treated with 100% and 67%. Leaf widths of *Solanum scabrum* treated with duduthrin to (control aphids) on 69th day did not differ statistically ($p \geq 0.05$) with 100%. On 83rd day, leaf widths from plants treated with duduthrin did not differ statistically with leaf widths of plants treated with 67% and 33% pyrethrum root extracts but a significantly higher width (7.33cm) was obtained from plants treated with 100%. The use of 33%, 67%, 100% pyrethrum leaf extracts to control aphids did not show significant effects on leaf width of *Solanum scabrum* compared to control on 60th, 69th, 76th and 83rd days. Leaf width of *Solanum scabrum* were significantly different only on day 62nd where plants treated with 100% recorded higher leaf width (4.33cm) and was significantly different from plants treated with 67%, 33%, 0% and duduthrin. Leaf widths obtained from plants treated with 33% and 67% pyrethrum leaf extracts did not differ significantly compared to duduthrin (Table 7a).

Overally, Statistical analysis showed significant difference ($p \leq 0.05$) in leaf length and width in plants treated with pyrethrum flowers, roots and leaves extracts to control aphids (Table 7b). A significantly higher leaf length and width was obtained from plants treated with pyrethrum flower

extracts (6.18cm and 5.02cm respectively). The control of aphids using 100% pyrethrum extracts resulted into a significantly higher leaf length and width of *Solanum scabrum* (Table 7b) as compared to other treatments. The size of leaf increased significantly from 60th day to 83rd day (Table 7b)

Table 7a: Anti-aphid effect of different concentration (33%, 67% and 100%) of pyrethrum flower, leaf and root extracts on leaf length and width of *Solanum scabrum* from 60th to 83rd day

Parameter	Plant Part	Treatment Extract concentrations	Days				
			Day 60	Day 62	Day 69	Day 76	Day 83
Leaf length (cm)	Flower	0%	4.33a	4.53b	4.83b	4.87b	5.00b
		33%	5.33a	6.00ab	5.67b	7.00ab	8.17ab
		67%	5.73a	6.00ab	6.67ab	8.67a	8.83ab
		100%	6.50a	7.67a	8.43a	9.33a	10.33a
		Duduthrin	6.17a	6.33ab	6.50ab	7.83a	9.47a
		LSD	2.75	2.57	2.64	2.63	3.85
	Root	0%	3.00a	3.37a	4.33a	4.70b	5.97b
		33%	4.30a	4.67a	5.33a	6.40ab	6.80b
		67%	5.00a	5.80a	6.77a	7.00ab	8.30ab
		100%	5.50a	6.00a	7.33a	9.57a	10.17a
		Duduthrin	5.67a	6.00a	6.33a	6.73ab	7.23b
		LSD	2.84	2.93	3.08	3.83	2.87
	Leaf	0%	3.00a	3.57b	4.83a	5.67a	5.67a
		33%	4.00a	5.33ab	5.70a	6.00a	6.67a
		67%	4.33a	5.83ab	6.17a	6.27a	7.17a
		100%	4.50a	6.00a	6.50a	6.17a	7.33a
		Duduthrin	4.50a	5.33ab	7.17a	7.37a	8.33a
		LSD	1.92	2.42	2.89	2.37	2.78
	Flower	0%	3.50a	2.63a	2.63b	3.50b	3.60b
		33%	4.07a	4.00a	5.70ab	5.07ab	6.07ab

Leaf width (cm)		67%	4.17a	4.50a	5.50ab	6.07ab	6.33ab
		100%	5.67a	5.00a	6.60a	7.17a	7.77a
		Duduthrin	4.67a	4.60a	5.17ab	5.50ab	5.93ab
		LSD	2.66	2.79	3.08	2.76	2.96
	Root	0%	2.17a	2.10a	3.23b	3.00a	3.57b
		33%	2.67a	2.87a	3.67ab	4.27a	4.60ab
		67%	3.60a	3.83a	4.47ab	4.93a	5.27ab
		100%	3.93a	3.50a	5.53a	5.67a	7.33a
		Duduthrin	3.90a	3.57a	5.33a	5.33a	4.60ab
		LSD	2.29	2.74	1.98	3.80	3.09
	Leaf	0%	2.00a	1.90b	3.83a	4.10a	3.53a
		33%	2.10a	3.73ab	3.93a	4.17a	4.83a
		67%	2.63a	3.03ab	3.53a	4.37a	5.50a
		100%	3.33a	4.33a	5.00a	4.53a	5.33a
		Duduthrin	3.00a	3.80ab	4.83a	5.50a	5.77a
		LSD	1.34	1.93	2.59	2.23	2.48

Means with the same letter down the column are not significantly different at $p \leq 0.05$.

Table 7b: Overall anti-aphid effects of different concentration (33%, 67% and 100%) of pyrethrum flower, leaf and root extracts on leaf length and width of *Solanum scabrum* from 60th to 83rd day

Variables	Leaf length(cm)	Leaf width(cm)
Pyrethrum plant parts		
Flower Extracts	6.81a	5.02a
Leaf Extracts	5.74c	3.95b
Root Extracts	6.08b	4.11b
LSD	0.27	0.34
Extract concentration		
100%	7.42a	5.38a
67%	6.57b	4.52bc

33%	5.82c	4.12c
0%	4.49d	3.00d
Duduthrin	6.73b	4.77b
LSD	0.35	0.44
Day		
60	4.79e	3.43c
62	5.52d	3.56c
69	6.17c	4.60b
76	6.90b	4.88ab
83	7.65a	5.32a
LSD	0.35	0.44

Means with the same letter down the column are not significantly different at $p \leq 0.05$.

4.3.4 Leaf Area

The control of aphids by application of the various concentrations (33%, 67%, 100%) of pyrethrum flower extracts had no significant effects ($p \geq 0.05$) on leaf area of *Solanum scabrum* as compared to control (0%) on 60th day (Table 8a and 8b). The effect of controlling aphids using 100% pyrethrum flower extracts resulted into a higher leaf area on 62nd day (38.93cm^2), 69th day (57.58 cm^2), 76th day (68.16 cm^2) and 83rd day (80.58 cm^2). Leaf area from *Solanum scabrum* treated with 67% and 33% pyrethrum flower extracts did not show any significant difference from each other on 62nd,69th,76th and 83rd days. On 62nd, 69th, 76th and 83rd days, the least leaf area (12.03cm^2, 13.16 cm^2, 17.93 cm^2 and 18.82 cm^2 respectively) (Table 8a) was obtained from plants under control (0%). Leaf area increased with increase in treatment concentration. Leaf area obtained from *Solanum scabrum* treated with duduthrin (standard check) was not significantly different from leaf area obtained from plants treated with 33% and 67% pyrethrum flower extracts on 62nd, 69th, 76th and 83rd days. Aphid control using 33%, 67%, 100% pyrethrum root extracts did not show significant effect ($p \geq 0.05$) on leaf area of *Solanum scabrum* compared to control on 60th, 62nd and 76th days. The control of aphids by application of the various concentrations of pyrethrum root extracts had significant effect ($p \leq 0.05$) on leaf area of *Solanum scabrum* on 69th and 83rd days. The effect of controlling aphids using 100% pyrethrum root extracts resulted into a higher leaf area on 69th day (38.90 cm^2) and 83rd day (80.58 cm^2) as compared to control where significantly least leaf area was obtained. Plants treated with 67% and 33% pyrethrum root extracts recorded leaf area of 31.00 cm^2 and 21.06cm^2 respectively. Leaf area increased with increase in treatment concentration. Leaf area obtained from plants treated with duduthrin (standard check) was not significantly different from *Solanum scabrum* treated with 0% and 33% pyrethrum root extracts

on 83rd day. The effect of controlling aphids using 33%, 67% and 100% pyrethrum leaf extracts showed no significant effect ($p \geq 0.05$) on leaf area on 60th, 69th 76th and 83rd days as compared to control (0%). The control of aphids by application of the various concentrations of pyrethrum leaf extracts had significant effect ($p \leq 0.05$) on leaf area of *Solanum scabrum* on Day 62 (Table 8a). The use of 100% pyrethrum leaf extracts to control aphids resulted into a higher leaf area of 28.03cm^2 compared to other treatments. *Solanum scabrum* treated with 33% and 67% pyrethrum leaf extracts did not significantly differ in leaf area compared to leaf area from plants treated with duduthrin. The least leaf area was recorded from *Solanum scabrum* under control (0%).

Generally, from Table 8b, statistical analysis showed significant difference ($p \leq 0.05$) in leaf area in plants treated with pyrethrum extracts to control aphids with plants sprayed with pyrethrum flower extracts recording a significantly higher leaf area (37.55cm^2). The control of aphids using 100% extracts resulted into a significantly higher leaf area (43.72cm^2) of the plant. Leaf area on 60th and 62nd days were not significantly different ($p \geq 0.05$) having an area of 18.36 cm^2 and 21.46cm^2 respectively (Table 8b). The following days, leaf area increased significantly ($p \geq 0.05$) with 83rd day recording the highest leaf area of 44.31cm^2.

Table 8a: Anti-aphid effect of different concentration (33%, 67% and 100%) of pyrethrum flower, leaf and root extracts on leaf area of *Solanum scabrum* from 60th to 83rd day

Parameter	Plant Part	Treatment Extract concentrations	Days				
			Day 60	Day 62	Day 69	Day 76	Day 83
Leaf Area (cm^2)	Flower	0%	15.74a	12.03b	13.16b	17.93b	18.82b
		33%	24.01a	26.01ab	32.83ab	37.31ab	50.82ab
		67%	26.72a	30.33ab	38.75ab	54.98ab	58.24ab
		100%	37.29a	38.93a	57.58a	68.16a	80.58a
		Duduthrin	29.56a	29.81ab	22.76ab	43.46ab	61.86ab
		LSD	25.57	26.56	33.49	37.17	47.05
	Root	0%	6.97a	7.98a	14.06c	15.33a	21.30b
		33%	11.75a	13.99a	20.26bc	27.57a	31.71b
		67%	18.83a	23.67a	31.13ab	35.90a	45.46ab

		100%	23.01a	19.71a	38.90a	60.01a	80.58a
		Duduthrin	25.24a	25.26a	32.81ab	41.74	34.03b
		LSD	23.59	25.22	16.17	49.26	44.95
	Leaf	0%	6.67a	7.26b	19.59a	23.57a	20.12a
		33%	8.44a	20.75ab	23.18a	25.23a	33.49a
		67%	11.19a	17.48ab	21.33a	29.05a	40.30a
		100%	15.70a	28.03a	35.08a	29.70a	42.56a
		Duduthrin	14.29a	20.62ab	36.65a	41.40a	48.76a
		LSD	10.05	19.39	28.68	25.68	33.11

Means with the same letter down the column are not significantly different at $p \leq 0.05$.

Table 8b: Overall anti-aphid effects of different concentration (33%, 67% and 100%) of pyrethrum flower, leaf and root extracts on leaf area of *Solanum scabrum* from 60th to 83rd day

Variables	Leaf area(cm^2)
Pyrethrum plant parts	
Flower Extracts	37.55a
Leaf Extracts	24.82b
Root Extracts	28.13b
LSD	3.73
Extract concentration	
100%	43.72a
67%	32.22b
33%	25.82c
0%	14.44d
Duduthrin	34.62b
LSD	4.81
Day	
60	18.36d
62	21.46d
69	29.94c

76	36.76b
83	44.31a
LSD	4.81

Means with the same letter down the column are not significantly different at $p \leq 0.05$.

4.3.5 Number of Fruits

The control of aphids using varying concentrations (33%, 67% and100%) of pyrethrum flower, root and leaf extracts had significant effect ($p \leq 0.05$) on number of fruits of *Solanum scabrum* as shown in Table 9a and 9b.

The control of aphids by application of 33%, 67% and 100% pyrethrum flower extracts had significant effects ($p \leq 0.05$) on number of fruits of *Solanum scabrum* as compare to control on 62nd and 69th day (Table 9a). Results in Table 9a, showed that plants treated with 100% pyrethrum flower extracts recorded a significantly higher fruit number (46.33, 68.33, 89.00 and 109.33 fruits) for 62nd, 69th, 76th and 83rd day respectively while a comparatively least number of fruits was recorded from plants treated with control (0%) across all days. The effect of controlling aphids using duduthrin showed no significant difference in number of fruits of *Solanum scabrum* from plants treated with control (Day 76) and all treatments except plants treated with 100% pyrethrum flower extracts (Day 83). The control of aphids by application of 100% pyrethrum root extracts had significant effects ($p \leq 0.05$) on number of fruits of *Solanum scabrum* compared to control (0%) on 62nd Day (Table 9a). Results presented in Table 9a showed a comparatively higher number of fruits on Day 62nd (36.67 fruits) and 83rd day (113.33 fruits) in plants treated with 100% root extracts. On Day 69th and Day 76th, there is no significant difference in number of fruits treated with different concentrations as compared to control. From Table 9a, a significantly higher number of fruits was recorded from plants treated with 100% leaf extracts in all days except Day 76th where plants treated with duduthrin recorded a higher number of fruits (75.67 fruits). A significantly lower number of fruits was obtained from plants treated with 33% pyrethrum leaf extracts but the lowest was recorded from control (0%).

Overall results shown in Table 9b indicated that the use of both pyrethrum flower extracts and pyrethrum root extracts to control aphids did not show any significantly effect in number of fruits. Additionally, the control of aphids using 33%, 67% and 100% pyrethrum extracts had significant effect on number of fruits of *Solanum scabrum* as compared to control (0%). Generally, a

significantly higher ($p \geq 0.05$) number of fruits (68.61) was recorded from plants treated with 100% pyrethrum extracts and the least number of fruits (25.19) was recorded from plants treated with 0% pyrethrum extracts (Table 9b). The effect of controlling aphids using pyrethrum (flower, root and leaves) extracts significantly lead to increased number of fruits from 62nd to 83rd day (Table 9b).

Table 9a: Anti-aphid effect of different concentration (33%, 67% and 100%) of pyrethrum flower, leaf and root extracts on number of fruits of *Solanum scabrum* from 60th to 83rd day

Parameter	Plant Part	Treatment Extract concentrations	Days			
			Day 62	Day 69	Day 76	Day 83
Number of Fruits	Flower	0%	13.33c	20.00c	39.33b	52.00b
		33%	20.00bc	22.33c	61.33ab	59.00b
		67%	27.33bc	30.67bc	65.67ab	89.33ab
		100%	46.33a	68.33a	89.00a	109.33a
		Duduthrin	37.00ab	45.33b	49.67b	88.33a
		LSD	18.94	21.44	30.80	42.41
	Root	0%	15.67b	24.00a	39.00a	58.00b
		33%	18.33b	36.67a	56.00a	61.67b
		67%	19.00b	49.00a	64.67a	71.00b
		100%	36.67a	52.33a	66.67a	113.33a
		Duduthrin	29.33ab	48.33a	63.33a	76.67b
		LSD	15.49	29.86	35.83	28.64
	Leaf	0%	4.67c	9.67d	25.33c	9.33c
		33%	17.33bc	25.00cd	45.67bc	34.67b
		67%	20.00bc	28.67bc	38.33c	43.67b
		100%	22.33b	70.67a	66.67ab	81.67a
		Duduthrin	42.67a	44.00b	75.67a	68.00a
		LSD	15.85	18.28	26.73	15.64

Means with the same letter down the column are not significantly different at $p \leq 0.05$.

Table 9b: Overall anti-aphid effects of different concentration (33%, 67% and 100%) of pyrethrum flower, leaf and root extracts on number of fruits of *Solanum scabrum* from 60th to 83rd day

Variables	No. of fruits
Pyrethrum plant parts	
Flower Extracts	51.86a
Leaf Extracts	38.70b
Root Extracts	49.58a
LSD	3.69
Extract concentration	
100%	68.61a
67%	45.61c
33%	38.17d
0%	25.19e
Duduthrin	55.69b
LSD	4.77
Day	
62	24.67d
69	38.33c
76	56.42b
83	67.20a
LSD	4.27

Means with the same letter down the column are not significantly different at $p \leq 0.05$.

4.3.6 Number of Damaged Fruits

The control of aphids using 67% and 100% of pyrethrum flower extracts showed significant effect ($p \leq 0.05$) on number of damaged fruits of *Solanum scabrum* as compared to control (0%) on Day 62nd. The control of aphids by application of the various concentrations of pyrethrum flower extracts had no significant effect ($p \geq 0.05$) on number of damaged fruits of *Solanum scabrum* as compared to number of damaged fruits from plants treated with control and duduthrin on Day 69th and Day 83rd (Table 10a). A significantly least number of damaged fruits from plants treated with 100% pyrethrum flower extracts on Days 62nd (4.00 fruits) as compared to number of damaged

fruits obtained from other treatments. The control of aphids by application of 33%, 67% and 100% pyrethrum root extracts had significant effects ($p \leq 0.05$) on number of damaged fruits of *Solanum scabrum* in all days except for 69th day (Table 10a). Results in Table 10a showed a comparatively least number of damaged fruits (0.67 and 0.33) from 76th and 83rd days respectively. From Table 10a, there was no significant difference in number of damaged fruits in plants under 33%, 67% and 100% pyrethrum leaf extracts as compared to control (0%) from Day 62 to Day 83.

Generally, plants treated with pyrethrum flower extracts showed a significant effect ($p \leq 0.05$) on number of fruits as compared to both pyrethrum root and pyrethrum leaf extracts. In addition, there was no significant difference ($p \geq 0.05$) in number of damaged fruits between plants treated with pyrethrum root and leaf extracts (Table 10b). The control of aphids using 33%, 67% and 100% pyrethrum extracts had significant effect on damaged fruits of *Solanum scabrum* as compared to control (0%). The least number of damaged fruits (1.92) was recorded from plants which were sprayed with 100% followed by 67% with 3.47 damaged fruits. A slightly higher number of damaged fruits (4.72) were recorded from plants treated with 33% pyrethrum extracts (Table 10b). Highest number of damaged fruits (6.75) was recorded from plants under control (0%). There was significant difference ($p \leq 0.05$) in the number of damaged fruits in day 62nd to day 83rd. A significantly higher ($p \leq 0.05$) number of damaged fruits (7.29 fruits) was recorded on day 62nd different and the number reduced and 83rd day recorded the least damaged fruits (3.16) as observed in Table 10b.

Table 10a: Anti-aphid effect of different concentration (33%, 67% and 100%) of pyrethrum flower, leaf and root extracts on damaged fruit of *Solanum scabrum* from 60th to 83rd day

Parameter	Plant Part	Treatment Extract concentrations	**Days**			
			Day 62	Day 69	Day 76	Day 83
Number of Damaged Fruits	Flower	0%	16.33a	7.67a	10.00a	6.67b
		33%	13.33a	3.67b	7.33a	4.33a
		67%	10.67ab	3.67b	2.67a	3.33a
		100%	4.00b	1.67b	1.33a	2.33a
		Duduthrin	8.00ab	3.67b	2.67a	4.33a

		LSD	9.20	3.52	9.18	8.04
	Root	0%	14.00a	2.00a	4.00a	4.33a
		33%	6.67ab	1.33a	2.33abc	1.67bc
		67%	4.67b	1.67a	1.00bc	0.67bc
		100%	2.33b	0.33a	0.67c	0.33c
		Duduthrin	4.33b	1.67a	3.67ab	2.33b
		LSD	7.60	2.20	2.70	1.76
	Leaf	0%	6.00a	2.00a	4.00a	4.67a
		33%	6.00a	1.67a	3.67a	4.67a
		67%	5.67a	1.67a	3.00a	3.00a
		100%	5.00a	0.67a	2.00a	2.33a
		Duduthrin	2.33a	1.00a	1.33a	3.00a
		LSD	7.87	2.44	4.28	3.25

Means with the same letter down the column are not significantly different at $p \leq 0.05$.

Table 10b: Overall anti-aphid effects of different concentration (33%, 67% and 100%) of pyrethrum flower, leaf and root extracts on number of damaged fruits of *Solanum scabrum* from 60th to 83rd day

Variables	No. of damaged fruits
Pyrethrum plant parts	
Flower Extracts	5.88a
Leaf Extracts	3.18b
Root Extracts	2.97b
LSD	1.11
Extract concentration	
100%	1.92d
67%	3.47bc
33%	4.72b
0%	6.75a

Duduthrin	3.19cd
LSD	1.45
Day	
62	7.29a
69	2.29b
76	3.31b
83	3.16b
LSD	1.28

Means with the same letter down the column are not significantly different at $p \leq 0.05$.

4.3.7 Shoot and root fresh Weights

Application of pyrethrum flower extracts had significant effect ($p \leq 0.05$) on shoot and root fresh weights of *Solanum scabrum* as compared to control (Table 11a). Plants treated with 100% pyrethrum flower extracts recorded a significantly higher shoot fresh weight (54.47g) and root fresh weight (9.43g) compared to 33% and 67% pyrethrum flower extracts. Shoot and root fresh weights of plants treated with duduthrin did not differ significantly with weights obtained from plants treated with 100% flower extracts. From Table 11a, the control of aphids by application of 67% and 100% pyrethrum root extracts had significant effects ($p \leq 0.05$) on shoot and root fresh weights of *Solanum scabrum* compared to control. Plants treated with 100% pyrethrum root extracts recorded the highest shoot fresh weight (70.00g) and root fresh weight (14.47g) compared to fresh weights obtained from plants treated with duduthrin and 33% pyrethrum root extracts. Least shoot and root fresh weight were obtained from control (0%). Results in Table 11a indicate that the control of aphids by application of 100% pyrethrum leaf extracts had significant effect on shoot and root fresh weights of *Solanum scabrum* compared to the fresh weights seen in plants treated with 33% and 67%. Shoot fresh weight from plants under duduthrin did not differ from the weights observed from plants under 100%. Table 11b, indicate an overall effects of controlling aphids using different concentrations (33%, 67% and 100%) of pyrethrum flower, root and leaf extracts on the shoot and root fresh weights of *Solanum scabrum*. A significantly higher ($p \leq 0.05$) shoot fresh weight (36.13g) and root fresh weight (8.88g) was recorded from plants treated with pyrethrum root extracts. Statistical analysis showed significant difference ($p \leq 0.05$) in shoot and root fresh weight from plants treated with 100% as compared to control. Generally, a significantly

higher ($p \leq 0.05$) shoot fresh weight of 54.73g and root fresh weight of 10.68g was recorded from plants sprayed with 100% treatment.

Table 11a: Anti-aphid effect of different concentration (33%, 67% and 100%) of pyrethrum flower, leaf and root extracts on shoot and root fresh weights of *Solanum scabrum*

Plant Part	Treatment Extract Concentration	Shoot fresh weight (g)	Root fresh weight (g)
Flower	0%	11.70b	2.10b
	33%	6.10b	2.25b
	67%	21.63ab	4.95ab
	100%	54.47a	9.43a
	Duduthrin	49.17a	7.37a
	LSD	33.16	4.65
Root	0%	13.67c	2.77b
	33%	19.53c	3.70b
	67%	31.30bc	10.90a
	100%	70.00a	14.47a
	Duduthrin	48.10ab	12.90a
	LSD	22.24	5.88
Leaf	0%	5.13b	1.60c
	33%	6.20b	3.80bc
	67%	11.53b	4.70bc
	100%	39.73a	8.13ab
	Duduthrin	48.53a	10.30a
	LSD	18.07	4.98

Means with the same letter down the column are not significantly different at $p \leq 0.05$.

Table 11b: Overall anti-aphid effect of different concentration (33%, 67% and 100%) of pyrethrum flower, leaf and root extracts on shoot and root fresh weight of *Solanum scabrum*

Plant parts Extracts	Treatment	Shoot Fresh weight (g)	Root fresh weight (g)
Pyrethrum plant parts			
Flower Extracts		28.61ab	5.22b
Leaf Extracts		22.23b	5.71b
Root Extracts		36.13a	8.88a
LSD		10.38	2.13
Extract concentration			

100%	54.73a	10.68a
67%	21.49b	6.85b
33%	10.61b	3.25c
0%	9.52b	2.04c
Duduthrin	48.60ab	10.19a
LSD	13.41	2.76

Means with the same letter down the column are not significantly different at $p \leq 0.05$.

4.3.8 Shoot and Root Dry Weights

The control of aphids by application of 33%, 67% and 100% pyrethrum flower extracts had significant effect ($p \leq 0.05$) on shoot and root dry weights of *Solanum scabrum* (Table 12a). A significantly higher shoot and root dry weight is observed on plants treated with 100% pyrethrum flower extracts with 14.30g and 3.00g respectively as shown in Table 12a. The control of aphids by application of 33%, 67% and 100% pyrethrum root extracts had significant effects ($p \leq 0.05$) on shoot and root dry weights of *Solanum scabrum*. Plants treated with 100% and 67% pyrethrum root extracts showed no significant difference in root dry weights. Least dry weights were obtained from control (0%). The use of 100% pyrethrum leaf extracts to control aphids resulted into a significantly higher shoot and root dry weights (12.53g and 3.40g respectively) (Table 12a). Plants treated with 100% leaf extracts showed no significant difference in plant shoot and root dry weights compared to Duduthrin. The results presented in Table 12b showed the overall effect of controlling aphids using pyrethrum (flower, leaf and root) extracts on shoot and root dry weights. A significant effect ($p \leq 0.05$) on shoot and root dry weights was seen from plants treated with pyrethrum flower, root and leaf extracts (Table 12b). One hundred percent pyrethrum extracts applied the greatest effect with significantly higher ($p \leq 0.05$) shoot and root dry weight compared to control. Shoot and root dry weights from plants treated with Duduthrin showed no significant difference from dry weights obtained from plants treated with 100% pyrethrum extracts (Table 12b).

Table 12a: Anti-aphid effect of different concentration (33%, 67% and 100%) of pyrethrum flower, leaf and root extracts on shoot and root dry weights of *Solanum scabrum*

Plant Part	Treatment Extract Concentration	Shoot dry weight (g)	Root dry weight (g)
Flower	0%	4.85ab	0.77c
	33%	2.23b	1.30bc
	67%	7.57ab	2.33ab
	100%	14.30a	3.00a
	Duduthrin	14.37a	3.23a
	LSD	9.85	1.50
Root	0%	2.90c	1.77b
	33%	6.70bc	2.47ab
	67%	17.40ab	3.63a
	100%	21.07a	3.47a
	Duduthrin	14.03ab	4.20a
	LSD	11.01	1.79
Leaf	0%	3.30b	0.90c
	33%	3.33b	1.47bc
	67%	4.00b	2.77ab
	100%	12.53a	3.40a
	Duduthrin	14.06a	3.47a
	LSD	8.22	1.85

Means with the same letter down the column are not significantly different at $p \leq 0.05$.

Table 12b: Overall anti-aphid effect of different concentration (33%, 67% and 100%) of pyrethrum flower, leaf and root extracts on shoot and root dry weight of *Solanum scabrum*

Plant parts Extracts Treatment	Shoot dry weight (g)	Root dry weight (g)
Pyrethrum plant parts		
Flower Extracts	8.66ab	2.13b
Leaf Extracts	7.45b	2.40ab
Root Extracts	12.33a	3.04a
LSD	4.00	0.74
Extract concentration		
100%	15.97a	3.29a
67%	9.66b	2.91a
33%	4.09c	1.75b
0%	3.54c	1.03b

Duduthrin	14.15ab	3.63a
LSD	5.17	0.94

Means with the same letter down the column are not significantly different at $p \leq 0.05$.

4.3.9 Yield /ha

Yield was obtained from the cured leaves. The control of aphids by application of the various concentrations of pyrethrum flower extracts had significant effect ($p \leq 0.05$) on yields of *Solanum scabrum* (Table 13a). A significantly higher yield (778.00g) was recorded from plants treated with 100% pyrethrum flower extracts compared to yields obtained from plants under control (0%). There was no significant difference on yields from plants treated duduthrin and control (0%) (Table 13a). The control of aphids by application of 100% pyrethrum root extracts showed no significant effect on yield of *Solanum scabrum* as compared to 33% and 67% (Table 13a). From Table 13a, a higher yield was (450.00 kg/ha) obtained from plants treated with Duduthrin as compared to yield obtained from plants under control (54.00kg/ha). Table 13b shows the overall effect of controlling aphids using different parts of pyrethrum on yields of *Solanum scabrum*. Plants sprayed with the pyrethrum flower and root extracts showed no significant difference ($p \geq 0.05$) on plant yields. The highest leaf yield of 591.13 kg/ha (Table 13b) was harvested from plants sprayed with 100% pyrethrum extract followed by 67% (531.17kg/ha) and finally 33% (440.22kg/ha). Plants sprayed with synthetic insecticide Duduthrin recorded a significantly ($p \leq 0.05$) lower leaf yield as compared to the yield from plants under different concentrations of pyrethrum (33%, 67% and 100%). Whereas the lowest leaf yield of 122 kg/ha was harvested from plants sprayed with control (0%).

Table 13a: Anti-aphid effect of different concentration (33%, 67% and 100%) of pyrethrum flower, leaf and root extracts on yield/ha of *Solanum scabrum*

Plant Part	Treatment Extract Concentration	Yield/ha (Kg)
Flower	0%	222.00b
	33%	560.10a
	67%	669.90a
	100%	778.00a
	Duduthrin	309.90b
	LSD	247.26
Root	0%	80.70b

	33%	579.90a
	67%	673.40a
	100%	673.40a
	Duduthrin	480.00a
	LSD	266.39
Leaf	0%	54.00c
	33%	180.67bc
	67%	250.20b
	100%	322.00b
	Duduthrin	450.00a
	LSD	153.16

Means with the same letter down the column are not significantly different at $p \leq 0.05$.

Table 13b: Overall anti-aphid effect of different concentration (33%, 67% and 100%) of pyrethrum flower, leaf and root extracts on yield/ha of *Solanum scabrum*

Plant parts Extracts	Treatment	Yield/ha (kg/ha)
Pyrethrum plant parts		
Flower Extracts		507.98a
Leaf Extracts		251.37b
Root Extracts		499.34a
LSD		93.30
Extract concentration		
100%		591.13a
67%		531.17ab
33%		440.22b
0%		122.00c
Duduthrin		413.30b
LSD		120.45

Means with the same letter down the column are not significantly different at $p \leq 0.05$.

4.4 Determination of Anti-aphid effect of pyrethrum extracts on chlorophyll and phytonutrient contents of *Solanum scabrum*

4.4.1 Chlorophyll Content

The control of aphids by application of 33%, 67% and 100% pyrethrum flower extracts showed no significant effect ($p \geq 0.05$) on leaf chlorophyll content of *Solanum scabrum* as compared to

control on 54th day (Table 14a). On 62nd day, a significantly higher chlorophyll content (72.60mg) was recorded from plants treated with 100% pyrethrum flower extracts. Chlorophyll content from plants treated with 33%, 67% and duduthrin did not vary significantly. There was no significant variation in chlorophyll content of *Solanum scabrum* on 54th and 62nd days on plants treated with various concentrations of pyrethrum root extracts and duduthrin as compared to control (Table 14a). The different concentrations (33%, 67% and 100%) of pyrethrum leaf extracts showed no significant effect ($p \geq 0.05$) on leaf chlorophyll content of *Solanum scabrum* as compared to control on 54th day (Table 14a). Significant difference in chlorophyll content was observed on 62nd day among treatments.

The results presented in table 14b indicate the overall effect of controlling aphids using pyrethrum (flower, root and leaf) extracts on leaf chlorophyll content of *Solanum scabrum.* Plants sprayed with pyrethrum leaf extracts recorded a significantly higher chlorophyll content of 59.66mg which was followed by chlorophyll (57.81mg) recorded from plants treated with pyrethrum root extracts. The highest chlorophyll content (57.65mg) was recorded from plants treated with 100% and the least chlorophyll content was recorded from control (42.17mg).

Table 14a: Anti-aphid effect of different concentration (33%, 67% and 100%) of pyrethrum flower, leaf and root extracts on chlorophyll content (mg) of *Solanum scabrum* on 54th and 62nd day

Plant parts	Treatment Extract Concentrations	Days	
		Day 54	Day 62
Flower	0%	45.69a	41.68b
	33%	49.54a	64.03ab
	67%	55.39a	67.12ab
	100%	52.32a	72.60a
	Duduthrin	54.96a	69.30ab
	LSD	23.87	28.30
Root	0%	26.03a	41.53a
	33%	29.47a	23.80a

	67%	44.18a	40.35a
	100%	50.08a	50.12a
	Duduthrin	45.01a	46.32a
	LSD	48.61	62.68
Leaf	0%	55.67a	61.17c
	33%	47.92a	64.73bc
	67%	52.93a	68.05ab
	100%	48.34a	72.41a
	Duduthrin	52.68a	72.72a
	LSD	9.73	6.27

Means with the same letter down the column are not significantly different at p≤0.05

Table 14b: Overall anti-aphid effect of different concentration (33%, 67% and 100%) of pyrethrum flower, leaf and root extracts on chlorophyll content (mgg-1) of *Solanum scabrum*

Plant parts extracts	Treatment	Leaf chlorophyll content (mgg-1)
Pyrethrum plant parts		
Flower Extracts		57.26b
Leaf Extracts		59.66a
Root Extracts		57.81c
LSD		2.22
Extract concentration		
100%		57.65a
67%		54.69b
33%		46.58c
0%		42.17d
Duduthrin		56.83ab
LSD		2.87
Time/ Day		
Day 54		47.35b
Day 62		55.81a
LSD		1.82

Means with the same letter down the column are not significantly different at p≤0.05.

4.4.2: Phytonutrient content

Results in Table 15 showed that the use of 33%, 67% and100% pyrethrum flower, root and leaf extracts did not show any significant difference on the amount of sodium in *Solanum scabrum* as compared to control. Additionally, plants treated with pyrethrum extracts did not vary significantly in amount of sodium as compared to the amount of sodium in plants treated with duduthrin. Results in Table 15 showed that the use of 33%, 67% and100% pyrethrum flower, root and leaf extracts to control aphids did not show any significant effect on the amount of calcium in *Solanum scabrum* as compared to control (Table 15). Additionally, the control of aphids using varying concentrations (33%, 67% and100%) of pyrethrum (flower, root and leaf) extracts had significant effect ($p \leq 0.05$) on the amount of potassium of *Solanum scabrum* as compared to control. A higher amount of potassium (100.46ppm and (90.28ppm) was obtained from plants treated with 100% pyrethrum flower and root extracts respectively (Table 15). A significantly higher amount of potassium was recorded from plants treated with duduthrin followed by the amount of potassium obtained from 100% pyrethrum leaf extracts. Least amount of potassium was recorded from plants under control (0%).

Table 15: Anti-aphid effect of different concentration (33%, 67% and 100%) of pyrethrum flower, leaf and root extracts on phytonutrient content (Na, Ca and K) of *Solanum scabrum*

Pyrethrum Plant Part	Treatment Extract concentration	Sodium (ppm)	Calcium (ppm)	Potassium (ppm)
Flower	0%	0.03a	0.00a	43.85d
	33%	0.03a	0.08a	61.36c
	67%	0.07a	0.02a	67.67bc
	100%	0.03a	0.01a	100.46a
	Duduthrin	0.07a	0.13a	74.80b
	LSD	0.07	0.15	12.76
Root	0%	0.03a	0.03a	40.73d
	33%	0.33a	0.03a	57.29c
	67%	0.07a	0.03a	66.45bc
	100%	0.03a	1.22a	90.28a
	Duduthrin	0.07a	0.72a	73.38b
	LSD	0.05	1.19	14.71
Leaf	0%	0.03a	0.02a	30.00d
	33%	0.03a	0.06a	44.26c

	67%	0.03a	0.30a	51.18bc
	100%	0.07a	0.23a	59.12ab
	Duduthrin	0.06a	0.39a	66.45a
	LSD	0.06	0.49	10.31

Means with the same letter down the column are not significantly different at $p \leq 0.05$.

CHAPTER FIVE

DISCUSSION

5.1 Phytochemical constituents of pyrethrum (*Chrysanthemum cinerariaefolium* Vis.)

In the present study, the extracts of flower, leaf and roots of pyrethrum showed the presence of alkaloids, flavonoids, phytosterols, phenols, tannins, triterpenoids and saponins. The concentration of the bioactive compounds varied in pyrethrum plant parts with each part showing a distinct amount. Pyrethrum flower extracts recorded the highest amount of phenols, flavonoids and triterpenoids, average amount of saponins, alkaloids, tannins and little amount of phytosterols. Pyrethrum leaf extracts showed average amount of phytosterols, alkaloids, phenols, flavonoids, tannins, little amount of saponins but triterpenoids were absent. Pyrethrum root extracts indicated a higher amount of saponins, alkaloids and phytosterols but showed little amount of phenols, flavonoids and tannins. The distinct insecticidal potency of each pyrethrum plant part against aphids might have been attributed to the variation in phytochemicals. Pyrethrum plant extracts and its phytoconstituents have been reported for anti-feedant, repellant and insecticidal activities (Haouas *et al*., 2008). Basically when botanicals are used for insecticidal purpose, their value is dependent on the phytochemicals they possess (Okwu, 2001).

Alkaloids have been reported as the most important group of natural substances playing an important role in plant defenses. Their presence in pyrethrum flower and root extracts may have contributed largely to anti-aphid activity of these extracts. This is attributed by their plant defensive mechanisms against phytophagous insects. It has been suggested that they constitute part of plant defense along with terpenoids, phenols, flavonoids, tannins and steroids (Cox, 2002). Pyrethrum leaf extracts showed anti-aphid activity despite the fact that the amount of the phytochemicals they possess were little to average. This may be due to the fact that these compounds are insecticidal even at low concentrations. This is in agreement with the finding of Rattan (2010) who reported that insecticidal compounds are effective in low levels and their mechanism of action differ, but many affect acetylcholine receptors in the nervous system or membrane sodium channels of nerves. In tomato, alkaloids, phenolics, proteinase inhibitors and the oxidative enzymes when ingested separately result in a reduced affect, but act together in a synergistic manner, affecting the insect during ingestion, digestion and metabolism.

Flavonoids present in pyrethrum extracts may have contributed largely to anti-aphid activity through their cytotoxicity and interaction with different enzymes through complexation. This is in line with the finding of Hare (2011) who reported that both flavonoids and isoflavonoids protect the plant against insect pests by influencing the behavior, and growth and development of insects Shripad *et al.* (2003) reported insecticidal and antimicrobial activities of flavonoids isolated from *Ricinus communis* against *C. chinensis*. The anti-aphid activity may be attributed to the synergistic effects of phenolic compounds which include flavonoids (Keerti *et al.*, 2013).

Both pyrethrum flowers and root extracts had average amount of tannins. Tannins may have played a larger role in destroying aphids as they are known to have a strong deleterious effect on phytophagous insects. This agrees with those of Sharma *et al*. (2009) who found out that tannins affect the insect growth and development by binding to the proteins, reduce nutrient absorption efficiency and cause midgut lesions. Tannins are astringent (mouth puckering) bitter polyphenols and act as feeding deterrents to many insect pests. When ingested, tannins reduce the digestibility of the proteins thereby decrease the nutritive value of plants and plant parts to herbivores. Role of tannins in plant defense against various stresses and their induction in response to insect damage has been studied in many plants (Roitto *et al.*, 2009).

In a study done by De Geyter *et al.* (2011), the insecticidal and deterrent activity of crude fruit sap extract of *S. incanum* was attributed to the presence of saponins, which were associated with the alterations in the feeding behaviour, molting process, interaction with hormones that regulated the growth and caused death at different stages of development. Additionally, in a study carried out by Marianna *et al*. (2012), the pesticidal activity of saponins was attributed to their wide spectrum of action and its amplitude of physiological impacts therefore saponins may have played an important role in control of aphids.

5.2 Effects of pyrethrum extracts on aphid population on *Solanum scabrum*

Results indicate that pyrethrum root extracts caused significant reduction in aphid population (61.27 aphids) as compared to flower extracts and leaf extracts. The reduction in number of aphids after application of different parts of pyrethrum (flower, roots and leaf) extracts depicted the presence of bioactive compounds which may have caused the death of aphids. *Solanum scabrum* treated with 100% pyrethrum flower extracts recorded zero (0.00 aphids) number of aphids on 69th and 83rd days while 67% recorded zero count (0.00 aphids) on 76th and 83rd days The zero count

of aphids observed in plants treated with 100% flower extracts on Day 69 and 67% on day 76 indicated the effectiveness of pyrethrum extracts at different forestated concentration levels. Alkaloids have plant defensive mechanisms against phytophagous insects which affect acetylcholine receptors in the nervous system or membrane sodium channels of nerves. Terpenoids, phenols and steroids ingested separately act together in a synergistic manner, affecting the insect during ingestion, digestion and metabolism, flavonoids cause mortality of phytophagous insects through their cytotoxicity and interaction with different enzymes through complexation and tannins possess a strong deleterious effect on insects. These results are in agreement with the findings of Pavela (2009) who reported that the extracts derived from *Chrysanthemum cinerariifolium* caused 100% mortality rate against *M. persicae* after 12 days of treatment. This could be due to one or more groups of active principle(s) present in the extracts (Adeniyi *et al.*, 2010). Qualitative phytochemical screening revealed that a higher amounts of alkaloids, saponins, phytosteroids, phenols and flavonoids were detected in the pyrethrum root and flower extracts. These phytochemicals are important for mediating interactions between plants and their biotic environment and do not have apparent function in physical or biochemical processes (Umar, 2016). A number of these phytochemicals have been shown to have insecticidal and also deterrent activities against various insects (Adeniyi *et al.*, 2010).

In addition, the use of root extracts of pyrethrum showed better anti-aphid effects than leaf extracts of pyrethrum and this may be attributed to the presence of pyrethrins intermediates in roots which are absent in leaf extracts of pyrethrums. In a study done by Ramirez (2012), pyrethrins intermediates in pyrethrum root extracts reduced mycelial growth rates of *R. solani*. This is possible owing to the intercellular transport mechanisms of monoterpenoid intermediates which occur in the biosynthesis of pyrethrins (Ramirez, 2012).

In addition, Pyrethrum flower extracts also showed a drastic mortality of aphids, this may be attributed to a higher recorded amount on phenols and flavonoids. The known active ingredients pyrethrins present in the flower extracts might have resulted into a knockdown effect, hyperactivity, and convulsions hence death of aphids due to blockage of the voltage gated sodium channels in nerve axon. This occurs when the aphids ingest or are exposed to these botanicals.

Pyrethrum is a contact poison and the nervous system of the insect body is disturbed by pyrethrins, which first leads to a tremor and finally to the death of the animal

(http://www.kpic.eu/biochemical-effect-characteristics.html). The molecular structure of the pyrethrins allows the long lasting connection to the receptors and thus causes an enduring excitation of the nerve cells by a permanent uncontrolled inflow of Na^+ ions into the cell. This causes the symptom tremor in the insects which leads to a quick death. Insect susceptibility to pyrethrin is attributable to cuticular permeability and sensitivity of internal tissues that control oxidative enzyme systems.

The results indicate that both pyrethrum flower and root extracts may be good remedy for aphid control owing to their effectiveness against the intended pest. It is noticeable that aphids were less affected by pyrethrum leaf extracts as compared to fore mentioned extracts. This may be depicted by reduced amount of saponins and average quantity of phenols, alkaloids, flavonoids and phytosteroids present in pyrethrum leaf extracts. This is also in line with the study by Michuki (1994) that, the majority of the active constituents are present in the mature fully opened flower head, whereas the stem/leaves contains only about 0.1% as much. This may be due to translocation of pyrethrins to the intercellular space of leaves and achenes being an adaptation to accumulate larger pyrethrin quantities, or to preserve the bioactivity of these compounds, as pyrethrins are sensitive to UV degradation hence shadowed or greatly reduced in leaves.

With the 100% showing the highest mortality of aphids and 33% with least mortality, this may be attributed to the concentration of bioactive compounds. Undiluted extracts were highly toxic as compared to diluted ones. However, secondary metabolites from different plant species cause physiological and cellular disturbances that include inhibition of acetylcholinesterase, disruption of sodium and potassium ion exchange (by pyrethrin), and interference of mitochondrial respiration (Usta *et al*., 2002).

5.3 Anti-aphid effect of pyrethrum extracts on growth parameters and yields of *Solanum scabrum*

5.3.1 Plant Height

The results from the present study showed that pyrethrum flower, leaf and root extracts had no significant effect ($p \geq 0.05$) on plant heights of *Solanum scabrum* on 60^{th}, 62^{nd}, 69^{th}, 76^{th} and 83^{rd} days as compared to control (0%). Pyrethrum flower extracts and root extracts recorded a higher plant height compared to plants treated with leaf extracts. The results might be due to the fact that the botanicals by themselves do not increase plant height rather they decrease the negative impact

of the pest, stunting nature of the pest. This is in accordance with Alemu *et al* (2014), who reported that some aqueous plant extracts increased plant growth parameters including plant height at varying degrees under field experiment. Taller plants indicated a higher sensitivity of aphids to bioactive compounds in the extracts which may have destroyed them hence allowing the vegetable grow tall normally. Aphids were sensitive to 100% and 67 % pyrethrum flower extracts and to 100% root extracts as compared to leaf extracts. This may be attributed to a lesser amount of bioactive compounds present in leaf as compared to those in flowers and roots. Michuki (1994) reported that the majority of the active constituents are present in the mature fully opened flower head, whereas the stem/leaves contains only about 0.1% as much. Additionally, the increase in height was slow at the beginning of the experiment but progressively increased as more cells were formed (Salisbury and Ross, 1992), this is not in line with the findings of the current study, flower extracts may be an instant contact poison which needs continuous application to control aphids hence and nutrients accumulated as days increased with regular spraying of pyrethrum extracts to curb aphid population. The constant plant height may be attributed to the impacts of aphids even after they are sprayed. The results are in line with those of Lo *et al*. (1999) who reported that large populations of *Aphis fabae* developing on leaves of peach and nectarine plants will stunt or kill young shoots if left unchecked. Aphid populations can grow to extremely high levels under favorable environmental conditions in a short time, covering sprouts, leaves, flowers, and fruits which reduces the availability of photosynthetic active radiation to the plants hence resulting into stunting. The occurrence of stunting reduced main stem height probably due to the overall effect of aphid infestation. In addition, Lawal, (2015) reported that *aphis fabae* is an important insect pest of the Solanaceae family and can attain very high densities on young plant tissue, causing water stress, wilting, and reduced growth rate of the plant (Lawal, 2015). The reduction in plant height at control may be due aphid populations growing to extremely high levels under favorable environmental conditions causing stunting.

5.3.2 Curled leaves

In this study leaf curling resulted from aphid infestation and formation of hiding folds under the leaves. This may be attributed to the provision of hiding pockets for aphids even after being cured or remain curled even after aphids are destroyed. According to Iguchi *et al*. (2012) and Ashilenje *et al*. (2011), aphids curl the leaves thereby forming pockets and folds which is known to provide

shelter to the aphids but consequently unknown to give protection against insecticide treatments. Overally, Pyrethrum flower and root extracts caused significant reduction in number of aphids hence observed least number of curled leaves as compared to pyrethrum leaf extracts. This may be attributed by a lesser amount of bioactive compounds present in leaf extracts as compared to those in flowers and roots extracts which reduced greatly the aphid population. The results agreed with those reported by Michuki (1994) that the majority of the active constituents are present in the mature fully opened flower head, whereas the stem/leaves contains only about 0.1% as much.

Plants treated with duduthrin showed a higher number of curled leaves. This may be attributed to aphid resistance to synthetic pesticide which agreed with the report of Lawal (2015) who stated that, over time, aphid populations in pepper developed resistance against compounds of synthetic insecticides including organochlorines, organophosphates, carbamates and synthetic pyrethroids. Due to repeated usage of these insecticides aphids may have evolved several insecticide resistance mechanisms, including the detoxification of insecticides by elevated esterases (Khan *et al*., 2011). Anstead *et al*. (2005) reported development of resistance in aphids to more insecticides than any other insect. Therefore, results from this study showed that pyrethrum extracts may be utilized as an alternative for aphid control perhaps their use may yield great success without aphid resistance.

5.3.3 Leaf length and width

The results from this study showed better performance of undiluted pyrethrum extracts as compared to control. The reduction in leaf size in control may be due to phloem feeding by aphids which directly removed nutrients from the plant. These results are in agreement with those of Chander *et al*. (2006) who reported that direct feeding of aphids results into reduced growth rates of green leaves hence low leaf yields. The interruption of one plant process through phloem feeding by aphids affected other processes like leaf photosynthesis, transpiration, stomatal conductance of leaf and root respiration as reported by La Rossa *et al*. (2013) and Chander *et al.* (2006).

The pyrethrum extracts may lower the negative impact of aphids because of their insecticidal effects and aphid mortality therefore leading to observed high leaf length and width. A reduced leaf size in plants treated with duduthrin may as well be attributed to aphid resistance or the detoxification of insecticides by elevated esterases produced by aphids as reported by Khan *et al*. (2011).

5.3.4 Leaf area

The control of aphids using pyrethrum flower and root extracts resulted into a significantly larger leaf area as compared to the use of pyrethrum leaf extracts. Plants in the untreated plots (control) showed a significant reduced leaf growth which lead to reduced leaf area. Pyrethrum extracts reduced the negative impact of aphids which is attributed to retarded leaf development processes, leaf senescence and death, delayed leaf emergence which overall effect is distorted leaves that offers least leaf area. Aphids being phloem feeders additionally contribute to primarily reducing leaf area through assimilate sapping therefore the reduced leaf area in the study under aphid infestation could be due to disturbed and imbalance nutrition. Higher leaf area may be associated with the control of aphids using pyrethrum flower and root extracts though leaf extracts performance was lower which is in line with the report by Michuki (1994).

5.3.5 Number of fruits

The results from this study showed that the number of fruits differed significantly ($p \leq 0.05$) in all treatments with 0% recording the least fruits and 100% treatment recording the higher number of fruits. This is attributed to sensitivity of aphids to pyrethrum extracts. Vegetative growth of crops under pest infestation may result into plant stress which culminates into reduced fruit production (La Rossa *et al*., 2013). Management of the pest may allow the crop to recover though not to its optimal potential as initially uninfected plant. The high fruit number shown by plants at 76^{th} and 83^{rd} days of treatment application indicate effectiveness of aphid control using 100% and 67% pyrethrum flower and root extracts. The results agree with those of Michuki (1994). Pyrethrum extracts influenced the fruit number indirectly by causing mortality in aphid thereby reducing the impact caused by these aphids.

5.3.6 Number of damaged fruits

The results from this study showed that the number of damaged fruits differed significantly ($p \leq 0.05$) in all treatments with 0% recording the highest damaged fruits and 100% treatment recording the least number of fruits. The observed reduced number of damaged fruits may be attributed to sensitivity of aphids to pyrethrum extracts which contained a vast array of phytochemicals ranging from alkaloids, saponins, phenols, and the known pyrethrins. These results are in agreement with the findings of Pavela (2009) who reported that the extracts derived from

Chrysanthemum cinerariifolium had 100% mortality rate against *M. persicae* after 12 days of treatment. Aphid infestation starts from young leaves and apices until the population is high where they migrate to new sites including new apices, flowers or infests the fruits. In contrast, pyrethrum leaf extracts treatments showed no significance in all days. This may be attributed to reduced amount of bioactive compounds present in leaf/stem of pyrethrum as compared to higher amounts in flower heads.

The results from this study are in agreement with those of La Rossa *et al*. (2013) who reported that aphid populations can grow to extremely high levels under favorable environmental conditions in a short time, covering sprouts, leaves, flowers and fruits. All parthenogenetic aphids are born as wingless nymphs, but can develop into either alate (winged) or apterous (wingless) morphs at maturity (Blackman and Eastop, 2000). According to Lawal (2015), the formation of alate forms within a population is usually triggered by overcrowding, limited food, need for migration or the presence of genetic plant resistance. Heavy attack on older plants may cause crop lose by decreasing flower, intensive injury to the fruit internal contents and complete destruction of the fruit and lowered seed production. Damage may also cause seed loss (La Rossa *et al*., 2013).

5.3.7 Shoot and root fresh weights

The results from this study showed that plants treated with 100% pyrethrum flower extracts recorded a significantly higher shoot fresh weight (54.47g) and root fresh weight (9.43g) compared to 33% and 67% pyrethrum flower extracts and the least weight was recorded from 0% (negative control). This showed direct relationship between concentration and fresh weight. At higher concentration level (100%) a high aphid mortality is recorded which resulted into more biomass, comparatively at a lower level (0%) high aphid survival was recorded which may have led to a number of pest damage mechanisms cutting across alterations of several plant physiological processes hence observed reduction in the fresh and dry weights.

This indicate that *Solanum scabrum* infested by aphids are highly affected but can tolerate stress up to some extent, but as population increases, significant reduction in fresh weight was observed. The reduction in fresh might be due to phloem feeding which hindered availability of food necessary in building the plant biomass, directly removed nutrients or covered the leaf surface hence the photosynthetic active radiation to the plants and closely agree with findings of Chander *et al.* (2006). Assessing assimilation rates of wheat, Chander *et al.* (2006) found that aphids are

assimilate sappers which greatly reduce plant assimilation rate and together with the direct feeding results into reduced stem reserves and storage organs resulting to reduced caloric weight of the plant which is in line with the results of this study.

The findings from this study corroborates with those of Lawal (2015), who found out that prolonged aphid infestation can cause appreciable reduction in plant yield in pepper because they consume plant nutrients and their sucking behavior can cause chlorosis and distortion of the leaves, abscission of blooms, and plant stunting and wilting. Chlorosis results into reduced concentrations of chlorophyll Additionally, wilting is a test of a plant losing a higher percentage of water which points out the intensity of water loss in relation to increasing aphid population.

5.3.8 Shoot and root dry weights

A significantly higher shoot and root dry weight was observed on plants treated with 100% pyrethrum flower extracts with 14.30g and 3.00g respectively while least dry weights were obtained from control (0%). Reduction in root and shoot dry weight as seen from plants treated with 0% could be associated with reduced rate of leaf production as a result of leaf curling caused by aphids as well as sooty molds covering the leaf surface which reduced photosynthesis and accumulation of dry matter. This lead in to reduction in food manufactured by the plant which automatically leads to reduced fresh and dry weights of the plant. The results are in agreement with the findings reported by Lawal (2015). Extracts depicted anti-aphid effects which resulted into a higher recorded root and shoot weight. The anti-aphid activity may be due to naturally occurring combination of secondary compounds which were present in pyrethrum flower and root extracts.

5.3.9 Yield/ ha

The results from the current study indicated a significantly higher yield (778.00g) from plants treated with 100% pyrethrum flower extracts compared to yields obtained from plants under control (0%). Additionally, a significantly higher yield is obtained in plants treated with 100% of pyrethrum flower and root extracts as compared to lower yields obtained from leaf extracts with similar concentration. This may be explained in relation to indirect effect of extracts on yield. Aphids being well known assimilate sappers automatically duced plant assimilates which include important plant nutrients both obtained via absorption or by photosynthetic activity. The control

of aphids using pyrethrum flower, root and leaf extracts resulted into a reduced number of aphids based on the toxicity of extracts. Zero count obtained from plants treated with flower and root extracts. This is in agreement with findings of Michuki (1994) who stated that a lesser amount of bioactive compounds is present in leaf as compared to those in flowers and roots but the majority of the active constituents are present in the mature fully opened flower head.

From the results in this study the synthetic insecticide Duduthrin® (Lambda-cyhalothrin 17.5g/litre) sprayed plot recorded a significantly ($p \leq 0.05$) lower leaf yield compared to the different concentrations of pyrethrum (100%, 67%, and 33%). This may be attributed to aphid resistance to synthetic pesticide which is in agreement with the report of Lawal (2015). The lowest leaf yield of 122 kg/ha was harvested from plants treated with 0% (control); this may be mainly due to the intense infestation of aphids on the crop as the aphid population increased inherently. Chander *et al*. (2006) reported that the direct feeding of aphids results into reduced growth rates of green leaves hence low leaf yields. These studies (Khan *et al*., 2011, Chander *et al*., 2006 and Ashilenje *et al*., 2011) are in agreement with the findings from this study.

5.4 Anti-aphid effect of pyrethrum extracts on chlorophyll and phytonutrient content of *Solanum scabrum*

5.4.1 Chlorophyll content

The control of aphids by application of 33%, 67% and 100% pyrethrum flower, root and leaf extracts showed no significant effect ($p \geq 0.05$) on leaf chlorophyll content of *Solanum scabrum* as compared to control on 54^{th} day. This may be attributed to unaffected amounts of nitrogen owing to the fact that aphids had not been introduced. Aphid feeding adversely affect the plants and directly affect chlorophyll content. Interestingly, the chlorophyll concentration in pea plant tissues at 17 days of infestation was similar to the level in the respective uninfested plants (Goławska *et al*., 2010). This indicates that aphid feeding may have less effect on chlorophyll loss in this species in the long term. This may also apply to the plant species under the study.

Pyrethrum flower and root extracts (100%) recorded a high chlorophyll value due to their potency to the intended pest hence the plant physiological processes were not affected greatly as compared to control which showed least amount of chlorophyll. Chlorophyll content of leaves is a useful indicator of both potential photosynthetic productivity and general plant vigour (Alonso *et al*., 2002, Li *et al.*, 2018). Chlorophyll is widely used as a basis for the determination of photosynthesis

because the reaction components essential for photosynthesis (such as the reaction centers PS1 and PS11, electron carriers and enzymes elated to ATP synthesis and CO_2 fixation) are present in chloroplast at fixed molar ratios to chlorophyll (Kura Hotta *et al*., 1987). The reduction in chlorophyll in plants treated with 0% may be attributable to different pest damage mechanisms caused by aphids. Heavily infested plants (under control) showed chlorosis as a result of heavy feeding by aphids.

Fanizza *et al.* (1991) reported that leaves of stressed plants synthesize less chlorophyll pigment. The exact mechanism by which aphids affect plant metabolism is not fully understood, but Heng-Moss et al. (2003) speculated that by feeding mainly on phloem tissue the aphids change the pH either on luminal side of the thylakoid membrane, preventing the formation of zeaxanthin, or on the stromal side where regeneration of violaxanthin takes place. Burd and Elliot (1966) showed that aphid feeding could reduce protein synthesis, making photo inhibition irreversible as well as blocking electron transport on the acceptor site of photosystem II reaction center, causing Ove reduction in the system.

The use of 100% pyrethrum flower extracts on 62^{nd} day resulted into a significantly higher chlorophyll content (72.60mg) from plants treated with 100% pyrethrum flower extracts. The utilization of this extract at 100% may enable farmers to control aphids realize increased production.

Haile *et al*. (1999) and Heng-Moss *et al*. (2003) found a significant decline in chlorophyll in aphid-injured leaves and speculated that it may have resulted increased synthesis of chemical defense compounds in response to herbivory. The decline in chlorophyll concentration found in this study may also be due to increased production of defensive compounds.

The plant physiological processes affected by pests include assimilation rate reduction, assimilate sucking and tissue consumption (Chander *et al*., 2006). Assimilate sucking by these aphids may have led to consumption of a larger percentage of plant nitrogen and carbon. The abundance and population growth of many phytophagous insects depends on the dietary requirement which entail total amount of nitrogen and composition of nitrogenous compounds of food ingested (Schoonhoven *et al*.,1998). Nitrogen is the main building block of chlorophyll pigment, nitrogen stealing over time through phloem feeding by aphids may lead to chlorosis.

5.4.2 Phytonutrient content

Results from this study indicate the use of 33%, 67% and100% pyrethrum flower, root and leaf extracts did not show any significant difference on the amount of sodium and calcium in *Solanum scabrum* as compared to control. Additionally, plants treated with pyrethrum extracts did not vary significantly in amount of sodium that the amount of sodium remained constant in all treatments. availability was not affected by aphid infestation. The amount of sodium obtained from this study is low and this is in agreement with the findings of Smedley and Eisner, (1995) who found out that sodium concentration is very low in most plants. Sodium plays important roles in osmotic balance, in neuromuscular system, and in digestion and excretion process in animals. In contrast, the herbivores that eat them maintain much higher sodium levels. In vertebrates, sodium concentration was 100- to 1000-fold more than that in plants. In insects, the sodium concentrations in the haemolymph of phytophages in Orthoptera, Phasmida and Lepidoptera are much higher than that in plants. Moreover, the adult haemolymph of most holometabolous insects have higher sodium concentrations than that of the larvae. This may be a reason as to why aphids did not consume much of the sodium. Sodium ions accumulate in cystol of cells, particularly transpiring cells.

Aphid species are a group of highly specialized phloem feeding with multi-level adaptations facilitating exploitations of resources provided by their host. They cause a wide spectrum of detrimental effects in attacked organs, including mechanical disruption of penetrated tissues, depletion of photoassimillates and intensification of many intracellular processes (Galawska *et al*., 2012). The results from the current study indicate no significance in calcium of *Solanum scabrum* in plants treated with different concentrations of pyrethrum flower, root and leaf extracts. This may be explained in relation to indirect effect of extracts on yield. Muthomi and Musyimi, 2009 found out that the leaves of African nightshades consist of 87.2 g water, 1.0 mg iron, 4.3 g protein, 38 kcalories, 5.7 g carbohydrates, 1.4 g fibre, 442 mg calcium, 20 mg ascorbic acid, 3660 µg ß-Carotene, 75 mg phosphorus, and 0.59 mg riboflavin per 100 g fresh weight. Aphids being well known assimilate sappers and phloem feeders might have in one way or the other interfered with the plant nutritional balance. Aphid infestation may have induced stress leading to lowered counts of calcium in the plant. The control of aphids using pyrethrum flower, root and leaf extracts resulted into a reduced number of aphids generally but calcium distribution is influenced by slight

physiological changes developed by the plant in order to adjust to the external factors hence the observed lower amount of calcium.

The results from the study indicated that a higher amount of potassium (100.46ppm and (90.28ppm) was obtained from plants treated with 100% pyrethrum flower and root extracts respectively. This may be explained in relation to indirect effect of extracts on potassium. Aphids being well known assimilate sappers automatically reduced plant assimilates which include important plant minerals like potassium. The control of aphids using pyrethrum flower, root and leaf extracts resulted into a reduced number of aphids based on the toxicity of extracts. Zero count observed from plants treated with flower and root extracts. This is in agreement with findings of Michuki (1994) who stated that a lesser amount of bioactive compounds is present in leaf as compared to those in flowers and roots but the majority of the active constituents are present in the mature fully opened flower head.

CHAPTER SIX

CONCLUSIONS, RECOMMENDATIONS AND SUGGESTIONS FOR FURTHER RESEARCH

6.1 Conclusions

i. The present investigation revealed that pyrethrum flower, root and leaf extracts have varying concentration of phytochemical compounds which are anti-aphid. They include: alkaloids, saponins, flavonoids, phytostreroids, phenols, triterpenes, tannins and pyrethrins.

ii. Aphid control can be achieved using 100% and 67% pyrethrum flower and root extracts. It can be harnessed by smallholder farmers instead of depending on expensive synthetic pesticides.

iii. Pyrethrum flower and root extracts reduced aphid population hence promoting growth and yields of *Solanum scabrum*.

iv. Pyrethrum flower and root extracts reduced aphid population resulting to increased chlorophyll and phytonutrient content of *Solanum scabrum*.

6.2 Recommendation

This study recommends pyrethrum flower and root extracts (100%) to be used as an alternative for the management and control of aphids instead of synthetic pesticides.

6.3 Suggestions for Further Research

i. Further studies should quantify the amount of phytochemicals present in different pyrethrum parts.

ii. Further research should be done to find out the anti-aphid effects of different solvents pyrethrum extracts on African.

iii. Further research should determine the effect of control of aphids using pyrethrum extracts on other phytonutrients of African nightshades.

iv. A study be carried out to determine residual effect of crude pyrethrum extracts on African nightshades.

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APPENDICES

APPENDIX 1: Field experimental layout and trials

Appendix 1.0: Forty-five (3m by 1m) plots set in Randomized Complete Block Design

Appendix 1.1: A week old *Solanum scabrum*

Appendix 1.4: Aphid rearing in the green house

Appendix 2: Analysis of variance (ANOVA) for parameters

Appendix 2.1: ANOVA for aphid population

Number of aphids

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	42	573175.2533	13647.0298	116.05	<.0001
Error	32	3763.0933	117.5967		
Corrected Total	74	576938.3467			

R-Square	Coeff Var	Root MSE	No__of_Aphids Mean
0.993477	15.73752	10.84420	68.90667

Source	DF	Type II SS	Mean Square	F Value	Pr > F
Concentration	4	367518.2133	91879.5533	781.31	<.0001
Day	4	31873.0133	7968.2533	67.76	<.0001
Replication	2	1074.4267	537.2133	4.57	0.0180
Day*Concentration	16	160939.7867	10058.7367	85.54	<.0001
Replicati*Concentrat	8	7976.1067	997.0133	8.48	<.0001
Replication*Day	8	3793.7067	474.2133	4.03	0.0021

Appendix 2.2: ANOVA for plant growth parameters

Plant height

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	42	26683.71600	635.32657	6.39	<.0001

Error	32	3179.99947	99.37498
Corrected Total	74	29863.71547	

R-Square	Coeff Var	Root MSE	Plant_Height Mean
0.893516	16.54904	9.968700	60.23733

Source	DF	Type II SS	Mean Square	F Value	Pr > F
Concentration	4	6404.33813	1601.08453	16.11	<.0001
Day	4	4658.36480	1164.59120	11.72	<.0001
Replication	2	3067.42747	1533.71373	15.43	<.0001
Day*Concentration	16	146.55253	9.15953	0.09	1.0000
Replicati*Concentrat	8	11585.94587	1448.24323	14.57	<.0001
Replication*Day	8	821.08720	102.63590	1.03	0.4326

Curled Leaves

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	42	5116.613333	121.824127	2.85	0.0014
Error	32	1368.373333	42.761667		
Corrected Total	74	6484.986667			

R-Square	Coeff Var	Root MSE	Curled_Leaves Mean
0.788994	75.56904	6.539241	8.653333

Source	DF	Type II SS	Mean Square	F Value	Pr > F
Concentration	4	790.986667	197.746667	4.62	0.0046
Day	4	1688.720000	422.180000	9.87	<.0001
Replication	2	734.106667	367.053333	8.58	0.0010
Day*Concentration	16	485.946667	30.371667	0.71	0.7633
Replicati*Concentrat	8	850.693333	106.336667	2.49	0.0319
Replication*Day	8	566.160000	70.770000	1.65	0.1484

Leaf length

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	42	315.8609627	7.5204991	12.09	<.0001
Error	32	19.9041760	0.6220055		
Corrected Total	74	335.7651387			

R-Square	Coeff Var	Root MSE	Leaf_Length Mean
0.940720	11.58428	0.788673	6.808133

Source	DF	Type II SS	Mean Square	F Value	Pr > F
Concentration	4	113.6762453	28.4190613	45.69	<.0001
Day	4	75.1886453	18.7971613	30.22	<.0001

Replication	2	31.6052027	15.8026013	25.41	<.0001
Day*Concentration	16	17.4108480	1.0881780	1.75	0.0871
Replicati*Concentrat	8	55.7336107	6.9667013	11.20	<.0001
Replication*Day	8	22.2464107	2.7808013	4.47	0.0010

Leaf width

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	42	224.6365333	5.3484889	6.96	<.0001
Error	32	24.6042667	0.7688833		
Corrected Total	74	249.2408000			

R-Square	Coeff Var	Root MSE	Leaf_Width Mean
0.901283	17.48126	0.876860	5.016000

Source	DF	Type II SS	Mean Square	F Value	Pr > F
Concentration	4	83.06480000	20.76620000	27.01	<.0001
Day	4	32.71013333	8.17753333	10.64	<.0001
Replication	2	38.70960000	19.35480000	25.17	<.0001
Day*Concentration	16	10.35253333	0.64703333	0.84	0.6337
Replicati*Concentrat	8	39.91040000	4.98880000	6.49	<.0001
Replication*Day	8	19.88906667	2.48613333	3.23	0.0083

Leaf area

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	42	37755.77134	898.94694	7.04	<.0001
Error	32	4083.47905	127.60872		
Corrected Total	74	41839.25039			

R-Square	Coeff Var	Root MSE	Leaf_Area Mean
0.902401	30.08267	11.29640	37.55120

Source	DF	Type II SS	Mean Square	F Value	Pr > F
Concentration	4	13155.40330	3288.85082	25.77	<.0001
Day	4	8195.63789	2048.90947	16.06	<.0001
Replication	2	3278.18429	1639.09214	12.84	<.0001
Day*Concentration	16	2129.99381	133.12461	1.04	0.4422
Replicati*Concentrat	8	7985.14729	998.14341	7.82	<.0001
Replication*Day	8	3011.40478	376.42560	2.95	0.0137

Appendix 3: ANOVA Tables

Apendix 3.1: ANOVA Table of number of aphids

Source	DF	Sum of squares	Mean square	F Value	Pr > F
Model	160	1364275.40	8526.72	31.30	<.0001
Error	64	17432.60	272.38		
Corrected Error	224	1381708.00			

Appendix 3.2: ANOVA Table of growth parameters of *Solanum scabrum*

	Source	DF	Sum of squares	Mean square	F Value	Pr > F
Plant height	Model	160	80096.33	500.60	8.64	<.0001
	Error	64	3706.22	57.91		
	Corrected Error	224	83802.55			
Curled leaves	Model	160	12319.45	77.00	2.80	<.0001
	Error	64	1761.30	27.52		
	Corrected Error	224	14080.75			
Leaf length	Model	160	904.07	5.65	8.33	<.0001
	Error	64	43.41	0.68		
	Corrected Error	224	947.48			
Leaf width	Model	160	618.61	3.87	3.55	<.0001
	Error	64	69.71	1.09		
	Corrected Error	224	688.32			
Leaf area	Model	160	96562.72	603.52	4.62	<.0001
	Error	64	8359.61	130.62		
	Corrected Error	224	104922.33			

Appendix 4.3: ANOVA Table of number of fruits and damaged fruits of *Solanum scabrum*

	Source	DF	Sum of squares	Mean square	F Value	Pr > F
No. of fruits	Model	131	131837.95	1006.40	9.93	<.0001
	Error	48	4862.69	101.31		
	Corrected Error	179	136700.64			
No. of damaged fruits	Model	131	2789.34	21.29	2.32	<.0001
	Error	48	440.63	9.18		
	Corrected Error	179	3229.97			

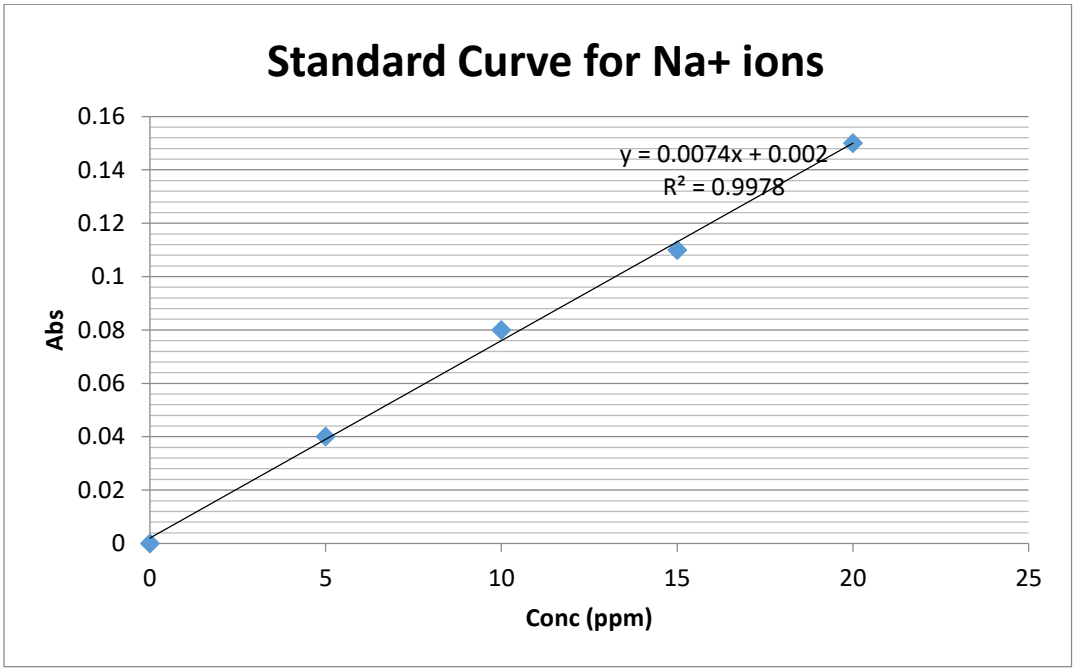
Appendix 3.4: ANOVA Table of fresh/dry weight and yield/ha of *Solanum scabrum*

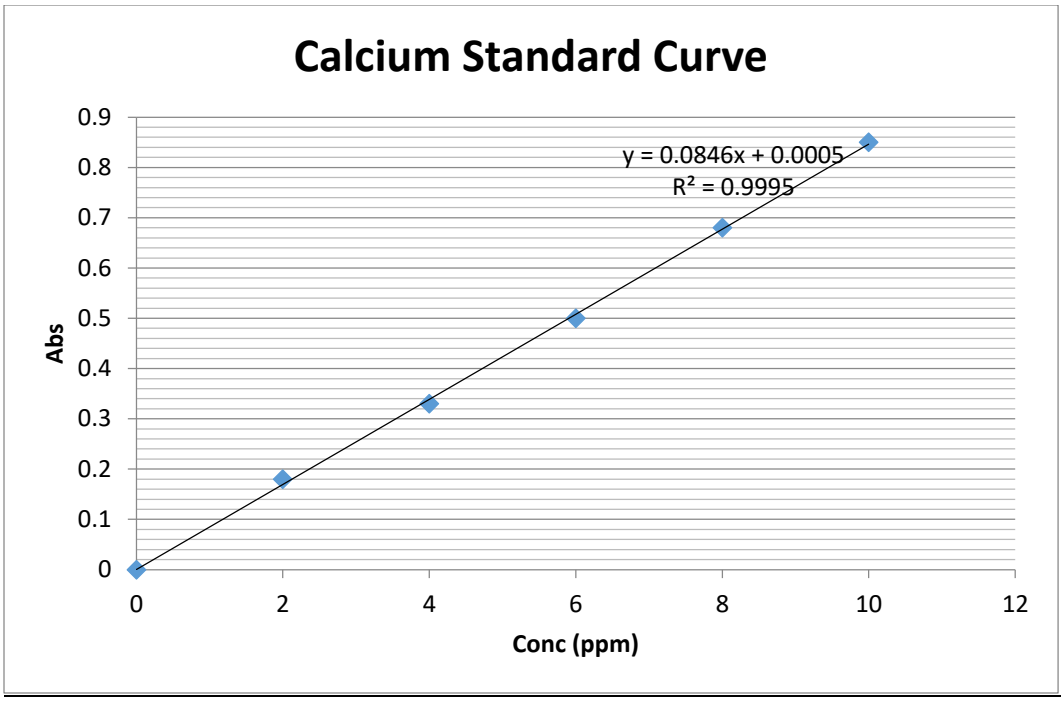
	Source	DF	Sum of squares	Mean square	F Value	Pr > F
Shoot fresh weight	Model	14	18790.10	1342.15	6.92	<.0001
	Error	30	5816.14	193.87		
	Corrected Error	44	24606.24			
Root fresh weight	Model	14	746.51	53.32	6.51	<.0001
	Error	30	245.76	8.19		
	Corrected Error	44	992.27			
Shoot dry weight	Model	14	1607.13	114.80	3.99	<.0001
	Error	30	863.77	28.79		
	Corrected Error	44	2470.90			
Root dry weight	Model	14	50.83	3.63	3.83	<.0001
	Error	30	28.44	0.95		
	Corrected Error	44	79.27			
Yield/ha	Model	14	2275892.98	162563.78	10.39	<.0001
	Error	30	469603.75	15653.46		
	Corrected Error	44	2745496.73			

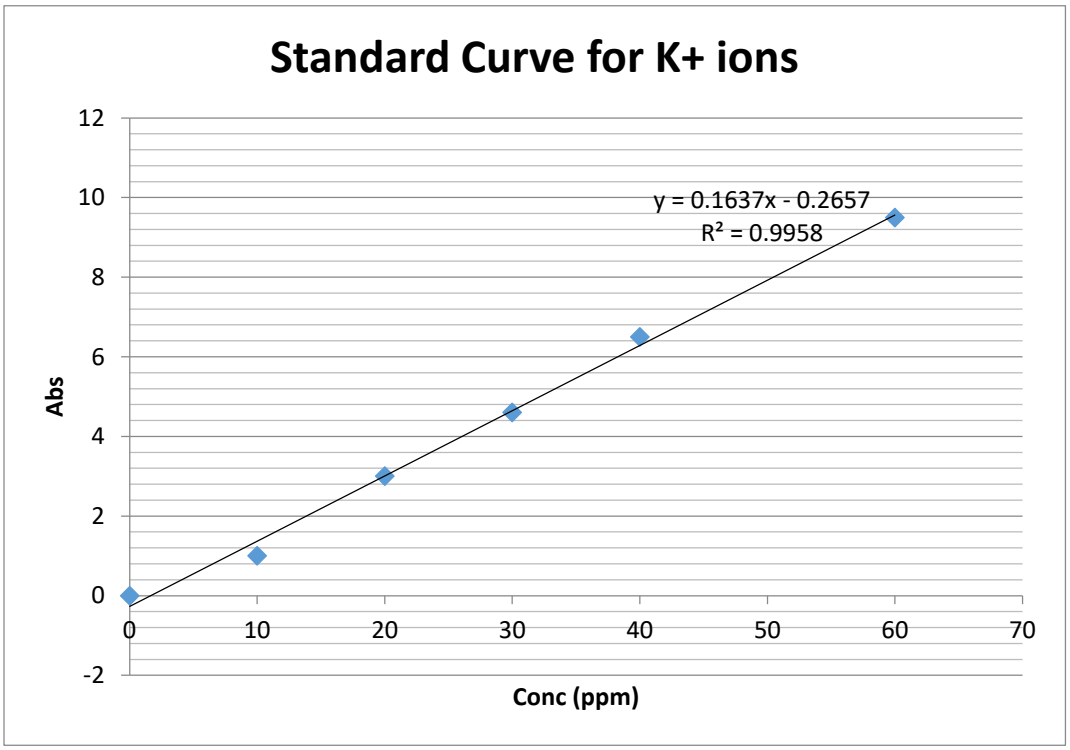
Appendix 3.5: ANOVA Table of chlorophyll content of *Solanum scabrum*

	Source	DF	Sum of squares	Mean square	F Value	Pr > F
Chlorophyll content	Model	73	40571.92	555.78	33.64	<.0001
	Error	16	264.35	16.52		
	Corrected Error	89	40836.28			

APPENDIX 4: Sample of standard curves used




Calcium Standard Curve
y = 0.0846x + 0.0005
R² = 0.9995
Abs
Conc (ppm)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
0
2
4
6
8
10
12


Standard Curve for K+ ions
y = 0.1637x - 0.2657
R² = 0.9958
Abs
Conc (ppm)
12
10
8
6
4
2
0
-2
0
10
20
30
40
50
60
70