

**EFFECTS OF STINGING NETTLE, AFRICAN SPIDER PLANT AND CHILLI
EXTRACTS ON TWO SPOTTED SPIDER MITE (*Tetranychus urticae* Koch)
POPULATION AND DAMAGE ON ROSES**

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**A thesis submitted to the Graduate School in partial fulfilment for the requirements of
the Master of Science Degree in Horticulture of Egerton University**



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X

DECLARATION AND RECOMMENDATION

DECLARATION

I hereby declare this thesis as my original work and has not been previously presented to any University for any degree or any other award.

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DEDICATION

To my loving parents Mr. P. A. C. Kapsoot & Mrs Beatrice Chepkurui, in memory of my late
sister Faith Cherop Kapsoot.

ACKNOWLEDGEMENT

I am very grateful to the Almighty God for the grace and strength He gave me to undertake and complete this Thesis. I thank Egerton University Department of Crops, Horticulture and Soils sciences, and the entire Egerton University fraternity for the academic support and advice they offered during my studies. This research would not have been complete without the support many people offered me. I'm thankful to my supervisors Dr. Mariam Mwangi and Prof. Alice W. Kamau who granted me consistent mentorship throughout my studies and deserves special respect; I gratefully acknowledge the financial support from my parents Mr. and Mrs P.A.C Kapsoot who financed the entire research project. My sincere gratitude goes to Fontana flowers limited (Akina) – Njoro for offering me the space and facilities to undertake my research. Last but not least my attention is drawn to the Director Mr Girish Appanna for his kindness and the support of the technical staff.

ABSTRACT

Tetranychus urticae Koch. is a major pest of cut rose flowers and causes high losses if not controlled effectively on time. Laboratory trials were conducted to evaluate and validate the repellent and toxic properties of three local plants *Cleome gynandra* (capparaceae), *Urtica dioica* (urticaceae) and *Capsicum frutescence* (solanaceae) against the two spotted spider mite *Tetranychus urticae* Koch (Acari; Tetranychidae) on rose flowers grown under greenhouse. 100g of each plant extracts was constituted in methanol and distilled water separately and rose leaves at 3 leaflet stage were immersed in it. Ten *T. urticae* mites were introduced onto the treated leaves and observations on repellence and mortality of mites was recorded. The experiment was laid out in a Completely Randomized Design with nine treatments replicated three times. Methanol was found to be the most effective solvent and *Cleome gynandra*, *Urtica dioica* and *Capsicum frutescence* methanoic extracts identified and recommended for further evaluation in the field trials for repellence and efficacy effects on the yield and quality of cut rose flowers. Results showed that these extracts from methanol were effective against *T. urticae* with the number of mites repelled significantly higher at $P \leq 0.05$. *Cleome gynandra* methanol extract, *Capsicum frutescence* methanol extract and *Urtica dioica* methanol extract were the most effective repellents in descending order. Significance of the treatments was more evident with exposure time. The crude extracts on the first day had moderate repellence in the second hour and higher repellence was found on the third and fourth hours after exposure. However, the observations also showed that plant extracts have a slow mortality effect on the two spotted spider mites over the six days period compared to Polytrin miticide which attained approximately 70% kill on the first day to sixth day. The powders were slow acting achieving up to 80% kill on the 6th day as observed in *Cleome gynandra* methanol extract. The three test plants have repellence and mortality effects which may be used as there is considerable acaricidal activity of *C. gynandra*, *C. frutescence* and *U. dioica* on *T. urticae*. Their use against the two spotted spider mites significantly reduced the population of mites on rose leaves within a period of six days. However, the level of mortality and repellence was dependent upon the period of exposure to the crude plant extracts. The potential benefits of methanoic plant volatile extraction in the control of mites in rose grown for export markets is evident.

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CHAPTER ONE

INTRODUCTION

1.1 Background Information

Horticulture is the third most important sub sector within the Kenyan economy, generating Ksh.217 billion in 2012 in an area of 662,835 ha with a total production quantity of 12.6 million tonnes. Cut flowers contribute about Ksh. 39.7 billion which is 18% of the domestic value. Roses are the dominant cut flower exported from Kenya which occupies an area of 2163.78ha producing 839,906 MT and 90% of the domestic value. (HCDA, 2012). The rose (*Rosa hybrida* L.) is regarded as the most important cut flower and accounts for about 75% of the total flower production in many world leading producer countries such as Kenya and Ecuador (Laws, 2005). The main destination for most of the cut flowers is the Netherlands Dutch auction where roses sold account for over 30% of the total turnover (Nyalala and Grout, 2007). Kenya has continuously attracted investors despite competition from other African countries like Ethiopia, Uganda and Tanzania.

Tetranychus urticae Koch. is a major threat to the increased production of high quality roses in Kenya and other rose growing countries, (Josvold and Chaney, 2001). This two spotted mite feeds by attacking the leaf cells and piercing them to extract the cell contents which lead to the collapse of the cells. Necrosis is observed by spotting on the upper side of the leaf. High infestation may cause severe necrosis and eventual leaf drop due to leaf desiccation (Nyalala and Grout, 2007). Cut roses are grown mainly for their aesthetic value and therefore, the market tolerance for any damage is low in market destinations.

Miticides have been used discriminatively by rose growers to control *T. urticae*, this amounts to 40% of pesticide volume applied to rose flower crops (Josvold and Chaney, 2001) and accounts for 25 % to 50% of the total cost of pest control depending on the rose variety, season and region (temperate or tropics). Use of miticide sprays is not satisfactory due to the high costs of miticides, logistics of regular application, development of Miticide- resistant mite strains and an increased residue level, unacceptable to the consumers and environment. Further, the pressure to consider bio-control and organic production is increased by the enforcement of the European codes of practice for export market aimed at reducing and eventually eliminating the use of synthetic pesticides.

Biological control of two spotted spider mites as an alternative to miticides is being advocated (Labuchagne, 2005). The bio-control using predatory mites *Phytoseiulus persimilis* and *Amblyseius californicus* (Cherian, 2003), is equally expensive and often difficult to sustain, implement and requires a high level of technical skill. Moreover, these predatory mites are highly specific and have a narrow target range which does not eliminate the pest entirely. They may not also be compatible with the use of chemical fungicides or bactericides (Armstrong, 2001). Currently the practice which farmers use to control the two spotted spider mite is the use of *Phytoseiulus persimilis*.

Traditional methods of control using local plant extracts have been tried. Nyalala and Grout (2007) intercropped African spider plant (*Cleome gynandra* L.) with rose crop and concluded that the treatment had a significant repellent effect to *T. urticae*. Integrated Pest Management systems using a combination of predatory mites, soapy water solutions chilli and garlic are being used by farmers although they have not been validated. Knapp and Kahenge (2003) found that neem formulations controlled two spotted spider mites on tomatoes. Dabrowsky, seredynska and Urszula (2007) concluded that various plant extracts had insecticidal repellent and acaricidal properties hence need for further research to find toxic and repellent plants to the two spotted spider mite. This research tested the use of crude extracts from African spider plant (*Cleome gynandra*) Stinging nettle (*Urtica dioica*) and hot chilli (*Capsicum frutescence*) on *T. urticae* Koch. on rose cut flower.

1.2 Statement of the Problem

Production of cut-rose for export and local markets is mainly under greenhouse conditions, which is important for obtaining high quality and yield. However, production has remained a challenge to many commercial rose producers mainly because of *Tetranychus urticae* Koch. the major pest attacking roses (Josvold and Chaney, 2001) resulting in low yields and poor quality roses. Losses due to damage caused by *T. urticae* are estimated to range between 20% and 80% annually (KFC, 2008). Most rose growers use synthetic miticides to control the two spotted spider mite. Although the use of synthetic miticides is recommended for effective control of *T. urticae*, their continued use has led to development of resistant mite populations. The cost of miticides is high and the maximum residue levels are unacceptable to consumers and market regulators requirements of minimum or zero tolerance to pesticide use. This has led to the need for alternative control measures that are

consumer and environmental friendly. Some local farmers have used traditional plant materials and concoctions, most of which are yet to be rationalized or validated scientifically.

1.3 Justification

Tetranychus urticae Koch is the pest responsible for a large number of rose flower consignment rejections at the exit and entry inspection points causing major losses to the exporters and producers. To avoid these losses, many rose growers are using synthetic miticides containing active ingredients such as abamectin, to control the two spotted spider mite. However, continued application of these miticides has led to high costs of production, logistics of regular application, development of miticides-resistant mite strains and an increased residue level dangerous to the consumers and environment. The EUREPGAP regulations also emphasize a minimum or zero tolerance to pesticides use and pest free produce exported to the European countries.

Due to these regulations, many growers have resorted to using solutions, e.g. the commercial formulation of plant extract from the seed kernels of the neem tree – NeemAzal®-T/S for control of sucking arthropod pests on ornamental plants in greenhouses namely; spider mites, white flies and aphids. Apart from the neem tree, a wide range of other tropical plant species e.g. chinaberry (*Melia* spp.); *Warburgia* spp., *Quillaja saponaria* Molina; *Gmelina arborea* L. are being extensively studied for their insect repellent and antifeedant properties (Oparaeke, 2005; Waligóra, 2006). In Kenya local farmers have used local preparations of soapy water, chilli pepper, garlic, African spider plant, black night shade stinging nettle and white oil to control these pests. However, these methods and solutions have not been verified for use as bio pesticides.

Therefore, there is need to test and validate the use of *Cleome gynandra*, *Urtica dioica* and *Capsicum frutescence* extracts as alternative to synthetic pest control solutions thereby increasing the quality and yield of cut rose flowers of Kenyan growers.

1.4 Objectives

1.4.1 Broad objective

To develop sustainable and environmental friendly ways of controlling *Tetranychus urticae* Koch using plant extracts to increase the yield and quality of cut-rose flower

1.4.2 Specific objectives

To determine the;

1. Repellent effect of crude plant extracts of *C. gynandra*, *U. dioica* and *C. frutescence* on *T. urticae* population.
2. Toxic effect of crude plant extracts of *C. gynandra*, *U. dioica* and *C. frutescence* on *T. urticae* population.
3. Effect of crude plant extracts of *C. gynandra*, *U. dioica* and *C. frutescence* on *T. urticae* damage and quality of rose flowers
4. Effect of crude plant extracts of *C. gynandra*, *U. dioica* and *C. frutescence* on the yield of rose flower.

1.5 Null Hypotheses

1. Crude plant extracts of *C. gynandra*, *U. dioica* and *C. frutescence* have no repellent effect on *T. urticae* population.
2. Crude plant extracts of *C. gynandra*, *U. dioica* and *C. frutescence* have no toxic effect on *T. urticae* population.
3. Crude plant extracts of *C. gynandra*, *U. dioica* and *C. frutescence* have no effect on *T. urticae* damage and quality of rose flowers
4. Crude plant extracts of *C. gynandra*, *U. dioica* and *C. frutescence* have no effect on the yield of rose flower.

CHAPTER TWO

LITERATURE REVIEW

2.1 Classification and Botany of the rose flower

The rose (*Rosa hybrida* L.) belongs to the family Rosaceae and is closely related to apple, pear, plum, cherry, blackberry, and strawberry, which includes over 200 species of the wild roses mostly from the temperate regions, mainly the northern hemisphere which serves as the primary origin. The rose can be recognised by its bushy and shrubby appearance with straight woody branches covered with prickles (Gerardo, 2007).

Rosa hybrida L. is a description used for most cultivated rose cultivars of the Hybrid tea or Floribunda types or classes. These cultivated roses have been derived over centuries through complex crosses involving a number of species of the genus *Rosa* (Phillips and Rix, 1988). The species are very variable and hybridize freely, making species delimitation difficult (Zieliński *et al.*, 2004). The chromosome number of rose varies from $2n=2x=14$ to $2n=8x=56$, with most species being diploid or tetraploid. Commercial rose cultivars (*Rosa x hybrida*) tend to be either triploid or tetraploid (Rout *et al.*, 1999). Classification is also problematic due to continuous variation of characters. In one section of *Rosa*, section Caninae, species are classified only by their unique meiotic system, not by visible characters. Apomixis has been reported in the species of section Caninae, in hybrids among section Caninae species (Gudin, 2000), as well as in diploid *Rosa x hybrida* (Crespel *et al.*, 2001). Apomixis would help to perpetuate inter-specific and intra-specific hybrids, adding further taxonomic confusion. The taxonomic difficulties of this species have resulted in reports of anywhere from 100 to 250 *Rosa* species, of which there are innumerable cultivars (Ross, 1991).

The rose leaves are deciduous and rarely persistent, are stipulate, composite and imparipinnate with 3-5 or 7 oval or oval-lanceolate shaped leaflets. The margins could more or less be serrated or not, with relief form of nerves on the underside. The flowers can be single or clustered forming a corymb. The sepals, petals, pistil and stamen are inserted at the edges and inside a cup-shaped receptacle, which contains numerous ovaries in its cavities. The sepals are 5 or more rarely 4; they are green and have photosynthetic and protective function for the flower before it opens (Gerardo, 2007).

2.2 Production and importance of roses

Rose production in Kenya started in the 1980's, and since then has continued to grow tremendously to the current 4,039 ha (HCDA, 2012). The most productive areas are at heights between 1400 and 2300 m a.s.l. (Above sea level), which includes areas around Kitale, Naivasha, Nakuru, Thika, Nairobi and Mount-Kenya (Plate 2.1). Sixty percent of the production area is found around Nairobi and Lake Naivasha. The leading flower growing counties are Kiambu 47% and Nakuru 35%. There are over 80 commercially grown flowers in Kenya and the roses contribute upto 75% of flower exports. (HCDA, 2012).

Kenya is ranked third exporter of flowers globally after the Netherlands and Columbia. Other exporters posing competition to market access are Ecuador, Ethiopia and Israel. In order for Kenya to remain competitive and expand markets, there is need for the industry to institute measures that will ensure the industry remains competitive especially compliance to phytosanitary measures. (HCDA, 2012)

2.3 Production constraints

Growing of roses in Kenya has experienced constraints which include the economic recession the world over during early 2009 which affected sales of cut roses in the auction and direct market. High costs of investment, high dependence on imported planting material, high taxation, climate change, high costs involved in compliance to standards such as the global gap hence cutting off the small holder farmers. A major constraint is pests which impact heavily on the industry since they affect market access due to interceptions abroad caused by non-compliance to phytosanitary requirements. (HCDA, 2012)

Other problems are pests mainly the two spotted spider mites (*Tetranychus urticae* Koch), *Agrobacterium tumefaciens*, whiteflies, aphids and caterpillars. These pests also contribute to the reduced quality of flowers especially if not controlled on time (HCDA, 2012). The two-spotted spider mite, *T. urticae* Koch, is considered a serious pest of fruits, vegetables and ornamental plants worldwide with more than 1200 species of host plants reported including 150 economically important species (Zhang, 2003). Diseases such as downy mildew that can wipe out all the roses growing in a greenhouse if not controlled early enough have affected rose producing farms in high altitude regions where the weather is usually wet and humid all year round. Powdery mildew also attacks roses and can cause losses in aesthetic value of the cut flower (Gerardo, 2007).

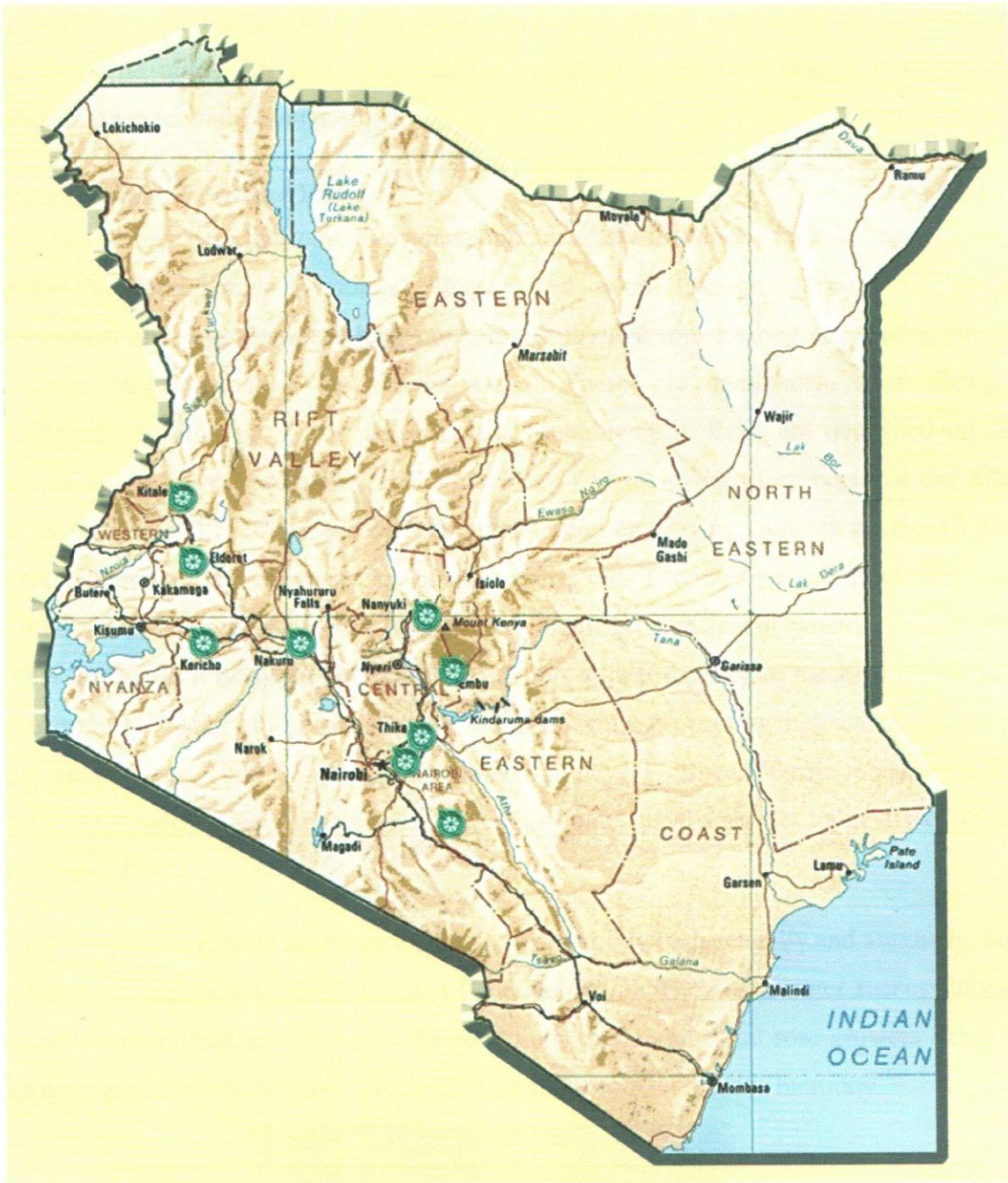
Another major constraint to production of roses in Kenya is mainly the competition from neighbouring countries. Ethiopia and Tanzania have wooed investors with low taxes on foreign investors and presence of a large workforce. Countries like Ecuador and Israel have raised the bar for roses through value addition that the Kenyan growers are striving to compete with, in terms of packaging and presentation to the consumers (HCDA, 2012). The European market requirements have also been hard on the Kenyan industry and some farms have had to close down due to non conformity of regulations of fair trade, workers welfare, environmental pollution and minimum use of chemicals.

2.4 Importance of *T. urticae*

The TSSM (*Tetranychus urticae* Koch: Tetranychidae) was first described by Robert Koch in 1836. It is a polyphagous pest of more than 900 plant species and a serious pest of more than 150 economically important crops among field and forage crops as well as horticultural crops (Najafabadi *et al.*, 2011). The spider mites are important pests in many agricultural systems and are the most important arthropod pest of fresh cut roses. Two spotted spider mite (*T. urticae*) is a major threat to the production of quality roses (Josvold and Chaney, 2001) and is known to cause up to 80% losses in crops.

Suski and Naegele (1996) conducted an experiment and concluded that the initiation of dispersal of *T. urticae* was a response to food shortage and desiccation on the host interacting with some unknown factors. Horizontal dispersal of spider mites is faster than vertical dispersal in tomato plants according to Krainacker and Carey (1990), which may also be the case on roses and suggests the behavioural response of mites to environmental conditions probably played an important role in determining the initial dispersal direction. Spider mites are also reported to avoid unfavourable conditions on over exploited leaves. Kielkiewicz, 1996; Nihoul *et al.*, 1991 reported that populations of *T. urticae* showed a greater tendency to increase on the upper ten leaves and the most marked increase in population was found in the middle leaves of the plants. Lack of correlation between mite density and leaf damage can be attributed to spider mites moving upwards to colonize youngest leaves (Kielkiewicz, 1996).

Spider mites tend to disperse by wind or by walking up a plant as the mite population and plant grows (Sabelis and Dicke, 1985). Sabelis (1985) reported that the vertical and horizontal course of mite movement may vary widely for different species of host plants and spider mite species.



KEY

⊗ Flower growing areas

Plate 2.1: Map showing rose growing areas in Kenya.

Kenya flower council-<http://www.kenyaflowercouncil.org/floricultureinkenya>

Experience has shown that infestation often starts on the outside (border rows) of a plot, therefore, other adjacent crops, wild plants and weeds can serve as a source of infestation. The mites can also be spread by irrigation water, dust storms, clothing and implements (Keizer and Zuubier, 2000).

2.5 Biology of the *T. urticae* Koch

The mites go through five development stages, which include egg, larvae, protonymph, deutonymph (Nymphal stages) and adult (Meyer, 1996). The larval, protonymphal and deutonymphal stages are further divided into feeding and resting stages, the active stage precedes the resting stage. These are nymphochrysalis (larvae), deutochrysalis (protonymph) and teliochrysalis (deutonymph). Eggs are deposited on the underside of the leaves, preferably at the junction of veins. Oviposition begins a day after adult emergence and the females reach their maximum egg laying capacity on the 4th day, then the oviposition rate decreases as the mite grows old. The eggs are spherical, clear and look like microscopic pearls about 0.14 mm in diameter, translucent when first laid then becomes opaque as it develops and two red eye spots appear just before hatching (Plate 2.2). The larvae have 3 pairs of legs and lasts for 3-5 days, while the protonymph and deutonymph have 4 pairs of legs. Total nymphal period lasts 6-10 days. Fertilized female longevity is 13-32 days while unfertilized females live 27-39 days; males have a shorter longevity of 24-30 days (Meyer, 1996).

Tetranychus urticae mites are vigorous, and multiply both sexually and asexually, and complete their life cycle within 7–39 days (Cherian, 2003). They overwinter successfully in protected environments, and thrive in the conditions that commercial rose growers strive to achieve i.e. temperatures between 25°C –28° C, and 60%–70% relative humidity.

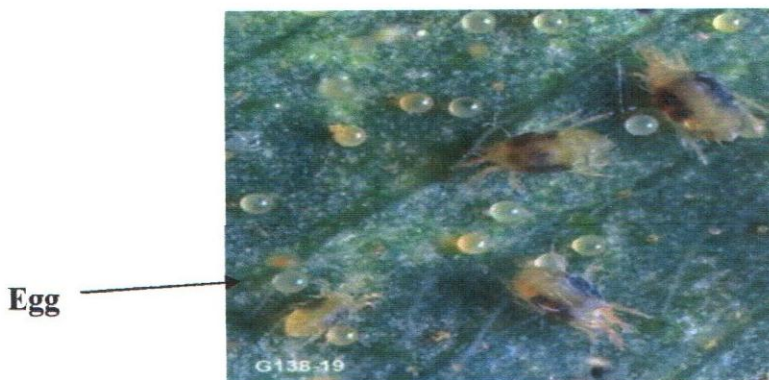


Plate 2.2: Adult female and eggs of two-spotted spider mite (*Tetranychus urticae* Koch.)

Source; University of California Cooperative Extension (UCCE)

2.6 Symptoms of damage of *T. urticae* infestation

Tetranychus urticae Koch population can expand rapidly up to 40% per day. It causes direct damage to plants including defoliation, russetting and in excessive outbreaks plant death and indirect damage by decreased photosynthesis and transpiration (Najafabadi *et al.*, 2011). The two spotted spider mite causes damage by piercing leaf cells and sucking sap, leading to lethal cell collapse and visible spotting (Plate 2.3) on the upper leaf surface, (Nyalala and Grout, 2007). Both immature and adults feed on the same plant and all stages are typically found on the under surface of leaves.

Heavy infestation can cause hyper-necrosis with significant desiccation and leaf fall (Landeros *et al.*, 2004) which usually results in the death of the plants due to reduced photosynthetic surfaces. These mites also cause damage through production of a gummy substance which is conducive for the growth of sooty mould resulting in blackening of the leaves and stem. They also cause small lesions and although the individual lesions are very small, commensurate with the small size of the mites, the frequently-observed attack of hundreds or thousands of spider mites can cause thousands of lesions and thus can significantly reduce the photosynthetic capability of plants, greatly reducing their production of nutrients and sometimes even killing the plants. Although this way of feeding could spread plant viruses, this is considered of secondary importance. *Tetranychus urticae* Koch. is considered to be the most important of the mites on roses. Cut flowers are grown for their appearance and aesthetic value (Nyalala and Grout, 2007) and the commercial tolerance of such damage is very low usually very close to zero in export markets



Plate 2.3: Damage caused by two spotted spider mites on rose leaf

2.7 Control Methods

Control of two-spotted spider mite, *T. urticae* Koch, populations mostly relies on the use of acaricides. However, this species rapidly develops resistance to acaricides owing to its high reproductive potential, extremely short life cycle and arrhenotokous reproduction (Jahangir *et al.*, 2011). Genetically fixed resistance mechanisms in spider mites are thought to be similar to those documented in insects and involve enhanced detoxification through the enzymatic activity of esterases, glutathione-S-transferases and P450 monooxygenases and/or modification of the acaricide target site (Van Leeuwen *et al.*, 2004). The risk of developing resistance is particularly high in greenhouses because of the relative isolation of mite populations, the extended growing season and the intensive use of pesticides (Jahangir *et al.*, 2011). The most threatening situation probably occurs in ornamental horticultural crops such as potted plants and cut flowers, where biological control is not well established owing to a zero pest tolerance.

In addition consumers insist on cut flowers that are pest and blemish free. Flowers are also not edible and thus are excluded from regulations on pesticide residues, resulting in intensive pesticide use. In rose greenhouses, pesticide use poses a threat to occupational safety and health, which is as important as the enhanced risk of resistance development. *Tetranychus urticae* is one of the economically most important pests on rose. Its quick resistance build-up to acaricides is threatening proper rose production in greenhouses.

2.7.1 Biological control

Biological control of red spider mites as alternative for miticides are being advocated (Labuchagne, 2005). The bio-control using predatory mites *Phytoseiulus persimilis* and *Amblyseius californicus* (Cherian, 2003) is the common method adopted by many farmers. There are also other predators available for use by rose growers like the *Neoseiulus fallacis* and the gall midge larva, *Feltiela acrisuga* (Cherian, 2003). However, these predatory mites are highly specific and have a narrow target range which does not eliminate the pest entirely. They may not also be compatible with the use of chemical fungicides or bactericides (Armstrong, 2001). Currently the farmers are controlling the two spotted spider mite using *P. persimilis* which is highly dependent on optimum relative humidity and temperature range between 70% and 75% and 25°C -30°C respectively.

Various insects are also important predators for example, the six spotted thrips *Scolothrips sexmaculatus* the larvae and adults of the spider mite destroyer lady beetle

Stethorus picipes, the larvae of certain flies including the cecidomyid *Feltiella acarivora*, and various general predators such as minute pirate bugs, big eyed bugs, and lacewings

2.7.2 Cultural control

Cultural practices can have a significant impact on spider mite population and some practices commonly exerted include the application of water to pathways and other dusty areas around the greenhouse at regular intervals which helps reduce the dust and hence mobility of the two spotted spider mite. Water-stressed plants are less tolerant to spider mite damage; hence it is necessary to provide adequate irrigation intervals. Washing of leaves or plants with forceful water through a hose pipe to remove dust may help prevent serious late-season mite infestations, this washes away mites present on the foliage to the ground.

2.7.3 Chemical control

Losses of crops due to insect pests vary between 10% and 30% for major crops (Ferry *et al.*, 2004). Management of agricultural pests over the past half century has been largely dependent on the use of synthetic chemical pesticides for field and post-harvest protection of crops. Different acaricides specially designed for the control of red spider mites are reported to have been used, some of the pesticides include the following chemical groups Organophosphates e.g. Profenofos like Polytrin, Pyrethroids e.g. Bifenthrin like seizer and Avermectins e.g. Abamectin.

Control of spider mites is normally done by use of granular systemic pesticides such as Aldicarb which may not be adequate for the late season pest buildup since the material may fail to be incorporated into the plant tissues. Sprays with Pentac, Dicofol, Tetradifon or Monocrotophos at large volumes per unit area lower mite populations and reduce flower losses (Wilfret *et al.*, 1973). Chemical control of *Tetranychus urticae* Koch is expensive and poses risk to growers, non-target organisms and the environment besides development of pesticide resistance (Motazedian *et al.*, 2012). The pest develops pesticide resistance very rapidly, commonly after few applications of a particular miticide (Nauen *et al.*, 2001). As reported on French bean by Monda *et al.* (2003), the pesticide budget per season tops KES 3,268.00 for 0.1 – 0.2 ha against KES 5,418.00 in net earnings at current exchange rates. Although 86% of farmers are aware of harmful pesticide residues in produce causing high rejection rates, they lack suitable alternative pest management strategies. They also lack information on efficacy of bio pesticides suited for safe crop protection

Potential problems associated with continued long-term use of toxic insecticides include pest resistance and negative impact on natural enemies. In addition, increasing documentation of negative environmental and health impact of synthetic toxic insecticides and increasingly stringent environmental regulation of pesticides (Isman, 2000) have resulted in renewed interest in the development and use of botanical pest management products by agrochemical companies.

2.7.4 Resistant varieties

Roses are of very many types and varieties or cultivars in the world, and it has been observed that even if the two spotted spider mite attacked almost all roses, they seem to prefer the red varieties more compared to the other colours. However, there are no known resistant varieties recorded or reported in roses (Gerardo, 2007).

2.7.5 Integrated pest management

Integrated pest management is defined as a pest management system that utilizes combinations of suitable techniques and methods in a compatible manner as possible and maintains the pest population levels below those causing economic injury. The principle is that selected cultural, biological, and chemical controls will be used together with an appropriate crop variety.

Identifying selective pesticides for IPM programmes is necessary to protect the natural enemies/ beneficial organisms and at the same time reduce environmental pollutants. A low mortality of organic pesticides and products to natural enemies is more important than a high mortality towards the target species. This is because the mode of action of a natural enemy on the target species has to be added to the effect of the selective pesticide. In fact, a selective pesticide should have a low or no mortality towards natural enemies. Moreover, it should degrade rapidly in the environment. At present, very few synthetic pesticides meet this criteria, but pesticides of plant origin seem to be good candidates, as they generally have a very short persistence on the plant (Cabras *et al.*, 2002) and up to now show high selectivity when used as extracts (Ragusa Di Chiara *et al.*, 2007). However, the selectivity of these products has to be strictly evaluated for different species of natural enemies as deleterious or sometimes positive effects were found among the natural enemies complex (Stark *et al.*, 1992).

Spider mite populations in roses can reach epidemic proportions in flower crops grown on the equator. Temperatures that fuel the production of flowers all year-round also

fuel the growth and reproduction of spider mites. While bending roses increases productivity, it also provides a safe haven for spider mites as chemical sprays cannot reach the underside of the leaf therefore use of the predatory mite *Phytoseiulus persimilis* as a biological control for spider mites is ideal because they can walk to the underside and feed on the mites. However this is limited to as long as enough *Phytoseiulus* are applied to the crop in relation to the number of spider mites present, also care is taken to integrate the predator introduction with compatible insecticides and fungicides. (Labuchagne, 2005). Pimentel *et al.*, (1992) reported that because of the problems with resistance to acaricides, it is vital to use integrated management programs whenever possible to maximize the regulatory effects of natural enemies.

2.7.6 Botanical extracts

Aromatic plants contain volatile compounds like essential oils and various plants have different bioactivity against important agricultural insect pests and mites (Wang and Liu, 1994, Park *et al.*, 2002.). Essential oils are naturally occurring terpene mixtures whose bioactivity against specific pests and micro organisms have received attention. These essential oils are generally composed of complex mixtures of monoterpenes, related to phenols and sesquiterpenes e.g. 1, 8-cineole the major constituent oils of *Rosemarinus officinale* commonly known as Rosemary, eugenol from clove oil *Syzygium aromaticum*, *Thymus vulgaris* garden thyme containing thymol (Isman, 2000). Essential oils are also believed to be allelopathic agents or irritants that protect plants from predation by insects and infestation by pathogens. Diterpenoids, saponins, isoflavan, alkaloids, phenolics have been identified as insect feeding deterrents. Presently they are especially recommended for ecological production of crops and small home gardens. Essential oils from accessions of *Lippia sidoides* Cham, were characterised and investigated for their acaricidal activity against *T. urticae* Koch and twenty nine compounds were found to have acaricidal effects (Cavalcanti *et al.*, 2010).

The neem tree has also been widely exploited and commercial formulations of plant extract from the seed kernels of the neem tree –NeemAzal® -T/S was tested against 140 species of mites (*Acari*), *Thysanoptera*, *Homoptera*, *Heteroptera*, *Coleoptera*, *Lepidoptera* and *Hymenoptera*. The producer – Trifolio-M GmbH obtained the first registration for NeemAzal in 1998 mainly for control of sucking arthropod pests such as: spider mites, white flies and aphids on ornamental plants in greenhouses. In addition to neem tree, a wide range

of other tropical plant species e.g.: chinaberry (*Melia* spp.); *Walburgia* spp., *Quillaja saponaria* Molina; *Gmelina arborea* L. were extensively studied for their insect repellent and antifeedant properties (Oparaeke, 2005; Waligóra, 2006). Neem seed kernel extracts and its formulation are reported to influence mortality, repellency, and fecundity of mites Monsuer *et al.*, 1993. It was found out that the two commercial preparations of neem seed extracts (Margosan-0 and Neem Azal S, Neem Azal T/S) were effective on *T. urticae* (Dimentry 1993). Several herbal extracts of *Achillea millefolium* L. (Asteraceae), *Taraxacum officinales* F. H. (Asteraceae), *Matricaria chamomilla* L. (Asteraceae), and *Salvia officinalis* L. (Lamiaceae) demonstrated strong inhibition of the feeding activity of mites (Tomczy *et al.*, 2011). It was determined that the extracts of yew showed a high mortality, decrease in female fecundity and shortened longevity (Furmanowa *et al.*, 2002). Shi *et al.*, 2006 also found that the extract of *Bassia scoparia* (L.) (Chenopodiaceae) showed contact and systemic effects, and it caused high rates of mortality in all the three mite species tested (*T. urticae*, *T. cinnabarinus*, and *T. viennensis*). Pure azadirachtin reduced the reproductive capacity and feeding of *T. urticae*.

In other tests crude foliar extracts of sixty seven species from six subfamilies of Australian Lamiaceae showed both contact and systemic Mortality to these mites (Rasikari *et al.*, 2005). The extracts of wild tomato leaf have also been found to have strong repellency effect on *T. urticae*. Antonious *et al.*, 2006 also tested the acaricidal activities of plant extracts on *T. urticae* and found that the mortalities were high in extracts of *Albizia coreana* Twig. *Pyracantha angustifolia* F. (Rosaceae), and *Ligustrum japonicum* Thunb. (Oleaceae) within 48 hours of treatment Attia *et al.*, 2011 revealed that the extract of garlic led to a rise in female mortality and a reduction in fecundity with the increasing of concentration.

Essential oils of *Artemisia absinthium* L. (Asteraceae) and *Tanacetum vulgare* L. (Asteraceae) were extracted by three methods, a microwave-assisted process (MAP), distillation in water (DW), and direct steam distillation (DSD), and tested for their mortality as contact acaricides on *T. urticae*. Direct steam distillation and distillation in water extracts of *T. vulgare* were more toxic (75.6 and 60.4% mite mortality, respectively, at 4% concentration) to *T. urtica* than to the microwave-assisted process extract (16.7% mite mortality at 4% concentration) (Chiasson *et al.*, 2001). The ethanol extracts of *Croton rhamnifolius* H.B.K. (Euphorbiaceae) *C. sellowi*, *C. jacobinensis*, and *C. micans* had a high

mortality on *T. urticae*, whereas *C. sellowi* extract showed the highest effect (Pontes *et al.*, 2011).

Garlic extracts have also been widely used and in research by Dabrowsky *et al.*, 2007 showed mortality at 48–57% on *T. urticae*. Wang *et al.*, 2007 revealed that the crude extract of walnut leaf had some contact and systemic effect on *T. cinnabarinus* and *T. viennensis*. It was found out that the extract of *V. album* and *T. parthenium* had high mortality rate and reduced fecundity for *T. urticae*. Essential oil extracted from *Tanacetum vulgare* and *Artemisia absinthium* strongly increased mortality of *Tetranychus urticae* (Chiasson *et al.*, 2001). Extracts from: *Matricaria recutita*, *Achillea millefolium*, *Taraxacum officinale*, *Salvia officinalis* have strongly reduced the *T. urticae* fecundity, longevity and intensity of feeding (Kawka and Tomczyk, 2002). Water extracts from needle surface of *Taxus baccata* strongly affected fecundity and oviposition period of two-spotted spider mite females.

Ginger contains a variety of compounds, which have insecticidal, oviposition, antifeedant, growth regulating, reduced fecundity, development modifying properties and repellent activity against many tested insects (Pitasawat *et al.*, 2003; Bandara *et al.*, 2005; Prajapati *et al.*, 2005). Insect activity included Lepidoptera such as *Spilosoma oblique* (Aggarwal *et al.*, 2001), Coleoptera, such as *Callosobruchus maculatus*, the pest of tomatoes, *Bemisia argentifolii* Bellows and Perring (Zhang *et al.*, 2004); the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann; the cowpea aphid, *Aphis craccivora* Koch (Ofuya and Okuku, 1994); neonate larvae of the pest, *Spodoptera littoralis* has been reported by Bambang *et al.*, (1996).

At Mashare ADI (Agricultural Development Institute) in Namibia, Natural (botanical) control methods are also currently being tested for their effectiveness against insect pests. Chilli, garlic and soap extract are being used and a mixture of buttermilk and flour. The results are not available. The Horticultural Section is also collaborating with the GTZ IPM Horticultural Project in several Southern African countries to obtain a bio control for the two spotted spider mite. Botanicals such as Neem (*Azadirachta indica*) and *Tephrosia sp.* are currently being evaluated in Malawi, Zimbabwe and Kenya.

2.8 Plant Species

2.8.1 *Cleome gynandra* (African spider plant)

Cleome gynandra is a branched and rather stout annual herb (Plate 2.4) that grows up to 1 m tall and is common in the tropical and subtropical climatic regions (Elfers, *et al.*, 1964). The plant is used as a vegetable, and are reported to have a high leaf protein content (Imbamba, 1973). The use of African spider plant *Cleome gynandra* L., a semi cultivated vegetable delicacy in Kenya and most East African countries, has been found to have acaricidal properties to the larvae, nymphs and adult of *Rhipicephalus appendiculatus* and *Amblyomma variegatum* ticks (Malonza *et al.*, 1992). This same plant according to Nyalala and Grout 2007, was found to have significant effect in the repellence of the red spider mite.



Plate 2.4: *Cleome gynandra* (African spider plant)

Source; http://commons.wikimedia.org/wiki/File:Cleome_gynandra.jpg

Essential oils from this plant have been used as a repellent against headlice, *Pediculus humanus capitis* (Siphunculata: Pediculidae) and as a general vermicide in hairdressing (Mitchell and Breyer-Brandwijk, 1962; Jacobson, 1975) while the seeds and oil have been reported to be anti-nematode (Usher, 1973). The petroleum ether extract at 2% concentration was reported to cause 100% mortality to insect pests of the cruciferous painted bug, *Bagrada cruciferanum* (Verma and Pandey, 1982). The plant has also been used in traditional medicine for the treatment of rheumatism, headache, epileptic fits, stomachache, conjunctivitis, stiffneck, scurvy, earaches and severe infection of threadworms (Mitchell and

Breyer-Brandwijk, 1962.). The whole plant has been reported to be a fish poison, alkaloids being stipulated to be the active principles (Smolenski, *et al.*, 1975).

2.8.2 *Capsicum frutescence* (Chilli or Hot pepper)

Chilli or hot pepper (Plate 2.5) is a commonly cultivated Solanaceous crop of India. Different cultivars of chilli are being grown for vegetables, spices, pickles, condiments, etc. It is mainly used for its pungency and colouring properties. The hotness or pungency of chilli is due to presence of a group of compounds called capsaicinoids (Estrada *et al.*, 1997). Among capsaicinoids, capsaicin and dihydrocapsaicin are present in major quantities, while homocapsaicin, nordihydrocapsaicin and homo-dihydrocapsaicin are present in very small quantities. (Tewari *et al.*, 2005). The capsaicin is produced by the glands at the junction of placenta and the pod wall, distributed unevenly throughout the pod and found maximum in placental tissues (Rowland *et al.*, 1984). It is a powerful and stable alkaloid which remains unaffected by cold or heat and remains unchanged despite of time, cooking or freezing. It has no colour, flavour or odour



Plate 2.5: *Capsicum frutescence* (Chilli)

Source; <http://www.abundantacres.net>

Dried plants or their extracts have been used by farmers in many developing countries to protect food and fiber from insects. Cowles *et al.*, 1989, reported that chili pepper powder deterred oviposition of the onion fly, *Delia antiqua*. Capsaicin in hot pepper has been reported to reduce larval growth of the spiny bollworm, *Earias insulana* (Weissenberg *et al.*,

1989) and the use of oleoresin from *Capsicum* as a repellent against cotton pests has been reported.

Plants produce a vast array of volatiles that play important role in plant defense (Aharoni *et al.*, 2003) Hot pepper accessions contain significant amounts of tannins. Breakdown products of tannins (phenols) behave as toxins and feeding deterrents. The potential of using hot pepper extracts for controlling spider mites is explored in this study.

2.8.3 *Urtica dioica* (Common Stinging Nettle)

Urtica dioica (Plate 2.6) is a dioecious, erect, perennial herb up to two meters tall, little branched parts with long stinging hairs, hence the name stinging nettle. *Urtica* comprises about 80 species and is almost cosmopolitan, with most species in temperate regions of the northern hemisphere and about 5 in Africa, 2 of which are introduced weeds. *Urtica simensis* Hochst ex A. Rich (Paulina, 2005) *Urtica dioica* L. has been reported from tropical Africa (DR Congo, Ethiopia), as an introduced weed in gardens, but its presence is not confirmed by herbarium specimens.

In South Africa and many other regions of the world, its leaves are used as a vegetable. It is widely used medicinally in treating asthma, allergies, coughs, rheumatism, symptoms of benign prostatic hyperplasia and paralyzed limbs, and has been recommended as a diuretic and antispasmodic and to stimulate hair growth. (Lopatkin *et al.*, 2005).

Stinging Nettle has a flavour similar to spinach when cooked and is rich in vitamins A, C, D, iron, potassium, manganese, and calcium. Young plants were harvested by Native Americans and used as a cooked plant in spring when other food plants were scarce. Nettle leaf is a herb that has a long tradition of use as an adjuvant remedy in the treatment of arthritis in Germany. Nettle leaf extract contains active compounds that reduce TNF- α and other inflammatory cytokines.

Nettles themselves contain a lot of nitrogen and so are used as a compost activator or can be used to make a liquid fertiliser which although somewhat low in phosphate is useful in supplying magnesium, sulphur and iron (Paulina, 2005). Recent experiments have shown that nettles may have some use as a companion plant to repel insect pests. (www.gardenwiseonline.ca).



Plate 2.6: *Urtica dioica* (Stinging nettle)

Source; <http://www.viladetora.net>

CHAPTER THREE

REPELLANCE AND MORTALITY EFFECTS OF CRUDE PLANT EXTRACTS ON TWO-SPOTTED SPIDER MITE (*Tetranychus urticae* Koch)

3.1 ABSTRACT

Laboratory trials were carried out to evaluate the repellence and mortality of crude extracts of *Cleome gynandra*, *Urtica dioica* and *Capsicum frutescence* on the two-spotted spider mite (*T. urticae* Koch) on rose flowers grown under greenhouse. 100g each of the plant extracts were constituted in 1 litre methanol and distilled water separately, and rose leaves at three leaflet stage were immersed in them. Ten *T. urticae* mites were introduced into the treated leaves and observations on repellence and mortality of mites were recorded. The crude extracts on the first day had moderate repellence in the second hour and higher repellence recorded on the third and fourth hours. Moderate and higher mortality was recorded on the third and sixth day after treatment respectively. It was also observed that extracts constituted from methanol had higher repellence and mortality rates compared to those extracts from distilled water. It was also observed that methanol as a solvent has both repellence and toxic effects on the mites.

3.2 INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch is a well known pest of greenhouse roses, and is controlled mainly with synthetic acaricides during the hot and dry seasons. Many studies have indicated the potential ecological damage due to widespread use of synthetic pesticides and the need to safeguard public health. Miticide spray is not satisfactory due to the high costs of miticides, logistics of regular application, development of Miticide-resistant mite strains and an increased residue level dangerous to the consumers and environment. Furthermore, the pressure to consider bio-control and organic production is increased by the enforcement of the European codes of practice for export market aimed at reducing and eventually eliminating the use of synthetic pesticides.

Biological control of two spotted spider mite as an alternative to miticides is being advocated (Labuchagne, 2005). The bio-control using predatory mites *Phytoseiulus persimilis* and *Amblyseius californicus* (Cherian, 2003), is equally expensive and often difficult to implement or sustain and requires a high level of technical skill. Moreover, these predatory mites are highly specific and have a narrow target range which does not eliminate the pest

entirely. They may not be compatible with the use of chemical fungicides or bactericides (Armstrong, 2001).

The use of pesticides and the increasing public concern on environmental pollution and health hazards created by synthetic pesticides generates a great interest. New classes of pest control agents with higher activity against the target pests, and lower impact on humans and environmental quality are advocated (Antonious and Snyder, 2006). Accordingly, there is a need to find an effective pesticide with low mammalian toxicity to control spider mites. The use of plant products for pest control may impart a selective advantage to plants by inhibiting, repulsing, and even killing non-adapted organisms that feed upon, or compete with the plant.

Repellence, which involves pushing pests away from growing plants, has three advantages; reduced reliance on synthetic pesticides, reduced chance for pesticide adverse environmental impacts and reduced pesticide residues on crops reaching the consumers. Plant-derived products have a broad spectrum of activity against insects and mites. Dried plants or their extracts have been used by farmers in many developing countries to protect food and fibre from insects (Antonious and Snyder, 2006). Cowles *et al.*, 1989 reported that chilli pepper powder deterred oviposition of the onion fly, *Delia antiqua*. The use of oleoresin from *Capsicum* as a repellent against cotton pests was also reported.

According to Nas (2004), interest in the application of botanical pesticides for crop protection is on the rise. Many researchers are experimenting and developing alternative plant extracts as pesticides to be used against insect pests. Plants have the richest source of renewable natural pesticides. Specifically, plant extracts provide a safe and viable alternative to synthetic pesticides and are compatible with the use of beneficial organisms, pest-resistant plants, and to preserving a healthy environment.

There are many benefits of using botanical pesticides such as reduced environmental degradation, increased safety for farm workers, increased food safety, reduction in pesticide resistance, and improved profitability of production. As a result, many plant compounds, the majority of which are alkaloids and terpenoids, have now been known to affect insects' behaviour, growth and development, reproduction, and survival (Warthen *et al.*, 1990). Many investigations have recently been performed in relation to effects of plants such as *Chrysanthemum roseum* Web. and Mohr. (Compositae), *Nicotiana tabaccum* L. (Solanaceae), *Derris elliptica* Benth (Fabaceae), neem tree, *Azadirachta indica* A. Juss (Meliaceae), *Melia azaderach* L. (Meliaceae), and *Xanthium strumarium* L. (Solanaceae) on insects (Erdogan

and Toros 2007). The seed kernel extract of neem, known as azadirachtin, has been most thoroughly tested, and it has been extracted in larger quantities than the other components of neem (Schmutterer and Zebitz, 1984). High rates of mortality have been found on the two spotted mites fed on the leaves treated with *A. indica* extract. In addition, the same extract significantly reduced the reproductive capacity of mites and the survival of the progeny of treated females greatly diminished in comparison to the control (Miranova and Khorkhordin 1996).

3.3 MATERIALS AND METHODS

3.3.1 Test plant materials

African spider plant *Cleome gynandra* L. and the common stinging nettle *Urtica dioica* were obtained from cultivated land in Kaptama Division Mount Elgon District. Whole plant samples including leaves, inflorescence and succulent stems were collected. Fresh chilli fruits *Capsicum frutescence* were obtained from the local market, harvested at physiological maturity (showing uniform red colour). Plant materials obtained were collected, cleaned and dried under shade for 3 days at 27°C - 30°C and further oven dried to a constant dry weight at 35°C for 48 hours (Wisniewski, 1999). The dried plant samples were then ground separately to fine powder using a laboratory grinder and divided into 100g portions for the experiments. The treatments were prepared by mixing 100g of each dry herb separately in 1 litre of distilled water to extract polar compounds and methanol to extract the non polar compounds. The extracts were left to stand for 24 hours while stirring from time to time, and then filtered through filter paper (Dabrowsky and Urszula, 2007) to obtain the filtrate which was used in the experiments.

3.3.2 Rearing of test mites

Cultures of *Tetranychus urticae* Koch were obtained locally from wild growing alternate hosts (*Solanum nigrum*) and reared on French beans *Phaseolus vulgaris* Variety 'monnel' at the Fontana Flowers Limited bio-control department tunnels (Plate 3.1). The two spotted mites were then obtained for use in the laboratory and field experiments. Identification of the two spotted spider mite *Tetranychus urticae* was confirmed by Entomologist Prof. Alice W. Kamau. of Egerton University



Plate 3.1: A tunnel with French beans (*Phaseolus vulgaris* variety 'monnel' where mites for the experiments were reared

3.3.3 Experimental design and treatment application

The experiment was laid out in a Complete Randomized Design (CRD) replicated three times with 9 treatments as follows; C = Control (distilled water); CG1 = *Cleome gynandra* (distilled water); CG2 = *Cleome gynandra* (methanol extract); CF1 = *Capsicum frutescence* (distilled water); CF2 = *Capsicum frutescence* (methanol); UD1 = *Urtica dioica* (distilled water); UD2 = *Urtica dioica* (methanol); P = Pesticide (Polytrin); M = Methanol

3.3.4 Methodology for Repellence

The treatments were constituted in distilled water and methanol as denoted by 1 or 2 on the treatment list given above. These were applied by immersing whole leaves at the three leaflet stage in the treatment solutions for 5 seconds to ensure uniform coverage of leaf surface. Ten *T. urticae* adults were introduced from the mass rearing colony with help of a fine Carmel brush onto treated rose leaves and untreated control leaves. The leaves were then placed inside Petri dishes and kept at room temperature in the laboratory.

Data for repellence were collected by counting the number of mites repelled on the leaf 1, 2, 3 and 4 hours after mites were placed on treated leaves.

3.3.5 Methodology for mortality

The treatments were constituted in distilled water and methanol as denoted by 1 or 2 on the treatment notations as given above. Ten *T. urticae* adults were introduced from the mass rearing colony with help of a fine Carmel brush onto the rose leaves. The treatments were applied by spraying the leaves at the three leaflet stage with the treatment solutions on both sides to ensure uniform coverage of leaf surface inside the Petri dishes and kept at room

temperature at 12hrD/12hrN photoperiod in the laboratory. A Vaseline ring was applied around the Petri dish to stop the mites from escaping.

Data were collected by counting the number of dead mites after one, three and six days (Dabrowsky and Urszula, 2007) under a laboratory light microscope. Mites were gently probed to ascertain mobility or not, if they did not move they were considered dead.

3.3.6 Data analysis

All parameters measured were subjected to Analysis of Variance (ANOVA) using GenStat Release fifteenth edition version 15.1.0.8035 Copyright 2012, VSN International Ltd. (Rothamsted Experimental Station). All means were separated using Least Significance Difference (LSD).

3.4 RESULTS

3.4.1 Repellent effects of crude plant extracts on *T. urticae*

The results summarised in table 3.1 below show average number of *T. urticae* repelled on the rose leaf (three leaflet stage) treated with crude plant extracts. It showed that the number of mites repelled differed significantly ($p \leq 0.05$). In the first hour the leaves treated with methanoic extracts of *Cleome gynandra* were observed to repel the highest number of mites significantly different from all other treatments. The lowest number of mites repelled on the leaves in the first hour was observed on the control (distilled water) treatment and was not significantly different from methanol, Polytrin and the distilled water extract of *Urtica dioica*.

In the second hour *Cleome gynandra* methanoic extract had the highest number of mites repelled on the leaves and was not significantly different from the distilled water extract of the same plant. The methanol extract of *Urtica dioica* was not significantly different from the distilled and methanol extract of *Capsicum frutescence* and distilled water extract of *Urtica dioica*. The control treatment had the lowest number of mites repelled on the leaves but was not significantly different from polytrin and methanol. Three hours after treatment methanoic extract of *Cleome gynandra* was observed to have the highest mites repelled from the leaf which was not significantly different from the distilled water extracts of *Cleome gynandra*. However, CG1 was not significantly different from CF1 and UD2. In the same hour polytrin treatment was observed to have the lowest number of mites repelled on the leaves and was not significantly different from control, methanol, UD2, UD1 and CF2. The fourth hour after treatment, methanol extract of *Cleome gynandra* was observed to have the highest number of mites repelled from the leaves and was not significantly different from *Cleome gynandra* distilled water extract. The lowest number of mites was observed on polytrin which was not significantly different from methanol, control, UD1 and CF2. The lowest percentage of mites repelled was recorded on CG2 (83.33%) followed by CG1 (60%) and the least percentage repelled recorded on polytrin (16.70%). (Figure 3.1)

Table 3.1: Average number of *T. urticae* repelled per rose leaf treated with crude plant extracts

Treatment	1 Hr	2 Hr	3 Hr	4 Hr	% Repelled at 4 Hrs
CG1	2.67 ^b	5.00 ^a	5.67 ^{ab}	6.00 ^{ab}	60.00
CG2	4.00 ^a	6.00 ^a	7.00 ^a	8.33 ^a	83.30
CF1	2.33 ^{bc}	2.67 ^b	4.33 ^{bc}	5.33 ^{bc}	53.30
CF2	1.67 ^{cde}	2.67 ^b	3.33 ^{cd}	3.33 ^{cd}	33.30
UD1	1.33 ^{def}	2.67 ^b	3.00 ^{cd}	3.33 ^{cd}	33.30
UD2	2.00 ^{bcd}	3.00 ^b	3.67 ^{bcd}	4.67 ^{bc}	46.70
Polytrin	1.00 ^{ef}	1.67 ^{bc}	1.67 ^d	1.67 ^d	16.70
Methanol	1.00 ^{ef}	2.33 ^{bc}	3.00 ^{cd}	3.33 ^{cd}	33.30
Control	0.67 ^f	1.00 ^c	2.33 ^{cd}	3.00 ^{cd}	30.00
LSD (0.05)	0.88	1.40	2.09	2.44	
CV (%)	27.50	26.90	32.00	32.50	

Means in the same column followed by the same letter are not significantly different at $P \leq 0.05$ using LSD

KEY

C = Control (distilled water); CG1 = *Cleome gynandra* (distilled water); CG2 = *Cleome gynandra* (methanol extract); CF1 = *Capsicum frutescence* (distilled water); CF2 = *Capsicum frutescence* (methanol); UD1 = *Urtica dioica* (distilled water); UD2 = *Urtica dioica* (methanol); P = Pesticide (Polytrin); M = Methanol

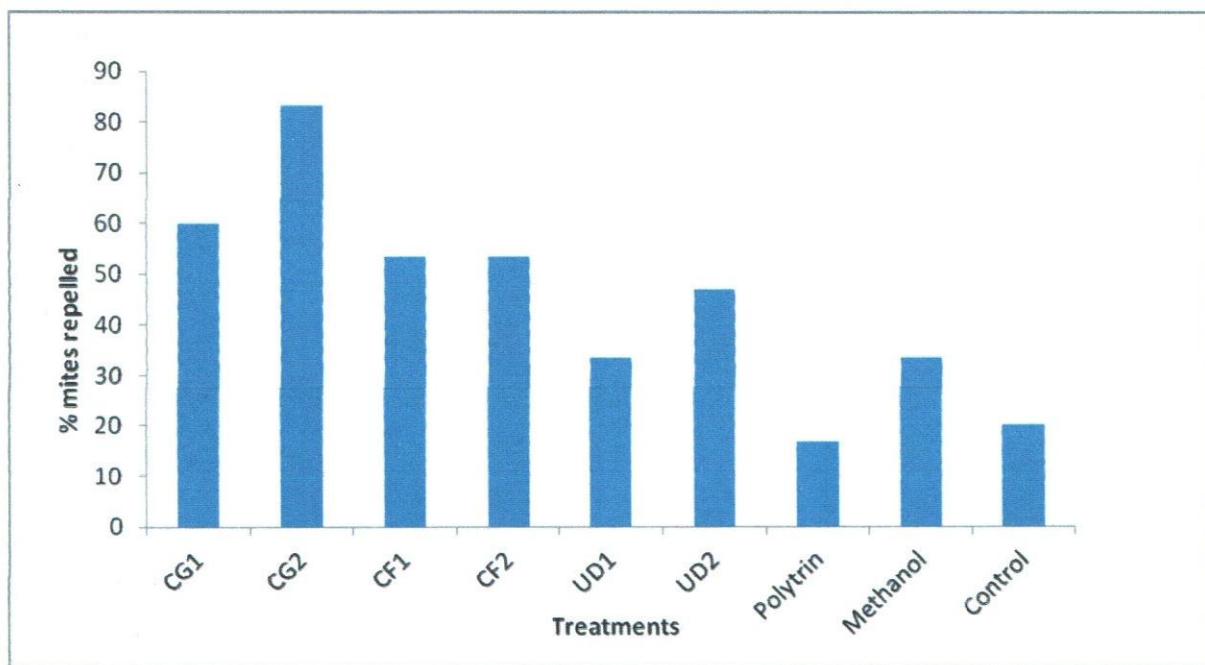


Figure 3.1: Percentage mites repelled four hours after introduction

KEY

C = Control (distilled water); CG1 = *Cleome gynandra* (distilled water); CG2 = *Cleome gynandra* (methanol extract); CF1 = *Capsicum frutescence* (distilled water); CF2 = *Capsicum frutescence* (methanol); UD1 = *Urtica dioica* (distilled water); UD2 = *Urtica dioica* (methanol); P = Pesticide (Polytrin); M = Methanol

3.4.2 Toxic effects of crude plant extracts on *T. urticae*

The results summarised in table 3.2 below show average number of *T. urticae* count killed on the rose leaf (3 leaflet stages) after spraying with crude plant extracts. It showed that the number of mites killed differed significantly at $P \leq 0.05$.

On the first day, significantly highest (7.0) number of dead mites was observed on leaves treated with Polytrin. The Methanol extract of *Cleome gynandra* had the second highest number of dead mites (2.67) and was not significantly different from the CG 1, methanoic and distilled water extracts of CF, UD and methanol. The lowest number of mites killed was recorded on the control treatment which was not significantly different from the methanol and distilled water extracts of *Urtica dioica*.

On the third day, Polytrin was observed to have the highest number of mites killed and was not significantly different from the methanoic extract of *Cleome gynandra*. This was followed by the distilled water extract of *Cleome gynandra* which was not significantly different from methanol, distilled water and methanol extracts of *Capsicum frutescence*, and *Urtica dioica*. The lowest number of mites killed was observed on the control treatment which was not significantly different from the distilled water extract of *Urtica dioica*.

Cleome gynandra methanoic extract was observed to have the highest (8.0) number of mites killed on the sixth day and was not significantly different from Polytrin. This was followed by *Cleome gynandra* distilled water extract which was not significantly different from methanol, polytrin and methanol extract of *Urtica dioica*. The lowest number of mites killed was observed on control which was not significantly different from the distilled water extract of *Urtica dioica*.

Cumulatively after six days *Cleome gynandra* methanoic extract had the highest (80%) percentage of mite mortality followed by Polytrin at 73%. The lowest mortality was recorded on the control treatment with 13% followed by *Urtica dioica* distilled water extracts at 33 %.(Figure 3.2). Methanol as a treatment had slightly over 50% of the mites killed which indicates that methanol as a solvent in the plant extract constitution may have contributed to mite mortality.

Table 3.2: Average number of *T. urticae* killed per rose leaf treated with crude plant extracts

Treatment	Day 1	Day 3	Day 6	% Mortality
CG1	2.00 ^b	3.67 ^b	5.67 ^{bc}	56.70
CG2	2.67 ^b	6.00 ^a	8.00 ^a	80.00
CF1	2.33 ^b	3.67 ^b	4.00 ^{cd}	40.00
CF2	2.33 ^b	3.33 ^b	5.00 ^{cd}	50.00
UD1	1.33 ^{bc}	2.00 ^{bc}	3.33 ^{de}	33.30
UD2	1.67 ^{bc}	3.33 ^b	5.33 ^{bcd}	53.30
Polytrin	7.00 ^a	7.00 ^a	7.33 ^{ab}	73.30
Methanol	2.33 ^b	3.67 ^b	5.33 ^{bcd}	53.30
Control	0.33 ^c	1.00 ^c	1.33 ^e	13.30
LSD (0.05)	1.53	1.93	2.03	
CV (%)	36.10	29.80	23.20	

Means in the same column followed by the same letter are not significantly different at $P \leq 0.05$ using LSD

KEY

C = Control (distilled water); CG1 = *Cleome gynandra* (distilled water); CG2 = *Cleome gynandra* (methanol extract); CF1 = *Capsicum frutescence* (distilled water); CF2 = *Capsicum frutescence* (methanol); UD1 = *Urtica dioica* (distilled water); UD2 = *Urtica dioica* (methanol); P = Pesticide (Polytrin); M = Methanol

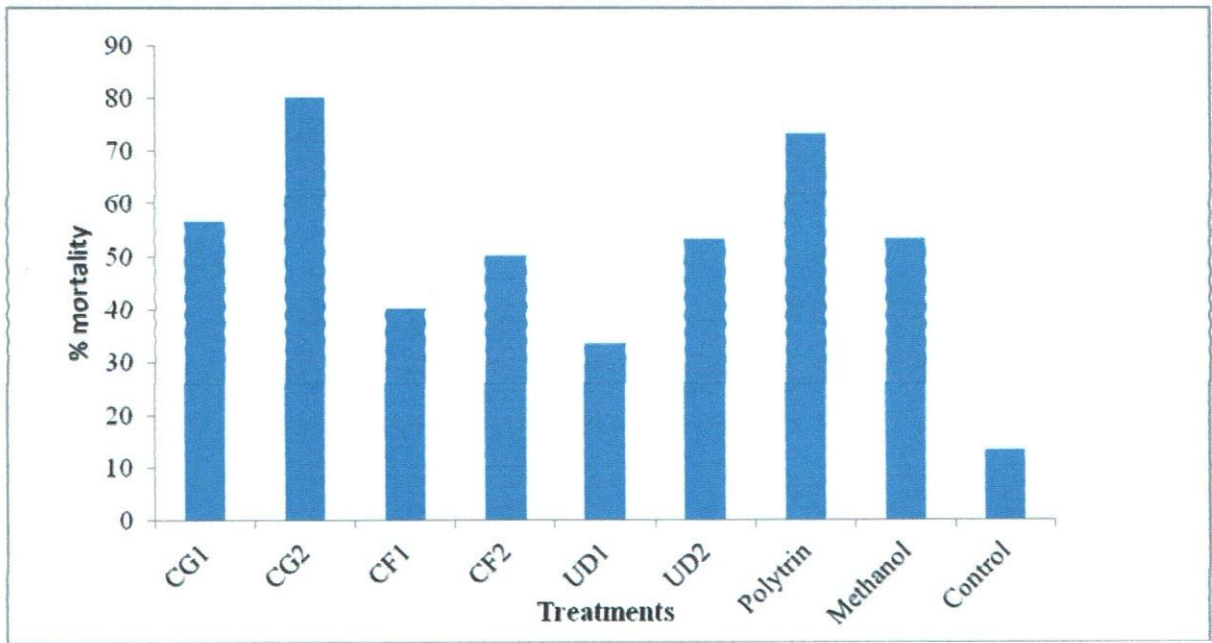


Figure 3.2: Cumulative *T. urticae* mortality per rose leaf treated with crude plant extracts

KEY

C = Control (distilled water); CG1 = *Cleome gynandra* (distilled water); CG2 = *Cleome gynandra* (methanol extract); CF1 = *Capsicum frutescence* (distilled water); CF2 = *Capsicum frutescence* (methanol); UD1 = *Urtica dioica* (distilled water); UD2 = *Urtica dioica* (methanol); P = Pesticide (Polytrin); M = Methanol

3.5 DISCUSSION

3.5.1 Repellent effects of crude plant extracts on *T. urticae*

The first three hours results showed that *Cleome gynandra*, methanol and distilled water extracts repelled lower number of mites. However the same treatment repelled higher numbers of mites by the fourth hour figure (3.1). These results indicate that these extracts are slow release repellents and work more effectively with exposure time. However the results contradict with those made by Saeedah *et al.* (2012) who in an experiment to test four methanoic extracts of *Anisosciadium orientale.*, *Scaligeria meifolia* Boiss, *Trigonella elliptica* Boiss and *Ptelea viscosa* L against the two-spotted spider mite observed that the lethal concentration of plant extracts faded within two or three days. In other closely related experiments neem extracts (*Azadirachtin indica*), *Allium sativum* and *Urtica dioica* were found to significantly inhibit the feeding characteristic of mites (Dabrowsky and Urszula, 2007). These sentiments are in tandem with the findings of this experiment which finds the methanoic extracts of *Cleome gynandra* to be potentially capable of keeping a check on the two-spotted mites.

The effectiveness of *Cleome gynandra methanol* extract as a repellent may also be attributed to *Gynandropsis gynandra* constituents of the essential oils which were evaluated against the livestock tick, *Rhipicephalus appendiculatus* and was found to repel ticks (Lwande *et al.*, 1999) which belong to the same class (Acari) as the two spotted spider mite. Nyalala *et al.*, 2010 also conducted trials to assess the bioactivity of Acetonitrile (methyl cyanide) emitted by *Gynandropsis gynandra* against spider mites and concluded that it showed a very strong indication that foliar emission of Acetonitrile by *Gynandropsis gynandra* depending on the concentrations could be repellent or toxic to spider mites. Hence, could be responsible to a significant degree, for the spider mite repellent activity of the plant when used as an intercrop with roses.

Adequate watering of plants during dry conditions can limit drought stress on mite infestation (Cranshaw and Scalar, 2014) Periodic hosing of rose plants with forceful jets of water can physically remove or dislodge and kill mites. This practise is also common with commercial rose growers to wash off mites and create a moist environment hence rendering mites immobile and environment uncondusive for their survival. This explains the low average number of mite count in the first and second hours of treatment application.

Variable levels of mortality have been reported in a number of other plants extracts. Amsalingam *et al.* (2010) revealed that neem kernel aqueous extract (NKAE) could cause more than 90 percent mortality on tea red spider mite compared to pongam kernel aqueous extract (PKAE) and garlic aqueous extract (GAE). These findings documented that all the adult mites were killed within four days, which conform to findings of this research. They also noted that the level of oviposition was significantly reduced by a range of 80 to 100 percent.

In other findings, Saeedah *et al.* 2013 showed that plant extracts from *S. meifolia* caused high ovicidal activity (45.84%) followed by *A. orientale* (41.40%), *T. elliptica* (40.11%) and *P. viscosa* (37.66%). They also indicated that the methanoic extracts of *A. orientale*, *S. meifolia*, *T. elliptica* and *P. viscosa* had high potential to be adopted as a management strategy for mites. These earlier findings corresponds with the results of this study and indicates a high potential of adopting methanoic extracts of *Cleome gynandra* and *Urtica dioica* as a control measure of the two-spotted mite in roses. The analysis done on a number of these plant extracts have revealed striking attributes geared towards the development of an environmentally safe strategy in dealing with crop pests. Chiasson *et al.*, (2001) showed that β -thujone, the major compound of the oil (more than 87.6%) of *T. vulgare* extracts could be the contributing factor to the acaricidal activity of this plant. Isman (2008) also demonstrated the lethal role of various plant oils on tobacco cutworm (*Spodoptera litura*) and the green peach aphid (*Myzus persicae*). These results were significantly important in developing pesticides which are free of environmental and human risks.

3.5.2 Toxic effects of crude plant extracts on *T. urticae*

Plant extracts had direct and slow contact mortality on the two spotted spider mites (Figure 3.2); shown through the cumulative adult insect mortality over the six days period. Polytrin Miticide, which attained approximately 70% kill on the first day to sixth day, indicates that Polytrin as a miticide kills within the first day of contact and has very minimal residual effect in comparison to the crude extracts which continued to cause reduced mite populations even after the second day. The crude extracts were slow acting achieving up to 80% kill cumulatively on the 6th day as observed in the CG2 extract, 56% in CG1, 53% in UD2 and Methanol. Similar results on mortality of plant extracts against the two-spotted spider mites have been reported. Yanar *et al.*, (2011) observed that plant extracts of *Lolium perenne* L., *Anthemis vulgaris* L. and *Chenopodium album* L. had significantly higher mortality rates than

azadirachtin (10 g/L) and the synthetic pesticides tested at 5% concentration in adhesive tape and residual film method. Furthermore, Tomczyk and Suszko, (2011) also reported significant acaricidal effects of the common sage (*Salvia officinalis* L.) and German chamomile (*Matricaria chamomilla* L.) plant extracts on the mortality of adult mites. They however noted reduced mortality rates from the German chamomile compared to the common sage. These findings indicate the hidden potential of plant extracts that could be harnessed to control various insect pests in flower production.

Other research also conducted using plant extracts have been investigated and the findings for *T. urticae* are similar to those of this study. Neem seed kernel extracts and its formulation are reported to influence mortality, repellency, and fecundity of mites (Monseuer *et al.*, 1993, Dimetry *et al.*, 1993). It was found out that the two commercial preparations of neem seed extracts (Margosan- 0 and Neem Azal S, Neem Azal T/S) were effective on *T. urticae* (Miranova *et al.*, 1996). Several herbal extracts of *Achillea millefolium* L. (Asteraceae), *Taraxacum officinales* F. H. (Asteraceae), *Matricaria chamomilla* L. (Asteraceae), and *Salvia officinalis* L. (Lamiaceae) demonstrated strong inhibition of the feeding activity of mites (Kawka and Tomczyk, 2002). It was determined that the extracts of yew showed a high toxicity, decrease in female fecundity and shortened longevity (Furmanowa *et al.*, 2002). Shi *et al.*, 2006 revealed that the extract of *Bassia scoparia* (L.) A. J. Scott. (Chenopodiaceae) showed contact and systemic effects, and it caused high rates of mortality in all the three mite species (*T. urticae*, *T. cinnabarinus*, and *T. viennensis*) while pure azadirachtin reduced the reproductive capacity and feeding of *T. urticae* (Sundaram *et al.*, 1995). The extracts of wild tomato leaf in an experiment by (Antonious *et al.*, 2006) which tested the acaricidal activities of plant extracts on *T. urticae* showed strong repellency effect on *T. urticae*. The mortalities were high in extracts of *Albizia coreana* Twig. *Pyracantha angustifolia* F. (Rosaceae), and *Ligustrum japonicum* Thunb. (Oleaceae) within 48 hour treatment. Attia *et al.*, 2011 revealed that the extract of garlic led to a rise in female mortality and a reduction in fecundity with the increasing of concentration. Essential oils of *Artemisia absinthium* L. (Asteraceae) and *Tanacetum vulgare* L. (Asteraceae) were extracted by three methods; a microwave assisted process (MAP), distillation in water (DW), and direct steam distillation (DSD), and tested for their toxicity as contact acaricides against *T. urticae*. DSD and DW extracts of *T. vulgare* were more toxic (75.6% and 60.4% mite mortality, respectively at 4% concentration) while MAP extract attained (16.7% mite mortality at 4% concentration) (Chiasson *et al.*, 2001). The ethanol extracts of *Croton rhamnifolius* H.B.K.

(Euphorbiaceae) *C. sellowi*, *C. jacobinensis*, and *C. micans* had a high toxicity on *T. urticae*, whereas *C. sellowi* extract showed the highest effect (Pontes *et al.*, 2011). Garlic extract showed a toxicity at 48– 57% on *T. urticae*. Wang *et al.*, (2007) revealed that the crude extract of walnut leaf had some contact and systemic effect on *T. cinnabarinus* and *T. viennensis*. It was found out that the extract of *V. album* and *T. parthenium* had high rate mortality and reduced fecundity for *T. urticae*. Ethanolic extracts of *V. album* and *T. parthenium* can be useful to control *T. urticae* populations on vegetable plants grown through Integrated Pest Management (IPM) and organic systems of agriculture.

CHAPTER FOUR

EFFECTS OF PLANT EXTRACTS ON TWO-SPOTTED SPIDER MITE (*T. urticae* Koch) POPULATION, DAMAGE AND YIELD OF ROSES

4.1 ABSTRACT

Trials were carried out to evaluate the effect of crude plant extracts of *Cleome gynandra*, *Urtica dioica*, and *Capsicum frutescense* on the two-spotted spider mite (*Tetranychus urticae* Koch) population, damage and yield on rose flowers grown under greenhouse. Rose bush variety, 'High and Delicate' was first planted after which the bushes were allowed to grow and establish for three months before the mites were introduced into each plant and allowed to establish for 45 days before the different treatments were applied. The experiment was laid out in a Completely Randomised Design (CRD) with nine treatments *Cleome gynandra* (CG), *Urtica dioica*, (UD), *Capsicum frutescense* (CF), and their combinations CG+CF, CG+UD, CF+UD, CF+UD+CG, Polytrin and Control, replicated three times. Extracts were constituted in methanol and sprayed on the experimental plots. Results showed that the crude extracts had a significant effect on the population of mites. The total lowest mite count was observed on polytrin followed by CG. At the end of the experiment it was observed that the highest mite population was observed on control followed by CF+UD and CF. The combination treatment of CG+UD+CF, CG and UD had the longest vase life days of between 12-13 days on an average. The yield of roses from week one to week four in the CF +CG + UD and polytrin treatments showed superior quality in terms of length and head size which are the main qualities used to determine flower prices in the markets. The longest lengths with large or big heads sell very expensively.

4.2 INTRODUCTION

Mites are one of the most important pests of roses grown in greenhouses throughout the world. Over 40% of the total pest and disease management cost for greenhouse cut roses was attributed to the control of two-spotted spider mites (*Tetranychus urticae* Koch). They are a major pest threat to the increased production of high quality roses in Kenya and other rose growing countries (Josvold and Chaney, 2001). This pest also commonly known as the two spotted spider mite (TSSM) feeds by attacking the leaf cells and piercing them to extract the cell contents which leads to the collapse of the plant cells, spider mites are significant

pests because they damage leaves and thereby cause loss of photosynthetic capacity. Their infestation can lead to foliage loss and plant death in extreme cases.

Spider mites have a wide host range, and can be introduced into a greenhouse from other crops or develop on weed species and subsequently move on to roses. They are tiny and often hide on the undersides of leaves, thereby escaping early detection. They have tremendous reproductive capacity in warm and dry conditions, and can rapidly develop resistance to pesticides. High infestation may cause severe necrosis and eventual leaf drop due to leaf desiccation (Nyalala and Grout, 2007). Cut roses are grown mainly for their aesthetic value and therefore, the market tolerance for any damage is low or even zero in European market destinations.

The two spotted spider mites multiply both sexually and asexually completing their life cycle within 24 days (Cherian, 2003) depending on the temperature. This pest thrives well in conditions that growers strive to achieve which are 60 % to 70% relative humidity and 25° C to 28° C, hence making them difficult to control completely.

Miticides have been used discriminatively by rose growers to control *T. urticae*, and this amounts to 40% of pesticide volume applied to rose flower crops (Josvold and Chaney, 2001) and accounts for 25 % to 50% of the total cost of pest control depending on the rose variety, season and region (temperate or tropics). Miticide sprays is not satisfactory due to the high costs of miticides, logistics of regular application, development of Miticide-resistant mite strains and an increased residue level dangerous to the consumers and environment. Further, the pressure to consider bio-control of mites and organic rose production is enhanced by enforcement of the European codes of practice for export market aimed at reducing and eventually eliminating the use of synthetic pesticides.

Biological control of two spotted spider mites as an alternative to miticides is being advocated (Labuchagne, 2005). The bio-control using predatory mites *Phytoseiulus persimilis* and *Amblyseius californicus* (Cherian, 2003), is equally expensive and often difficult to sustain, implement and requires a high level of technical skill. Moreover, these predatory mites are highly specific and have a narrow host range thereby not eliminating the pest entirely. They may also not be compatible with the use of chemical fungicides or bactericides (Armstrong, 2001). Currently the practice which farmers use to control the two spotted spider mite is the use of *Phytoseiulus persimilis*.

Traditional methods of control using local plant extracts have been tried. Nyalala and Grout (2007) intercropped *Cleome gynandra* L. with rose crop and concluded that the treatment had a significant repellent effect to *T. urticae*. Integrated Pest Management systems using a combination of predatory mites, soapy water solutions chilli and garlic are being used by farmers although they have not been validated. Knapp and Kahenge 2003 found that neem formulations controlled two-spotted spider mites on tomatoes. Dabrowsky and Urszula, 2007 concluded that various plant extracts had insecticidal repellent and acaricidal properties.

4.3. MATERIALS AND METHODS

4.3.1 Experimental site description

The research experiment to study crude plant extracts efficacy against two spotted spider mites was carried out at the Fontana flowers limited (Akina farm), which is located on the Njoro- Mau-Narok road 0° 20'S 35° 56'E, at an altitude of 2,180 m. a.s.l. The ecozone is lower Highland 3 (LH3) with an average temperature of 27°C, conducive for the production of cut rose flowers for export. The experiment was carried out in tunnels with greenhouse polyfilm covering containing anti drip properties, UV and infra red properties ideal for rose growing. (Plate 4.1) The growing media was artificial pumice stone mulched with sawdust filled on a 1m × 0.75m trough.

4.3.2 Crop establishment

Planting of the initial rose bushes was done manually three months prior to the introduction of mite colonies to allow for rose crop establishment and training for uniformity. Cultural practices namely bending, fertigation and weeding were applied uniformly. Drip lines were placed in between rows in the beds with emitter spacing similar to the intra plant spacing. Irrigation was done at 5 minute intervals for 12 cycles a day.

4.3.3 Mite pest introduction

Introductions of the two-spotted spider mites were done three months after planting (the first flush or harvest) for uniformity. The mites were allowed to multiply and establish for 45 days after introduction (Wekesa *et al.*, 2005). Sampling for the mites was done before treatments started. Mite density was estimated by selecting three leaves per rose plant; one from top and another from middle (Wekesa *et al.*, 2005) and another from the bended plant material. All mobile stages of mites and eggs were counted using a magnifying glass.

4.3.4 Experimental design and treatment application

Six plants were randomly assigned for each treatment, replicated three times in Completely Randomised Design (CRD) at a spacing of 2 metres between blocks. A spacing of 1m between plots and intra row and inter row spacing of 18cm and 20cm respectively were applied. The plants were planted on artificial media-(pumice stones) mulched with sawdust in troughs and drip irrigation system was used. The treatments were applied using a 1 litre hand sprayer. Common cultural practices like bending, irrigation, fertigation and weeding were uniformly applied to all the treatments. Treatments were as follows; Control (C) (distilled water), *Cleome gynandra* (CG), *Capsicum frutescence* (CF), *Urtica dioica* (UD), *Cleome gynandra* + *Capsicum frutescence* (CG+CF), *Cleome gynandra* + *Urtica dioica* (CG+UD), *Capsicum frutescence*+ *Urtica dioica* (CF+UD), *Capsicum frutescence* + *Urtica dioica*+ *Cleome gynandra* (CF+UD+CG), Pesticide (P) (Polytrin), and Methanol (M)



Plate 4.1: Experimental field layout

4.3.5 Parameters measured

a) Population density

The population density of *T. urticae* was recorded before treatments were applied as the pre-treatment and second, third and fourth weeks after treatment applications were conducted. Three sample leaves were randomly selected from each plant and the mites and eggs counted to give an average number of mites per plant (Wekesa *et al.*, 2005). Plants were sprayed immediately after pre-treatment sampling. Subsequent sprays were done once a week for four weeks.

b) Damage and quality

The aesthetic appearance and severity of damage was measured using a scale of 1-5 (Kamau, 1985)

1. No leaf damage due to mite feeding (Plate 4.2)
2. Slight damage; a few leaves showing slight yellowing and whitening due to mite feeding (punctures) (25% of the leaves damage due to mite attack) (Plate 4.3)
3. Moderate damage; many leaves showing yellowing and whitening due to mite feeding (punctures), (50% of the leaves damaged due to mite attack) Moderate symptoms of mite attack (Plate 4.4)
4. Severe damage; 75% of the leaves showing yellowing, whitening, desiccation, defoliation and webbing due to mite feeding (Plate 4.5)
5. Very severe damage; webbing, severe defoliation, death of leaves and entire plant (100% of leaves damaged due to mite attack) (Plate 4.6)



Plate 4.2: No damage

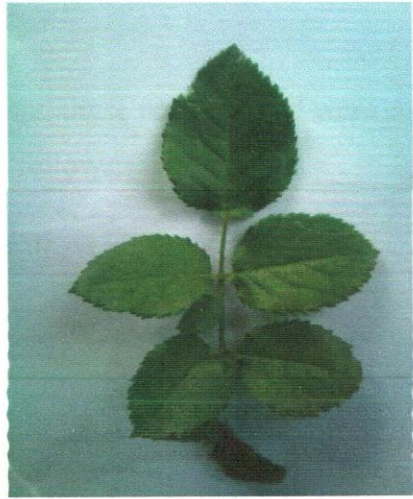


Plate 4.3: Slight damage

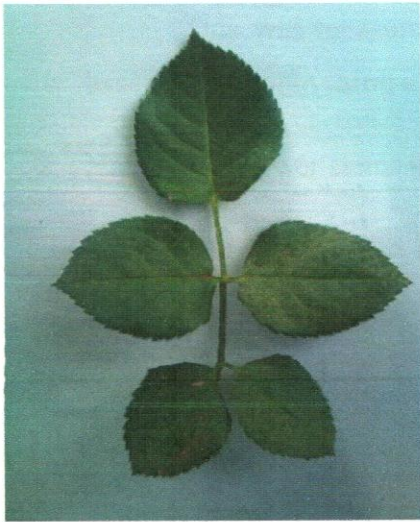


Plate 4.4: Moderate damage

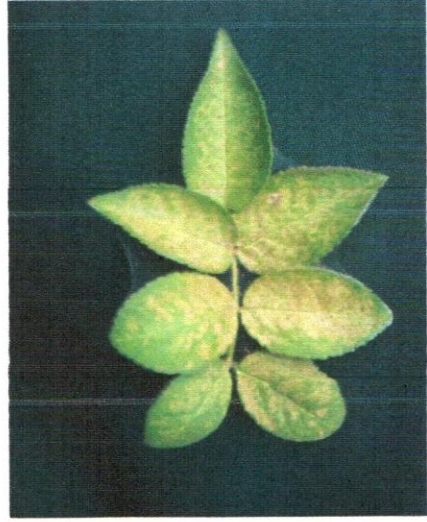


Plate 4.5: Severe damage



Plate 4.6: Very severe damage

c) Percentage leaves infested

All leaves showing symptoms of damage and those not damaged were counted to obtain the percentage leaves infested as follows;

$$\% \text{ leaves infested} = \frac{\text{No. of Infested leaves}}{\text{Total No. Leaves}} \times 100$$

d) Yield

Data collected for yield parameters during the study were as follows;

1. The flower stem length

This was measured in centimetres from the base at the harvest point to the base of the bloom using a metre rule or ruler.

2. Number of flower stems per plant

The flower stems harvested from the plant were counted.

3. Bloom size (Head size).

The height and width (diameter) of the longest point of the bloom was measured to ascertain its size in relation to the stem length

4. Chlorophyll content.

Three leaves were randomly selected and a chlorophyll meter used to measure the chlorophyll content of the plant

e) Vase life

The number of days to wilting of the flower in the vase (Senescence symptoms used were; change in colour/pigmentation, petal drop, petal blackening, and bent neck/drooping.) were recorded for each stem.

Stems from the treatment plots were harvested at cut stage two i.e. when the buds showed a pin sized hole on the tip of the bud. They were then immediately put in buckets containing post-harvest solution (calcium chloride + Aluminium sulphate) as a pulsing solution and transported to the cold stores at 4-6 °C to dissipate field heat, for a period of 12 hours (overnight). The flowers were then graded to equal lengths of 52cm and taken through transport freight simulation where flowers are packed in a standard carton box and stored at

2-4°C for 5 days. Recovery of the stems is then done on the fifth day by cutting the base of the stems 2cm and placing them in post-harvest solution containing chrysal solution (Sugar + bactericide + citric acid). The flowers were then displayed in shelves for 4 more days to simulate the supermarket display phase. The flowers were then put in the vase on the tenth day and the number of days to fifty percent flower termination was recorded.

4.3.6 Data analysis

All parameters measured were subjected to Analysis of Variance (ANOVA) using GenStat Release fifteenth edition version 15.1.0.8035 Copyright 2012, VSN International Ltd. (Rothamsted Experimental Station). All means were separated using Least Significant Difference (LSD). Percentage leaf infestation data was subjected to transformation using Vassar statistical package (website for statistical computations) www.vassarStats.net copyright @Richard Lowry 1998-2015

4.4 RESULTS

4.4.1 Effects of crude plant extract on *T. urticae* population

The results summarised in table 4.1 showed that the population of mites generally declined from week one to week four for all treatments except control. One week after treatment application there was no significant difference in the population of mites amongst the treatments, except the control which recorded a significantly ($P < 0.05$) higher number of mite population. The lowest number of eggs in the first week was observed on CF+UD+CG which was significantly different from UD and the control. The highest number of eggs was observed on control which was significantly different from all the treatment.

The second week of treatment, Polytrin was observed to have the lowest number of mites which was not significantly different from CG. The highest number of mites was recorded on control which was significantly different from all the other treatments. Polytrin was observed to have significantly lower number of eggs. CG, UD and CF+UD+CG was also observed to have no significant difference from CF+UD and CG+UD. The highest number of eggs was observed on control which was significantly different from all the other treatments.

After the third week of treatment, Polytrin had the lowest mite population and was significantly different from all treatments; followed by CG which was statistically similar to CF+UD and CF+UD+CG. The highest number of mites was recorded on control which was significantly different from all the treatments. During the same week of treatment application, Polytrin recorded the lowest number of eggs, significantly different from all treatments. The control had significantly the highest number of eggs.

In the last week of treatment polytrin was observed to have the lowest number of mites significantly different from all the other treatments. The highest mite population was observed on control which was significantly different from the rest of the treatments (Figure 4.1 and 4.2)

Table 4.1: Average number of *T. urticae* (adults, nymphs and larvae) and eggs per 3 leaf discs (d=10mm) per week after treatment

Treatment	Pre-					Pre-				
	Treatment	week 1	week 2	week 3	week 4	Treatment	week 1	week 2	week 3	week 4
CG	11.33 ^b	8.00 ^a	5.67 ^a	6.33 ^b	6.67 ^b	8.00 ^{ab}	7.00 ^{ab}	4.00 ^b	4.33 ^b	4.33 ^b
CF	10.33 ^b	8.67 ^a	8.00 ^{bc}	9.00 ^d	8.33 ^c	7.67 ^a	7.33 ^{ab}	6.33 ^c	5.67 ^b	5.00 ^{bc}
UD	11.33 ^b	9.67 ^a	7.67 ^{bc}	8.00 ^{cd}	8.00 ^{bc}	8.00 ^{ab}	9.00 ^b	4.00 ^b	4.67 ^b	4.67 ^{bc}
CG+CF	10.00 ^{ab}	8.00 ^a	7.00 ^b	8.33 ^{cd}	8.00 ^{bc}	8.67 ^{ab}	6.33 ^a	4.67 ^{bc}	5.67 ^b	5.33 ^c
CG+UD	10.33 ^b	9.00 ^a	8.33 ^c	8.33 ^{cd}	7.00 ^{bc}	8.67 ^{ab}	7.33 ^{ab}	5.33 ^{bc}	4.33 ^b	4.33 ^b
CF+UD	8.33 ^a	8.33 ^a	7.67 ^{bc}	6.67 ^b	8.33 ^c	7.33 ^a	6.67 ^{ab}	5.33 ^{bc}	4.67 ^b	4.67 ^{bc}
CF+UD+CG	10.00 ^{ab}	7.33 ^a	7.67 ^{bc}	7.33 ^{bc}	7.67 ^{bc}	8.33 ^{ab}	6.00 ^a	4.00 ^b	5.00 ^b	4.67 ^{bc}
Polytrin	11.00 ^b	8.33 ^a	5.00 ^a	4.33 ^a	5.00 ^a	8.33 ^{ab}	6.67 ^{ab}	1.67 ^a	2.67 ^a	1.33 ^a
Control	11.00 ^b	15.33 ^b	20.33 ^d	28.00 ^e	21.33 ^d	9.33 ^b	25.33 ^c	29.00 ^d	29.00 ^c	18.67 ^d
LSD (0.05)	1.88	2.45	1.11	1.24	1.56	1.38	2.53	1.75	1.37	1.00
CV %	10.40	15.40	7.50	7.40	10.10	9.60	16.10	14.20	10.80	9.80

Means in the same column followed by the same letter are not significantly different at $P \leq 0.05$ using LSD

KEY

Control (C) (distilled water), *Cleome gynandra* (CG), *Capsicum frutescense* (CF), *Urtica dioica* (UD), *Cleome gynandra* + *Capsicum frutescense* (CG+CF), *Cleome gynandra* + *Urtica dioica* (CG+UD), *Capsicum frutescense* + *Urtica dioica* (CF+UD), *Capsicum frutescense* + *Urtica dioica* + *Cleome gynandra* (CF+UD+CG), Pesticide (P) (Polytrin), and Methanol (M)

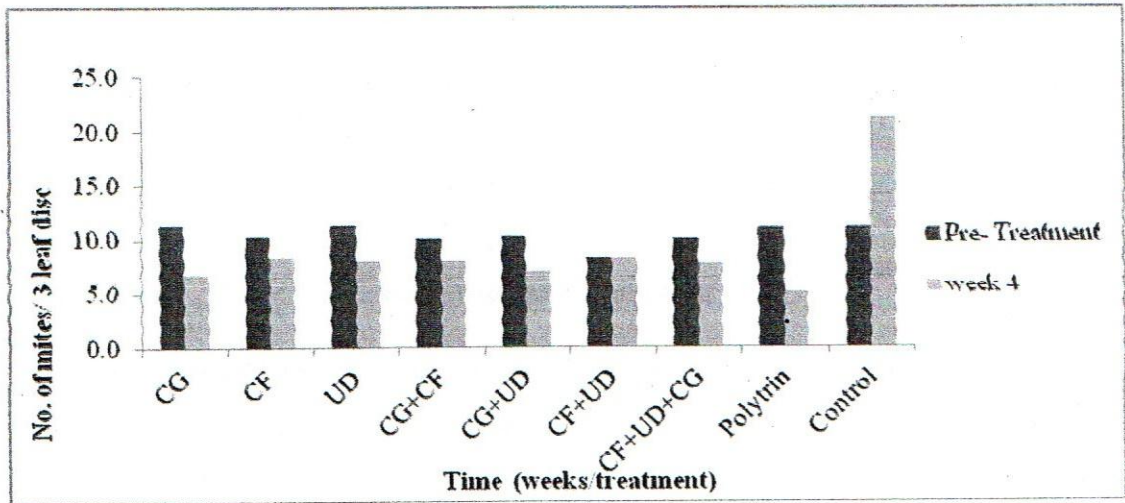


Figure 4.1: Number of *T. urticae* mites before and four weeks after treatment

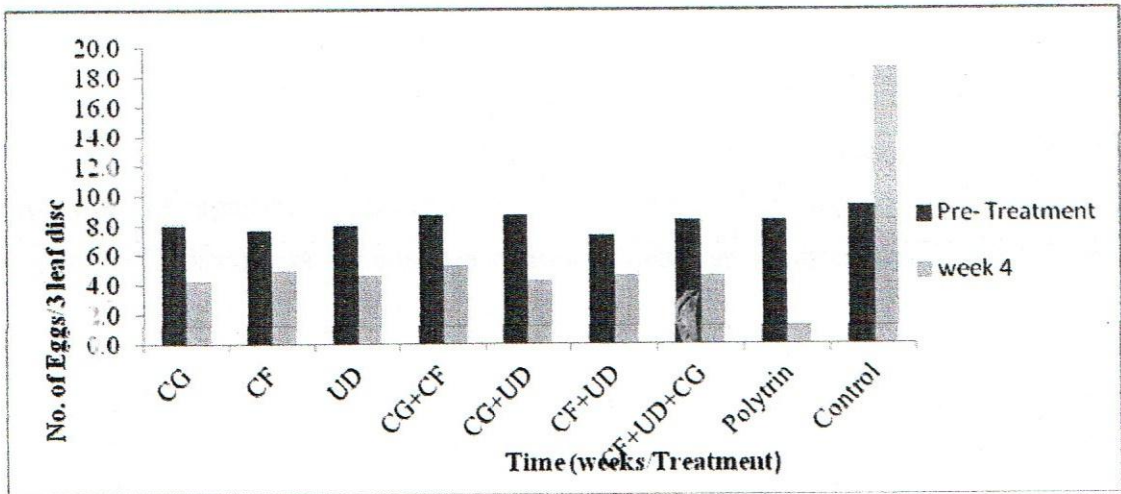


Figure 4.2: Number of *T. urticae* eggs before and four weeks after treatment

KEY

Control (C) (distilled water), *Cleome gynandra* (CG), *Capsicum frutescense* (CF), *Urtica dioica* (UD), *Cleome gynandra* + *Capsicum frutescense* (CG+CF), *Cleome gynandra* + *Urtica dioica* (CG+UD), *Capsicum frutescense* + *Urtica dioica* (CF+UD), *Capsicum frutescense* + *Urtica dioica* + *Cleome gynandra* (CF+UD+CG), Pesticide (P) (Polytrin), and Methanol (M)

4.4.2 Effects of crude plant extracts on damage and quality of rose flowers by mite infestation

Generally there was no significant difference in percentage leaf infestation amongst all treatments in both trial 1 and 2 (Table 4.2). The highest (22%) percentage leaf infestation was observed on CG+CF followed by CG+UD (21.30%) in trial 1 while in trial 2 Control (27.67%) was the highest. The lowest percentage leaf infestation in trial 1 was observed on CF+UD (10.33%) and in trial 2 CG+CF had the lowest leaf infestation (11.00%)

Aesthetic score of flowers harvested from the experiment showed significant differences in treatments ranked on a scale of 1-5.(Table 4.2). CG+CF had the highest aesthetic score which was not significantly different from CF and CG+UD in trial one and in trial two CF+UD had the highest aesthetic score which was significantly different from all the other treatments.

There were significant differences in vase life days amongst the treatments in trial one and two (Table 4.2). In trial one maximum vase life days was recorded on CG+UD+CF followed by CG and UD. The minimum vase life days was recorded on control which was significantly different from all the treatments. In trial 2, the longest vase life day (13 days) was recorded on UD which was not significantly different from CG, CG+CF+UD and polytrin. The shortest vasselife days (6.33 days) was recorded on control which was significantly different from all treatments.

This shows that damage incidence by mites on foliage affects the physiological processes of flowers not seen by physical expressions on the plant but observed later in the post-harvest life of the flowers.

Table 4.2: Average damage and quality parameters measured per treatment in trial land 2

Treatment	Trial 1			Trial 2		
	% leaf infestation	Aesthetic score	vase life (Days)	% leaf infestation	Aesthetic score	vase life (Days)
CG	15.00	4.00 ^b	12.33 ^b	17.00	4.00 ^b	12.33 ^a
CF	17.33	3.67 ^{ab}	9.33 ^f	18.67	4.00 ^b	8.67 ^d
UD	15.67	4.00 ^b	12.00 ^{bc}	18.33	4.00 ^b	13.00 ^a
CG + CF	22.00	3.33 ^a	11.00 ^{cd}	11.00	4.00 ^b	10.67 ^{bc}
CG + UD	21.33	3.67 ^{ab}	10.67 ^{de}	14.67	4.00 ^b	10.00 ^{cd}
CF+UD	10.33	4.00 ^b	9.33 ^f	14.33	3.67 ^a	9.33 ^{cd}
CG + CF + UD	14.33	4.00 ^b	13.67 ^a	17.00	4.00 ^b	12.33 ^a
Polytrin	11.67	4.00 ^b	9.67 ^{ef}	18.00	4.00 ^b	11.67 ^{ab}
Control	14.33	4.00 ^b	5.67 ^g	27.67	4.00 ^b	6.33 ^e
LSD (0.05)	NS	0.53	1.24	NS	0.33	1.39
CV %	37.7	7.90	6.90	29.3	5.00	7.70

Means in the same column followed by the same letter are not significantly different at $P \leq 0.05$ using LSD * NS= Not significant

KEY

Control (C) (distilled water), *Cleome gynandra* (CG), *Capsicum frutescence* (CF), *Urtica dioica* (UD), *Cleome gynandra* + *Capsicum frutescence* (CG+CF), *Cleome gynandra* + *Urtica dioica* (CG+UD), *Capsicum frutescence* + *Urtica dioica* (CF+UD), *Capsicum frutescence* + *Urtica dioica* + *Cleome gynandra* (CF+UD+CG), Pesticide (P) (Polytrin), and Methanol (M)

4.4.3 Effects of crude plant extracts application on yield and yield parameters on rose flowers infested by mites

Results in tables 4.3 and 4.4 below show the average yield parameters recorded per treatment in trial one and two. These parameters include chlorophyll content, number of stems harvested per rose bush per season, the stem length and the bloom size (height and width).

In trial 1, the chlorophyll content of rose plants treated with Polytrin recorded the highest chlorophyll content which was not significantly different from all the other treatments except control and CG+UD which recorded the lowest amount of chlorophyll content. In trial 2, the highest chlorophyll content was recorded on polytrin which was not significantly different from other treatments except CG+CF+UD and control which had the lowest content of chlorophyll. (Table 4.4)

The highest number of stems harvested per plant was recorded on polytrin treated plants which was significantly different from CG+CF and CG+UD. In trial two the highest number of stems harvested was recorded on CF which was not significantly different from UD, CG, and CG+CF+UD.

The longest (69.67cm) stem lengths of the plants harvested in trial one was recorded on Polytrin treated plants which was statistically different from CG+UD (52.00cm) In trial 2, there were no significant differences in the stem length among all the treatments.

The longest bloom (47.67) was recorded on CG and CG+CF+UD (47.67mm) treatments which was significantly different from CF, CG+UD and control. The shortest bloom (25.67mm) was recorded on CG+UD which were statistically similar to control.

Table 4.3: Average yield parameters per treatment in trial one

Treatment	Chlorophyll content (CCI)	No. of stems/plant	stem length (cm)	Bloom length (mm)	Bloom width (mm)
CG	46.43 ^{ab}	3.00 ^{ab}	61.33 ^{ab}	47.67 ^a	28.33 ^a
CF	40.10 ^{ab}	2.67 ^{ab}	57.67 ^{ab}	43.33 ^b	28.67 ^a
UD	42.33 ^{ab}	3.67 ^a	67.67 ^{ab}	47.00 ^a	28.67 ^a
CG + CF	38.50 ^{ab}	2.00 ^b	61.67 ^{ab}	47.00 ^a	29.33 ^a
CG + UD	33.80 ^b	2.00 ^b	52.00 ^b	25.67 ^c	24.00 ^b
CF + UD	42.20 ^{ab}	2.33 ^{ab}	66.00 ^{ab}	46.00 ^{ab}	29.33 ^a
CG + CF + UD	38.53 ^{ab}	2.67 ^{ab}	69.33 ^a	47.67 ^a	29.33 ^a
Polytrin	54.97 ^a	3.67 ^a	69.67 ^a	45.67 ^{ab}	28.67 ^a
Control	33.80 ^b	3.67 ^a	60.67 ^{ab}	28.33 ^c	30.67 ^a
LSD (0.05)	17.61	1.63	15.07	3.40	3.78
CV (%)	23.90	33.00	13.90	4.70	7.70

Means in the same column followed by the same letter are not significantly different at $P \leq 0.05$ using LSD

KEY

Control (C) (distilled water), *Cleome gynandra* (CG), *Capsicum frutescence* (CF), *Urtica dioica* (UD), *Cleome gynandra* + *Capsicum frutescence* (CG+CF), *Cleome gynandra* + *Urtica dioica* (CG+UD), *Capsicum frutescence* + *Urtica dioica* (CF+UD), *Capsicum frutescence* + *Urtica dioica* + *Cleome gynandra* (CF+UD+CG), Pesticide (P) (Polytrin), and Methanol (M)

Table 4.4: Average yield parameters per treatment in trial two

Treatment	Chlorophyll (CCI)	No. of stems/plant	stem length (cm)	Bloom length (mm)	Bloom width (mm)
CG	55.70 ^a	3.33 ^{ab}	59.67 ^a	46.67 ^a	26.67 ^{abc}
CF	50.97 ^{ab}	4.67 ^a	53.67 ^a	45.67 ^a	28.00 ^{ab}
UD	58.53 ^a	3.33 ^{ab}	57.33 ^a	46.33 ^a	28.67 ^a
CG + CF	58.13 ^a	3.00 ^b	65.00 ^a	45.33 ^a	29.33 ^a
CG + UD	58.10 ^a	3.00 ^b	54.00 ^a	34.00 ^b	24.00 ^{bc}
CF + UD	53.50 ^a	2.33 ^b	59.33 ^a	44.33 ^a	28.67 ^a
CG + CF + UD	37.47 ^b	3.33 ^{ab}	65.01 ^a	46.33 ^a	29.33 ^a
Polytrin	59.20 ^a	3.00 ^b	63.33 ^a	44.33 ^a	28.00 ^{ab}
Control	23.10 ^c	3.00 ^b	55.33 ^a	30.33 ^b	23.00 ^c
LSD (0.05)	14.20	1.66	NS	7.67	4.36
CV %	15.50	29.70	13.90	10.40	9.20

Means in the same column followed by the same letter are not significantly different at $P \leq 0.05$ using LSD *NS= Not significant

KEY

Control (C) (distilled water), *Cleome gynandra* (CG), *Capsicum frutescence* (CF), *Urtica dioica* (UD), *Cleome gynandra* + *Capsicum frutescence* (CG+CF), *Cleome gynandra* + *Urtica dioica* (CG+UD), *Capsicum frutescence* + *Urtica dioica* (CF+UD), *Capsicum frutescence* + *Urtica dioica* + *Cleome gynandra* (CF+UD+CG), Pesticide (P) (Polytrin), and Methanol (M)

4.5 DISCUSSION

4.5.1 Effects of crude plant extracts on *T. urticae* population

From the results shown in table 4.1 there was a significant decline in the number of mites from week one to week four in all treatments except for the control treatment. The control treatment's mite population gradually increased from week one to week three of treatment but experienced a decline in week four. This trend as observed in figure 4.1 could have been attributed to mite population build up to maximum numbers, colonizing the entire leaf and plant hence, reduced resources of food causing the mite population to starve or migrate to other leaves or plant due to the lack or shortage of food resources, mites are also known to cannibalise in times of reduced food supply as may have been the case in this situation.

Similar results of decline were also observed on the number of eggs laid, as the mite population decreased the egg numbers also decreased, this could be attributed to more eggs hatching to nymphs which were either repelled or died as a result of the plant extracts applied (Tomczyk and Susko, 2011, Yanar *et al.*, 2011). Also, the few number of eggs found or counted may have been hatched by the surviving few mites. At this point it is also important to note that the two spotted spider mites have been found to multiply through vivipary i.e. lays an egg that hatches into a nymph as soon as it is laid.

The table 4.1 also shows that at $P \leq 0.05$ the average number of mites recorded on plant extract treatments in week one, two and three after artificial introduction gradually reduced. Similarly the number of eggs laid in week one gradually reduced and recorded the lowest numbers on polytrin and plant extract treatment CG + UD. In week two, there was no significant difference in the treatments applied but these were significantly different from the control treatment. The sharp decrease in mite population in week two may be attributed to the strong miticide effect of the extracts and continued decrease due to the residual effect of the crude extracts. The slight increase in mite population on CF + UD, UD and CG + CF may be attributed to the reduced chemical extract residual effect due to volatilization. Polytrin miticide recorded at $P \leq 0.05$ significantly lower number of mites and eggs compared to other treatments. However, in week four, polytrin recorded the lowest number of mite population which was significantly different from CG, CF + UD, CG + CF + UD respectively (Table 4.1).

Generally, Polytrin had more effect in controlling the mite population. However, the crude extracts have also shown to have acaricidal effect and may be included in pest control strategies as bio - pesticides for mites in commercial rose growing.

The experimental data confirmed that plant extracts from various species evoke various negative responses from the two-spotted spider mite affecting their feeding behaviour and survival. Because intensive use of synthetic insecticides have caused outbreaks of spider mites on many crops and development of mite resistance to acaricides especially on treated ornamental crops, studies on the effect of extracts of various plant species on spider mites Tetranychidae have been intensified since the nineteen eighties and continue until the present time. Deterrent and toxic effect of seed extracts from *Abrus precatorius* was confirmed by Amer *et al.*, 1989. Essential oil extracted from *Tanacetum vulgare* and *Artemisia absinthium* strongly increased mortality of *Tetranychus urticae* (Chiasson *et al.*, 2001). Also extracts from *Matricaria recutita*, *Achillea millefolium*, *Taraxacum officinale*, *Salvia officinalis* have been found to strongly reduce the *T. urticae* fecundity, longevity and intensity of feeding (Kawka and Tomczyk 2002). Water extracts from needle surface of *Taxus baccata* have also been found to strongly affect fecundity and oviposition period of the two-spotted spider mite females (Furmanowa *et al.*, 2001).

4.5.2 Effects of crude plant extracts on damage and quality of rose flowers by mite infestation

The results on quality of flowers harvested from the experiment showed no significant differences in aesthetic score of crude extracts ranked on a scale of 1-5 (plate 4.2 – 4.6). The results in table 4.2 show that the treatment plants were infested with mites albeit different percentages this may be due to introduction of mites to each of the treatment plots for purposes of the experiment which were allowed to establish before treatments applied for control measure.

CF, CG+CF and CF+UD in trial 1 and 2 respectively had moderate damage and the others severe (see scale 1-5 and plates 4.4 - 4.5). The results seen may be attributed to the unpalatable nature of chilli extracts for the mites to feed on and hence better aesthetic value. Plants produce a vast array of volatiles and tannins that play an important role in plant defense (Aharoni *et al.*, 2003). The species *C. frutescens* also contains diterpenoids, flavonoids, saponins, and phenolic compounds having lethal effects, antifeedant effects, and parasite repellence. Hot pepper also contains significant amount of tannins (Antonious and Snyder, 2006) that break

down and behave as toxins. Similar reactions of various host plants have also been observed after their infestation by spider mites, which suggest the importance of phenolic compounds present in plant extracts in defence mechanisms of plants against the two spotted spider mite in this study.

The results further reflect on the vase life days where control was observed to have shorter number of days in the vase compared to all the treatments which were observed to have increased number of days. The vase life day's differences amongst the treatments in trial 1 and 2 with CG, CG+CF+UD and UD recording the highest number of days, this observation may be attributed to the feeding nature of the mites which feed by sucking the cell sap or chlorophyll of the plants hence plant unable to generate photosynthates as reserve energy. Hence faster loss of leaves and death of the stem earlier than the other treated rose stems. Furthermore, it is possible that mite feeding on the rose crops affects rose physiological processes within the plant resulting in reduced vase life days.

The difference in chlorophyll content may be explained by the level of percentage leaf infestation per treatment and further explain the pest preference in feeding on the plants hence observations seen on the aesthetic score. Spider mites tend to prefer the youngest, fully expanded leaves that have higher nitrogen content than older leaves (Chen *et al.*, 2007).

The other observation is that the experimental plots treated with crude plant extracts had better vase life days in trial 1 despite having lower percentage of leaf infestation by mites compared to the polytrin treated plants, this may be attributed to the systemic interference of the chemical active ingredients to the photosynthetic sites of the plant hence reducing or slowing down processes in the plant and affecting vase life in the long term.

The overall damage and quality rating in this experiment was assessed based on harvested stem plant appearance, including percentage leaf mite infestation expressed as an aesthetic score index which reflects the extent of leaf feeding damage by the mites. Opit *et al.*, (2005) found that overall plant quality on ivy geranium plants was negatively correlated with cumulative mite density. Therefore, plant quality ratings reflect the accumulation of mite feeding damage over time

The above observations suggest that damage by mites on foliage affects the physiological processes of flowers not seen by physical expressions on the plant but observed later in the post-harvest life of the flowers. Although plant-derived essential oils have been shown to be active against certain pests, their phytotoxicity has been questionable (Arnason *et al.* 1993). It has been reported that many plant extracts are phytotoxic to vegetables and herbaceous and foliar plant material (Isman, 2001). However, the extent of plant injury may be dependent on numerous factors, including the concentration of the compound, the rate at which it is applied, plant type and which plant parts are exposed during spray applications (Cloyd *et al.* 2009). Similarly, phytotoxic properties of some essential oils on *T. urticae* have been reported (Monsuer *et al.*, 1993). It has also been observed that pure rosemary oil can cause complete mortality of spider mites *T. urticae* at concentrations that are not phytotoxic to the host plants (Miresmaili and Isman, 2006).

4.5.3 Effects of crude plant extracts application on yield and yield parameters of rose flowers infested by mites

Results in table 4.3 and 4.4 showed the average yield parameters recorded per treatment in trial one and two. In trial one the rose plants in the control treatment plots had lowest levels of chlorophyll compared to all other treatments. This may be attributed to the feeding behaviour of two spotted spider mites which feed by sucking the cell sap contents which include the chlorophyll.

The Polytrin treated plants were observed to have the highest level of chlorophyll content compared to all the other treatments, this may be attributed to the knock down or instant killing of mites by the polytrin chemical hence reducing the number of mites feeding on the plant and allowing the plant time to regenerate more leaves and chlorophyll. In both trials also, the plant extract treated plots compare well with polytrin.

Chlorophyll is responsible for trapping light which is important in the light stage of photosynthesis. Reduced chlorophyll levels would mean reduced photosynthetic capacity which results in less carbohydrates being apportioned to the flowers sinks (buds and stems) hence reduced yields (head size, stem length etc.).

The number of flower stems per plant in trial one were the same amongst the treatments with CG+CF and CG+UD recording the lowest number of stems harvested per plant numerically. While in trial two the treatments had no effects on the number of stems harvested per plant this may be attributed to the genetic traits of the rose plant variety.

The stem lengths of the plants recorded in trial 1 and 2 were statistically the same except for small numerical variations. The longest stems harvested were recorded on CG+CF+UD in both trials and compared well with the polytrin treated plants. The shortest stems were recorded on CG+UD in both trials. The similarity in lengths may not have been affected by the treatments as this is also a genetic trait of the rose plant variety 'high and delicate'.

Lengths of the flower heads or blooms recorded were statistically the same except CG+UD and C (control) which recorded the lowest flower head/bloom lengths. As earlier discussed mite infestation on plant by mites affects photosynthetic ability of the plant hence reduced photosynthates apportioned to the flower sinks. During flowering all photosynthates are channelled to the flower buds for flower formation, therefore it can be concluded that the low chlorophyll content results in reduced photosynthates hence smaller bloom length.

The width of the blooms both in trial 1 and 2 were not observed to be statistically similar among the treatments. However, the plants treated with CG+UD and control were observed to have smaller flower head/bloom width. According to the Dutch Flower Auction and other commercial buying groups quality standards of 2013, the commercially accepted minimum bloom length and width in relation to stem length is as follows:

- 32cm and 42cm = 30 x 20mm,
- 52cm and 62cm = 35 x 20mm,
- 72cm and 82cm = 40 x 30mm.

In view of this standard the stems harvested in this research trial meet the market quality standards except for the stems of plants treated with CG+UD and control which had unproportional head sizes in relation to the stem length. CG+UD (25x24mm) and Control (28x30mm) in trial 1 and in trial 2 CG+UD (34x24mm) and control (30x23mm) these sizes on a head size measuring gauge are more of a square bud and do not meet the required standard of (35x20mm) reflected above.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

The repellent effects of crude plant extracts evaluated on *Tetranychus urticae* Koch had significant difference ($P < 0.05$) in terms of repellence and acaricidal activity of *Cleome gynandra*, *Capsicum frutescence* and *Urtica dioica* on *T. urticae* was evident. The use of these crude extracts as acaricides could significantly reduce the population of two-spotted spider mites on cut-flower rose leaves within a period of about six days. However, the level of their mortality and repellence is dependent upon the exposure period of the crude plant extracts dissolved in either methanol or distilled water. The methanol dissolved solutions proved more effective at 100 g of extract exposed between one and six days.

The total average mean number of both mites and eggs per 3 leaf discs treated with crude plant extracts was significantly ($P < 0.05$) less than the control four weeks after treatment applications suggesting that the crude plant extracts especially of African spider plant and chilli can be explored further for developing natural products for use as bio-pesticides. There is also need to further investigate the extracts dosage and frequency of application as well as refining the solution to reduce the extracts residual matter which may limit the aesthetic quality of cut rose flower for export in large scale commercial production greenhouses

The effect of the mite feeding and control using the crude plant extracts was not significant ($P < 0.05$) with regard to the number of stems per plant and their stem length. However it was noted that presence of few mites on the plant causes considerable reduction of chlorophyll on the foliage which may result in reduced number of marketable stems. It is therefore of commercial interest to maintain very low numbers of mites if none at all on the rose crops. Vaselife of the test plants harvested especially from the crude plant extracts treatment plots had longer vaselife days compared to those harvested from the polytrin or chemical which may have been affected by the persistence of the active ingredients on the plant tissues.

From this study we can conclude that crude plant extracts with repellent or toxic nature maybe of significant input as a supplement or incorporation in IPM mite control strategies. The potential benefits of methanoic plant volatile extraction for control of mites in rose growing for export markets is evident.

5.2 RECOMMENDATIONS

The following recommendations are made:

1. That crude plant extracts of *C. gynandra*, *U. dioica* and *C. frutescence* have repellent effect on *T. urticae* population and affect various phenomena of spider mite behaviour and survival, and therefore further studies should be done to ascertain their effects on spider mites
2. Crude plant extracts of *C. gynandra*, *U. dioica* and *C. frutescence* have toxic effect on *T. urticae* population. Therefore, identification of chemical compounds responsible for toxic/Mortality effects on spider mites and further studies should be done to ascertain their effects on spider mites
3. Crude plant extracts of *C. gynandra*, *U. dioica* and *C. frutescence* on *T. urticae* cause damage and quality of rose flowers and the presence of few mite numbers on rose crop can cause considerable damage on cut rose stems resulting in reduced vase life. Hence, growers need to always maintain a zero tolerance to mite pest presence on commercial cut rose greenhouses
4. Crude plant extracts of *C. gynandra*, *U. dioica* and *C. frutescence* have effect on the yield of the rose flower. Hence, growers need to always maintain a zero tolerance to mite pest presence on commercial cut rose greenhouses.
5. Identification of chemical compounds responsible for repellent, antifeedant and toxic effects on spider mites and other arthropod pests may provide additional tools in pro-ecological method of integrated pest control.
6. Further research on effective use of methanol as a solvent for extracting phenolic compounds may increase the effectiveness of crude plant extract compounds for pesticide use is required
7. Secondary metabolites from crude plant extracts can be very useful in plant protection, especially in combination with other non-chemical methods. More studies are needed on their negative influence and not only on pests but also on natural enemies

REFERENCES

- Aggarwal M, Walia S, Dhingra S, and Khambay BP. (2001). Insect growth inhibition, antifeedant and antifungal activity of compounds isolated/derived from *Zingiber officinale* Roscoe. (Ginger) rhizomes. *Pest Management Science journal* 57: 289–300
- Aharoni, A.; Giri, A.P. Deuerlein, S.; Griepink, F.; De Kogel, W.J.; Verstappen, F.W.; Verhoeven, H.A.; Jongsma, M.A.; Schwab, W.; and Bouwmeester, H.J. (2003). Terpenoid metabolism in wild-type and transgenic arabidopsis plants. *Plant Cell journal* 15, 2866–2884.
- Arnason JT, MacKinnon S, Durst A, Philogene BJR, Hasbun C, Sanchez P, Poveda L, San Roman L, Isman MB, Satasook C. (1993). Phytochemical potential of tropical plants. In: Downum KR, Romeo JT, Stafford HA, editors. Insecticides in tropical plants with non-neurotoxic modes of action, Chapter. 5. New York: Plenum. pp. 107–131.
- Amsalingam R., Mariappan S. R. S, Azariah B. and Narayanannair M. (2010) “Bioefficacy of certain plant extracts against the red spider mite, *Oligonychus coffeae* (Nietner) (Acarina: Tetranychidae) infesting tea in Tamil Nadu, India *International Journal of Acarology Volume 36, Issue 3, pages 255-258*
- Antonious G. F and J. C. Snyder, (2006) “Natural products: repellency and toxicity of wild tomato leaf extracts to the two-spotted spider Mite, *Tetranychus urticae* Koch,” *Journal of Environmental Science and Health B*, vol. 41, No. 1, pp. 43–55.
- Armstrong, H., (2001). Increasing ways to biologically control pests. *Flower Technology Journal* 4 (7): 22–25.
- Attia, S., K. L. Grissa, A. C. Mailleux, G. Lognay, S. Heuskin, and S. Mayoufi, (2011). “Effective concentrations of garlic distillate (*Allium sativum*) for the control of *Tetranychus urticae* Koch. (Tetranychidae),” *Journal of Applied Entomology*, vol. 136, No. 4, pp. 302–312.
- Bambang W, Nugroho, Schwarz B, Wray V and Proksch P. (1996). Insecticidal constituents from rhizomes of *Zingiber cassumunar* and *Kaempferia rotunda*. *Phytochemistry Journal* 41: 129–132.
- Bandara KA, Kumar V, Saxena RC and Ramdas PK. (2005). Bruchid (Coleoptera: Bruchida) ovicidal phenylbutanoid from *Zingiber purpureum*. *Journal of Economic Entomology* 98:1163–1169.

- Cabras, P., Caboni, P., Cabras, M., Angioni, A and Russo, M., (2002). Rotenone residues on olives and in olive oil. *Journal of Agricultural Food Chemistry*. 50, 2576–2580
- Cavalcanti SCH, Dos E, Niculau S, Blank AF, Camara CAG, Araujo IN and Alves PB.(2010). Composition and acaricidal activit of *Lippia sidoides* essential oil against two-spotted spider mite (*Tetranychus urticae* Koch.) *Elsevier Bio-resource technology* 101. pp 829-832.
- Chen, Y., G. P. Opit, V. M. Jonas, K. A. Williams, J. R. Nechols, and D. C. Margolies. (2007). Two spotted spider mite population level, distribution, and damage on ivy geranium in response to different nitrogen and phosphorus fertilizer regimes. *Journal of Economic Entomology* 100: 1821-1830
- Cherian, S. (2003). Biological control of mites in roses. *FloraCulture International*. 13(9):20–23
- Chiasson H., Belanger A., Bostanian N., Vincent C., and Poliquin A. (2001). Acaricidal properties of *Artemisia absinthium* and *Tanacetum vulgare* (Asteraceae) essential oils obtained by three methods of extraction. *Journal of Economic Entomology*. 94 (1): 167–171.
- Cloyd R.A, Galle CL, Keith SR, Kalscheur NA and Kemp KE. (2009). Effect of commercially available plant derived essential oil products on arthropod pest. *Journal of Economic Entomology*. 102 (4):1567–1579.
- Cowles, R.S.; Keller, J.E. and Miller, J.R. (1989) Pungent spices, ground red pepper, and synthetic capsaicin as onion fly ovipositional deterrents. *Journal of Chemistry and Ecology* 15: 719–730.
- Cranshaw W.S and Scalar D.C. (2014) Spider mites. Colorado state University Bioagricultural sciences and Pest management. 12/98 Revised 7/14
- Crespel L, Zang D, Meynet J, Jacob Y, and Gudin S (2001) Indentification of appomictic plant in *Rosa hybrid* L. by RFLPs. In: Proceedings of the third International symposium on rose research and cultivation (eds. N. Zieslin and H. Agbaria) *Acta Horticulturae* 547:51-55.
- Dąbrowsky T. Z, and Urszula ,Seredyńska (2007) Characterisation of the two-spotted spider mite (*Tetranychus urticae* koch, Acari: Tetranychidae) response to aqueous extracts from selected plant species. *Journal of plant protection research* 47 (2), pp. 114–123

- Dimetry N.Z, S. A. A. Amer, and A. S. Reda, (1993) "Biological activity of 2 Neem Seed kernel extracts against the 2-spotted spider mite *Tetranychus urticae* Koch," *Journal of Applied Entomology*, vol. 116, no. 3, pp. 308–312,
- Elfers, J., Graham, R. A., and Dewolf, G. P. (1964). In: C. E. Hubbard & E. Milne-Redhead, *Flora of Tropical East Africa* (p. 18). London: Whitefriars Press
- Erdogan, P and Toros, S. (2007) "Investigations on the effects of *Xanthium strumarium* L. extracts on Colorado potato beetle, *Leptinotarsa decemlineata* Say (Col.: Chrysomelidae)," *Munis Entomology & Zoology*, vol. 2, no. 2, pp. 423–432, 2007.
- Estrada B, Pomar F, Diaz J, Merino and Bernal FA. (1997) Evaluation of capsaicinoids in *Capsicum annum* L.var. annum cv. Padron at different growth stages after flowering. *Capsicum Newsletter*,16:60-4
- Ferry N, M.G. Edwards, J.A. Gatehouse and A.M.R. Gatehouse, (2004) Plant–insect interaction: Molecular approaches to insect resistance.. In: T. Sasaki and Christou, Editors, *Biotechnology* volume 15, pp. 155–161
- Furmanowa M., D. Kropczyska and A. Zobel (2002) "Influence of water extracts from the surface of two yew (*Taxus*) species on mites (*Tetranychus urticae*)," *Journal of Applied Toxicology*, vol. 22, no. 2, pp. 107–109
- Gerardo mercurio (2007). Cut rose cultivation around the world. Aesse stampa, Benevento. Italy.
- Gudin S (2000) Rose Genetics and breeding. *Plant Breeding Reviews*, volume 17 (ed. J. Janick), John Wiley & Sons, Inc. pp.159-189.
- Horticultural Crops Development Authority (HCDA). (2012). Horticultural Crops Development Authority Validated Report for 2012.
- Imbamba, S.K., (1973). Leaf protein content of some Kenya vegetables. *East African Agriculture and Forestry Journal*. 38(3):246–251.
- Isman, M.B., (2000). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Review of Entomology* 51: 45–66.
- Jacobson, M. (1975). The neem tree: natural resistance par excellence. In *Natural resistance of plants to pests*; Green, M.B. and Hedin, P.A. Eds. American Chemical Society: Washington, D.C., pg 220–232.

- Jahangir Khajehali, Pieter Van Nieuwenhuysse, Peter Demaeght, Luc Tirry and Thomas Van Leeuwen. (2011) Acaricide resistance and resistance mechanisms in *Tetranychus urticae* populations from rose greenhouses in the Netherlands
- Josvold, S.A., and Chaney, W.E., (2001). Evaluation of reduced risk and other biorational miticides on the control of spider mite (*Tetranychus urticae*). *Acta Horticulturae*. 547, 93–96.
- Kamau A.W (1985) The biology and control of tomato russet mite *Acolops lycopersci* (Masse) (Acarina: Eryophidae) in Kenya, PhD Thesis, University of Nairobi.
- Kawka B., and Tomczyk A. (2002). Influence of extracts from sage (*Salvia officinalis* L.) on some biological parameters of *Tetranychus urticae* Koch feeding on Algerian Ivy (*Hederahelix variegata* L.). *IOBC/ WPRS Bull.* 25 (1): 127–130.
- Keizer, M. and Zuurbier, J. (2000): Integrated pest management manual for extension staff, Mashare Agricultural Development Institute, Rundu, Namibia.
- Kenya Flower Council.(KFC). (2008). Kenya Flower Council 2008 Annual Report
- Kielkiewicz, M. (1996). Dispersal of *Tetranychus cinnabarinus* on various tomato cultivars. *Entomologia Experimentalis and Applicata* 80 (1). 254-257.
- Knapp, M. and S.S Kahenge, (2003). Effect of different neem formulations on the two-spotted Spider mite *Tetranychus urticae* Koch, on the tomato (*Lycopersicon esculentum* Mill). *Insect Science Application*. 23: Pp 1-7
- Krainacker, D. A. and, J. R. Carey (1990). Amdsulaotry dispersal and life history to food deprivation in two-spotted spider mites. *Entomologia Experimentalis and applicata* 56: 139-144.
- Labuchagne, L., (2005). Integrated Pest Management of spider mite in roses. *Floraculture International*. 15 (2), 12.
- Landeros, J., Guevara, L.P., BadiiM, H., Flores, A.E., and Pa´manes, A., (2004). Effect of different densities of the two spotted spider mite *Tetranychus urticae* on CO₂ assimilation, transpiration, and stomatal behaviour in rose leaves. *Experimentalis applicata acarologia*. 32 (3), 187–198.

- Lopatkin N, Sivkov A, Walther C, Schlafke S, Medvedev A, Avdeichuk J, Golubev G, Melnik K, Elenberger N, and Engelmann U (2005). Long-term efficacy and safety of a combination of sabal and urtica extract for lower urinary tract symptoms: a placebo-controlled, double-blind, multi-center trial. *World Journal of Urology*. Ltd, London
- Lwande, A.J. Ndakala, A. Hassanali, L. Moreka, E. Nyandat, M. Ndungu, H. Amiani, P.M. Gitu, M.M. Malonza and D.K. Punyua, (1999). *Gynandropsis gynandra* essential oil and its constituents as tick (*Rhipicephalus appendiculatus*) repellents, *Phytochemistry* 50 (3) pp. 401–405
- Malonza, M.M., Dipeolu, O.O., Amoo, A.O., and Hassan, S.M., (1992). Laboratory and field observations on anti-tick properties of the plant *Gynandropsis gynandra* (L.) Brig. *Veterinary parasitological*. 42, 123–136.
- Meyer, M. K. D. (1996). Mite pests and their predators on cultivated plants in South Africa. Vegetables and berries. *Plant protection research institute handbook* No. 6, ARC – Plant Protection Research Institute. Pretoria.
- Miranova, M. K and Khorkhordin, E. G. (1996). “Effect of Neem Azal T/S on *Tetranychus urticae* Koch,” in Proceedings at the 5th Workshop, pp. 22–25, Wetzlar, Germany,
- Miresmailli, S., Bradburry, R and Isman, M. B. (2006). Essential Oil of *Haplophyllum robustum* Bge. From Iran. *Journal of Essential Oil Research*, 16 (16): 548 – 549.
- Mitchell, W. J. and Breyer-Brandwijk, M. G. (1962). *The medicinal and poisonous plants of Southern and Eastern Africa* (1st Ed.) (p. 164). London: E. & S. Livingstone Limited
- Monda, E. O., S. Munene and A. Ndegwa. (2003). French beans production constraints in Kenya. African Crop Science Society. African Crop Science Conference Proceedings, Vol. 6: Pp. 683-687. Kenyatta University, Botany Department, P.O. Box 43844, Nairobi, Kenya and Thika Horticulture Research Centre, P.O. Box 220 Thika, Kenya
- Monsuer, F.A., K. R. S. Ascher, and F. Abo- Moch, (1993) “Effects of margosan-o, azatin and RD9-repeling on spiders, and on predacious and phytophagous mites,” *Phytoparasitica*, vol. 21, No. 3, pp. 205–211,
- Motazedian, N., S. Ravan, and A. R. Bandani. (2012). Toxicity and Repellency Effects of Three Essential Oils against *Tetranychus urticae* Koch (Acari: Tetranychidae). *Journal of Agricultural Science and Technology*, Vol. 14: Pp. 275-284

- Najafabadi, S. S. M., R. V. Shoushtari, A. A. Zamani, M. Arbabi and H. Farazmand. (2011). Effect of Nitrogen Fertilization on *Tetranychus urticae* Koch. (Acari: Tetranychidae) Populations on Common Bean Cultivars. *American-Eurasian Journal of Agriculture and Environmental Science*. 11 (4): Pp. 568-576
- Nas, M. N (2004). "In vitro studies on some natural beverages as botanical pesticides against *Erwinia amylovora* and *Curobac- terium flaccumfaciensis* subsp. Poinsettiae," *Turkish Journal of Agriculture and Forestry*, vol. 28, no. 1, pp. 57–61,
- Nauen, R., N. Stumpf, A. Elbert, C. P. W. Zebitz and W. Krans. (2001). Acaricide toxicity and resistance in larvae of different strains of *Tetranychus urticae* and *Panonychus ulmi* (Acari: Tetranychidae). *Pest Management Science*, 57: Pp. 253 - 261.
- Nihoul, P, G. Van Impe, and T Hnace. (1991). Characterizing indices of damage to tomato by The Two-Spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) to achieve biological control. *Journal of Horticultural Science* 66:643-648.
- Nyalala, S. and Grout B, (2007) African spider flower (*Cleome gynandra* L./*Gynandropsis gynandra* (L.)Briq.) as a red spider mite (*Tetranychus urticae* Koch) repellent in cut-flower rose (*Rosa hybrida* L.) cultivation. *Scientia Horticulturae*, Volume 114, Issue 3, Pages 194-198
- Nyalala S.O, M.A Petersen and B.W.W Grout, (2010). Acetonitrile (methyl cyanide) emitted by the African spider plant (*Gynandropsis gynandra* L. (Briq)): Bioactivity against spider mite (*Tetranychus urticae* Koch) on roses. *Scientia Horticulturae* Volume 128, Issue 3, Pages 352–356
- Ofuya TI and Okuku IE. (1994). Insecticidal effect of some plant extracts on the cowpea aphid, *Aphis craccivora* Koch (Homoptera: Aphididae). *Anzeiger Schädlingskunde* 67: 127–129.
- Oparaeke A.M. (2005). Studies on insecticidal potential of extracts of *Gmelina arborea* products for control of field pests of cowpea, *Vigna unguiculata* (L.) Walp: the pod borer, *Maruca vitrata* and the coreid bug, *Clavigralla tomentosicollis*. *Journal of Plant Protection* 45: 1–7.

- Opit, G. P., Y. Chen, K. A. Williams, J. R. Nechols, and D. C. Margolies. (2005). Plant age, fertilization, and biological control affect damage caused by two spotted spider mite on ivy geranium: development of an action threshold. *Journal of American Society of Horticultural Science*. 130: 159 -166
- Park I.K, Lee SG, Shin SC, Park JD, and Ahn YJ. (2002). Larvicidal activity of isobutylamides identified in *Piper nigrum* fruits against three mosquito species. *Journal of agricultural food and chemistry* 50: 1866–1870.
- Paulina, P., (2005). HDRA *Encyclopedia of Organic Gardening*, pp 207, Dorling Kindersley
- Phillips R and Rix M (1988) *The Biology and Ecology of Rosa x hybrida* (Rose). Random. House, pp 224
- Pimentel, D. H M., Acuay. P., Blotnen. M., Rice J., Silva. V., Nelson S Lipner A., Diordano, and Horowitz M. D'Mmore, (1992). Environmental and economic costs of pesticide use. *Bio-Science*. 42,750-760.
- Pitasawat B, Choochote W and Tuetun B. (2003). Repellency of aromatic turmeric *Curcuma aromatica* under laboratory and field conditions. *Journal of Vector Ecology* 28: 234–240.
- Pontes J., J. Oliveira, C. Camara, C. Assis, M. Juniour, and R. Barros, (2011). “Effects of the ethanol extracts of leaves and branches from four species of the croton on *Tetranychus urticae* Koch (Acari: Tetranychidae),” *BioAssay*, vol. 6, pp. 3–14.
- Prajapati V, Tripathi AK, Aggarwal KK and Khanuja SP. (2005). Insecticidal, repellent and oviposition-deterrent activity of selected essential oils against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. *Biores Technology* 96: 1749–1757.
- Ragusa Di Chiara, S., Tsolakis, H., Ragusa, E., Alonzo, G. and Saiano, F., (2007) . Effects of some botanical pesticides on *Tetranychus urticae* Koch (Acariformes, Tetranychidae) and its predator *Cydnodromus californicus* (McGregor) (Parasitiformes, Phytoseiidae) in laboratory trials. *Sociedad Latinoamericana de Acarologi'a. Me'xico*, pp. 347–354
- Rasikari, H.L. D. N. Leach and P. G. Waterman. (2005) “Acaricidal and cytotoxic activities of extracts from selected genera of Australian lamiaceae,” *Journal of Economic Entomology*, vol. 98, no. 4, pp. 1259–1266.
- Ross D (1991) *The Ross guide to rose growing*. Lothian Publishing Company Pty Ltd, Port Melbourne, Victoria, pp 117.

- Rout GR, Samantaray S, Mottley J and Das P (1999) Biotechnology of the rose: a review of recent progress. *Scientia Horticulturae* 81: 201-228.
- Saeedah G, M. Kambiz, R. Vahid and M. Ghamadyari (2013) Toxicity and ovicidal activity of different plant extracts on two spotted spider mites *Tetranychus urticae* Koch (Acari; Tetranychidae) *Scientia Horticulturae* Volume 46 issue 1
- Sabelis, M. W. (1985). Sampling techniques. IN: W. Helle & M. W. Sabelis. Spider mites, their Biology, Natural Enemies and Control. Vol. 1A. *Elsevier*, Amsterdam: 337-350
- Sabelis, M.W. and M. Dicke, (1985). Long-range dispersal and searching behaviour. In: Helle, W and Sabelis, W. M. (eds). Spider mites, their biology, natural enemies and control, Vol. 1 B *Elsevier*, Amsterdam: 141-160.
- Schmutterer, H and Zebitz, C. (1984). "Effect of in ethanolic extracts from seeds of single Neem Trees of African and Asian origin, on *Epilachna varii vestis* and *Aedes aegypti*. In: natural pesticides from Neem Tree and other Tropical Plants," in Proceedings of the 2nd International Neem Conference, pp. 83– 90, Rauischholzhausen, Germany, 1984.
- Shi, G. L Zha L.L. Liu S. Q, cao H, Clarke S.R and Sun H.J (2006) 'Acaricidal activities of extracts of *Kochia scoparia* against *Tetranychus urticae*, *Tetranychus cinnabarinus* and *Tetranychus viennensis* (Acari: Tetranychidae) " *Journal of Economic Entomology* volume 99. No.3, pp 858-863.
- Smolenski, S.J., Silinis, H. and Farnsworth, N.R., (1975). Alkaloid screening. *VIII Lloydia* 38, p. 497
- Stark, J.D., Wong, T.T.Y., Vargas, R.I., and Thalman, R.K., (1992). Survival, longevity and reproduction of tephritid fruit fly parasitoids (Hymenoptera: Braconidae) reared from fruit flies exposed to azadirachtin. *Journal of Economic Entomology*. 84 (4), 1125–1129
- Suski. Z. W. and A. J. Naegele, (1996). Light response in the two-spotted mite. II: Behavior of the 'Sedentary' and dispersal' phases. In: Naegele, J, A. (eds) Recent advances in Acarology. Cornell Univ. Press, New York: 445-453.
- Tewari A, Kausik MP, Pandey KS, and Dangi RS. (2005) Adaptability and production of hottest chilli variety under Gwalior agro-climatic conditions. *Curr Sci* 88:1545-6

- Tomczyk, A and Suszko, M. (2011). The role of phenols in the influence of herbal extracts from *Salvia officinalis* L. and *Matricaria chamomilla* L. on two-spotted spider mite *Tetranychus urticae* Koch. *Biological Lett.* 48(2): 193 – 205.
- Tsolakis, H., Ragusa, S., (2004) . Laboratory evaluation of plant extracts on *Tetranychus urticae* Koch (Acariformes, Tetranychidae), In: Weigmann, G., Alberti, G., Wohltmann, A., Ragusa, S. (Eds.), *Acarine Biodiversity in the Natural and Human Sphere. Phytophaga* 16, 539–548.
- Usher, G. (1973). *Dictionary of plants used by Man*. New York: Hasner Press.
- Van Leeuwen, T., V. Stillatus and L. Tirry (2004) Genetic analysis and cross-resistance spectrum of a laboratory-selected chlorfenapyr resistant strain of two-spotted spider mite (Acari: Tetranychidae) *Experimentalis Applicata Acarologia*, 32 (4) pp. 249–261
- Verma GS and Pandey UK (1982) Insect Antifeedant Property of Some Indigenous Plant Products. *Zeitschrift fuer Angewandte Zoologie* 74, 113-6
- Waligóra D. (2006). Activity of saponin extract from the bark of *Quillaja saponaria* Molina, against Colorado potato beetle (*Leptinotarsa decemlineata* Say). *Journal of Plant Protection*. 46: 199–205
- Wang, W., and Liu, T.S., (1994). Toxicity of mixtures of several miticides with the fungicide triforine against the two-spotted spider mite on roses. *Bull. Taichun District Agriculture Improvement Station* 44, 1–11.
- Wang Y.N , G. L. Shi, and L. L. Zhao,(2007) “Acaricidal activity of *Juglans regia* leaf extracts on *Tetranychus viennensis* and *Tetranychus cinnabarinus* (acaris tetranychidae),” *Journal of Economic Entomology*, vol. 100, no. 4, pp. 1298–1303,
- Warthen, J. D., Morgan, E. D and Mandava, N. B. (1990). “Insect feeding deterrents,” in *CRC Handbook of Natural Pesticides*, vol. 6 of *Insect Attractants and Repellents*, pp. 23–134, CRC Press, Boca Raton, Fla, USA, 1990.
- Wekesa, V.W., Nguya, K.M., Knaap, M., and Hamadi, I.B (2005). Pathogenecity of *Beauveria bassiana* and *Metarhizium anosopliae* to tobacco spider mite *Tetranychus evansi*. *Journal of Experimental Acarology*. Volume 36. 41-50

- Wilfret, G. J., J. C. Raulston, S. L. Poe and A. W. Engelhard. (1973). Cultural Techniques for the Commercial Production of Annual Statice (*Limonium sinuatum* Mill) in Florida. IFAS Agricultural Research and education Center Bradenton. *Florida Agricultural Experiment Stations Journal Series* No. 5167.1: Pp. 399-403
- Wisniewski, G. (1999) Passive control of drying of herbs with utilisation of solar air type collectors and PV modules In: Renewable energy in agriculture: Proc. International conference, Lithuanian Institute of Agricultural Engineering, Raudonvaris, Lithuania, 16-17 September 1999. 79- 86; 5
- Yanar, D., Izzet, K and Ayhan, G. (2011). Acaricidal Effect of different plant parts extracts on two-spotted spider mite (*Tetranychus urticae* Koch). *African Journal of Biotechnology*, 10(55): 11745 – 11750
- Zhang Z. (2003) *Mites of greenhouses: identification, biology and control*, CABI Publishing, Wallingford, pp. 54–56
- Zhang W, McAuslane HJ and Schuster DJ. (2004). Repellency of ginger oil to *Bemisia argentifolii* (Homoptera: Aleyrodidae) on tomato. *Journal of Economic Entomology* 97: 1310–1318.
- Zieliński J, Petrova A and Tan K (2004) Taxonomic status of the roses (*Rosa*) described by S.G. Dimitrov from Bulgaria. *Annals of Botany*. Volume 41: 449-451.

APPENDICES

Appendix I: ANOVA tables for the efficacy of crude plant extracts on Mortality of *T. urticae*
1,3 and 6 days after treatment

Variate: 1 day

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	4.2222	2.1111	2.71	
Rep.*Units* stratum					
Treatment	8	82	10.25	13.18	<.001
Residual	16	12.4444	0.7778		
Total	26	98.6667			

Variate: 3 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.407	0.704	0.57	
Rep.*Units* stratum					
Treatment	8	79.852	9.981	8.01	<.001
Residual	16	19.926	1.245		
Total	26	101.185			

Variate: 6 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.074	0.037	0.03	
Rep.*Units* stratum					
Treatment	8	96.963	12.12	8.84	<.001
Residual	16	21.926	1.37		
Total	26	118.963			

Appendix II: ANOVA tables for the efficacy of crude plant extracts on repellence of *T. urticae*

Variate: 1 Hour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	3.1852	1.5926	6.14	
Rep.*Units* stratum					
Treatment	8	26.0741	3.2593	12.57	<.001
Residual	16	4.1481	0.2593		
Total	26	33.4074			

Variate: 2 Hours

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.8889	0.4444	0.68	
Rep.*Units* stratum					
Treatment	8	58.6667	7.3333	11.23	<.001
Residual	16	10.4444	0.6528		
Total	26	70			

Variate: 3 Hours

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.667	0.333	0.23	
Rep.*Units* stratum					
Treatment	8	66.667	8.333	5.71	0.002
Residual	16	23.333	1.458		
Total	26	90.667			

Variate: 4 Hours

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.889	0.444	0.22	
Rep.*Units* stratum					
Treatment	8	95.333	11.917	6	0.001
Residual	16	31.778	1.986		
Total	26	128			

Appendix III: ANOVA tables for effect of plant extracts on *T. urticae* mite population

Variate: Pre-treatment

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.519	0.259	0.22	
Rep.*Units* stratum					
TRT	8	21.185	2.648	2.25	0.079
Residual	16	18.815	1.176		
Total	26	40.519			

Variate: week 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	12.074	6.037	3.03	
Rep.*Units* stratum					
TRT	8	138.074	17.259	8.65	<.001
Residual	16	31.926	1.995		
Total	26	182.074			

Variate: week 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.4074	0.7037	1.71	
Rep.*Units* stratum					
TRT	8	494.519	61.8148	150.02	<.001
Residual	16	6.5926	0.412		
Total	26	502.519			

Variate: week 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.8519	0.9259	1.82	
Rep.*Units* stratum					
TRT	8	1190.52	148.815	292.22	<.001
Residual	16	8.1481	0.5093		
Total	26	1200.52			

Variate: week 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.2963	0.1481	0.18	
Rep.*Units* stratum					
TRT	8	546.519	68.3148	83.84	<.001
Residual	16	13.037	0.8148		
Total	26	559.852			

Appendix IV: ANOVA tables for effect of plant extracts on *T. urticae* Eggs population

Variate: Pre-treatment

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.5185	0.2593	0.41	
Rep.*Units* stratum					
TRT	8	8.5185	1.0648	1.68	0.18
Residual	16	10.1481	0.6343		
Total	26	19.1852			

Variate: week 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	29.852	14.926	6.99	
Rep.*Units* stratum					
TRT	8	909.852	113.731	53.29	<.001
Residual	16	34.148	2.134		
Total	26	973.852			

Variate: week 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	4.963	2.481	2.43	
Rep.*Units* stratum					
TRT	8	1652.07	206.509	201.84	<.001
Residual	16	16.37	1.023		
Total	26	1673.41			

Variate: week 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	2.6667	1.3333	2.13	
Rep.*Units* stratum					
TRT	8	1603.33	200.417	320.67	<.001
Residual	16	10	0.625		
Total	26	1616			

Variate: week 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0	0	0	
Rep.*Units* stratum					
TRT	8	583.333	72.9167	218.75	<.001
Residual	16	5.3333	0.3333		
Total	26	588.667			

Appendix V: ANOVA tables for quality parameters recorded per treatment in trial one**Variate: % Incidence**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	378	189	2.81	
Rep.*Units* stratum					
Treatment	8	370	46.25	0.69	0.696
Residual	16	1074.67	67.17		
Total	26	1822.67			

Variate: Aesthetic score

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.51852	0.25926	2.8	
Rep.*Units* stratum					
Treatment	8	1.40741	0.17593	1.9	0.131
Residual	16	1.48148	0.09259		
Total	26	3.40741			

Variate: Vaselife

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.5185	0.2593	0.51	
Rep.*Units* stratum					
Treatment	8	127.852	15.9815	31.38	<.001
Residual	16	8.1481	0.5093		
Total	26	136.519			

Appendix VI: ANOVA tables for quality parameters recorded per treatment in trial two

Variate: % Incidence

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	134.3	67.15	0.69	
Rep.*Units* stratum					
Treatment	8	499.19	62.4	0.64	0.732
Residual	16	1553.04	97.06		
Total	26	2186.52			

Variate: Aesthetic score

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.07407	0.03704	1	
Rep.*Units* stratum					
Treatment	8	2.74074	0.34259	9.25	<.001
Residual	16	0.59259	0.03704		
Total	26	3.40741			

Variate: Vaselife

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.2963	0.1481	0.23	
Rep.*Units* stratum					
Treatment	8	110.074	13.7593	21.23	<.001
Residual	16	10.3704	0.6481		
Total	26	120.741			

Appendix VII: ANOVA tables for yield parameters recorded per treatment in trial one

Variate: Chlorophyll

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	498.2	249.1	2.41	
REP.*Units* stratum					
TRT	8	895	111.9	1.08	0.423
Residual	16	1655.4	103.5		
Total	26	3048.6			

Variate: Stems per plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	1.8519	0.9259	1.05	
REP.*Units* stratum					
TRT	8	11.4074	1.4259	1.61	0.198
Residual	16	14.1481	0.8843		
Total	26	27.4074			

Variate: Stem length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	131.56	65.78	0.87	
REP.*Units* stratum					
TRT	8	777.33	97.17	1.28	0.319
Residual	16	1213.11	75.82		
Total	26	2122			

Variate: Bloom length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	2	1	0.26	
REP.*Units* stratum					
TRT	8	1812.67	226.583	58.47	<.001
Residual	16	62	3.875		
Total	26	1876.67			

Variate: Bloom width

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	2.889	1.444	0.3	
REP.*Units* stratum					
TRT	8	81.333	10.167	2.13	0.095
Residual	16	76.444	4.778		
Total	26	160.667			

Appendix VIII: ANOVA tables for yield parameters recorded per treatment in trial two

Variate: Chlorophyll

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum		2	270.96	135.48	2.01
REP.*Units* stratum					
TRT	8	1084.48	135.56	2.01	0.111
Residual	16	1076.56	67.29		
Total	26	2432.01			

Variate: Stems per plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum		2	0.6667	0.3333	0.36
REP.*Units* stratum					
TRT	8	9.3333	1.1667	1.27	0.323
Residual	16	14.6667	0.9167		
Total	26	24.6667			

Variate: stem Length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum		2	0.96	0.48	0.01
REP.*Units* stratum					
TRT	8	298.52	37.31	0.55	0.8
Residual	16	1079.7	67.48		
Total	26	1379.19			

Variate: Bloom length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum		2	25.41	12.7	0.65
REP.*Units* stratum					
TRT	8	875.19	109.4	5.58	0.002
Residual	16	313.93	19.62		
Total	26	1214.52			

Variate: Bloom Width

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum		2	37.852	18.926	2.98
REP.*Units* stratum					
TRT		8	128.296	16.037	2.53
Residual		16	101.481	6.343	0.054
Total		26	267.63		