GROWTH AND QUALITY OF ROSE (*Rosa hybrida* L.) CULTIVARS AS INFLUENCED BY POLY FILM COVERS AND DIFFERENT CONCENTRATIONS OF CALCIUM FOLIAR FEED

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A Thesis Submitted to Graduate School in Partial Fulfilment for the Requirements of the Doctor of Philosophy Degree in Horticulture of Egerton University

EGERTON UNIVERSITY

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DECLARATION AND RECOMMENDATION

Declaration

I declare that this is my original work and has not been presented in this or any other university for award of any degree.

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Recommendation

This thesis has been submitted with our approval as the university supervisors.

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DEDICATION

I dedicate this work to the almighty God, for he is Ebenezer and to my family.

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I thank the almighty God for the gift of life, guidance and protection. I will forever be grateful to God for the gift of provision and strength to pursue my postgraduate studies. Thus far the hand of God has been upon me.

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ABSTRACT

Rose cut flower production contributes significantly to Kenya's economy and its quality is therefore paramount for sustainability on the export market. Despite the tremendous contribution, profitable production of cut rose flower is limited by unsuitable growth conditions which lead to low quality produce. A study was set up at Egerton University to investigate sustainable ways to improve growth and quality of rose cultivars through integration of greenhouse growth conditions and calcium foliar feed. The experiment was laid down as a split split plot experiment in a randomized complete block design (RCBD) replicated three times. Poly film covers (UV-A clear, IR 504 and UV – A 205 / N) formed the main plot treatment with rose cultivars and calcium foliar feed forming the sub and sub-sub plot treatments respectively. Rose plants were established on raised bed at a spacing of 30 cm × 20 cm and mother shoot was bending done to induce sprouting of bottom breaks upon which production was based. Data collection involved measurement of growth variables and physiological parameters such as: physiological disorders, leaf chlorophyll content, dry matter partitioning and accumulation, anthocyanins and post- harvest physiology parameters. The collected data was subjected to analysis of variance (ANOVA) using SAS version 9.2 (SAS Inst., Inc., Cary, NC) computer package and significantly different means were separated using Tukey's honestly significant difference (HSD) at $P \leq 0.05$ %. The results showed that the poly film covers significantly affected the percent light transmission. The average light transmission over the production period was 1227 µmol .m^{-2}. s^{-1}, 840 µmol .m^{-2}. s^{-1} and 976 µmol .m^{-2}. s^{-1} under UV-A clear, IR 504 and UV-A 205/N respectively. Greenhouse microclimate significantly varied with the type of poly film. Mean air temperature of 41 °C, 35.2 °C and 32.8 °C were recorded under UV-A clear, IR 504 and UV – A 205 / N. Highest number of short stems < 40cm, was recorded under UV-A clear poly film for cultivar Red Calypso. Time taken between visible bud break and flowering differed a mong the cultivars between 43 and 50 for flushes I and II being 3-5 days shorter that the time taken under IR504 and UV-A 205/N. The combination of UV-A clear poly film, Red Calypso and 5.0 ml/L had the highest stem firmness (7.83). Red Calypso under the IR504 poly film with no calcium treatment had stems with the least firmness (5.0). The results of this study indicate that poly films and rose cultivars should be screened for suitability before adoption under different environmental conditions for production of quality rose cut flowers.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of variance
CV	Coefficient of variation
Chl a	Chlorophyll a
Chl b	Chlorophyll b
DAH	Days after harvest
DAP	Days after planting
DM	Dry matter
DW	Dry weight
EU	European Union
FR	Far red light
g	Grams
GDP	Gross Domestic Product
GOK	Government of Kenya
HCDA	Horticultural Crop Development Authority
HCD	Horticultural Crops Directorate
HPLC	High Performance Liquid Chromatography
IR	Infra-red
LDPE	Low Density Polyethylene
MOA	Ministry of Agriculture
NIR	Near infra-red light
P	Probability level
PAR	Photosynthetically active radiation
RH	Relative humidity
ROS	Reactive oxygen species
UV	Ultraviolet
UV - A	Ultraviolet A radiation
UV - B	Ultraviolet B radiation
UV - C	Ultraviolet C radiation

CHAPTER ONE
INTRODUCTION

1.1. Background Information

Horticulture is among the leading contributors to the Agricultural gross domestic product (GDP) in Kenya. It generates approximately US $ 1 billion and continues to grow between 15 % and 20 % annually (HCD, 2016). Evaluation of Kenya's horticultural exports in 2016 revealed that cut flowers, vegetables and fruits which are the main horticultural crops contributed 29 %, 31 % and 30 % respectively, to the total earnings in the sector (HCD 2016). The horticultural industry has continuously boosted the country's total earnings, for example Kenya shillings 305, 216 and 87.7 billion were earned in 2017, 2016 and 2015 respectively, from horticultural exports while Kenya shillings 82.24, 70.83 and 67.7 billion were earned from flowers in the respective years (Figure 1). Internationally, Kenyan cut flowers account for 32 % of flower exports to the European Union (EU) flower market one of the largest flower markets in the world (HCD 2015).

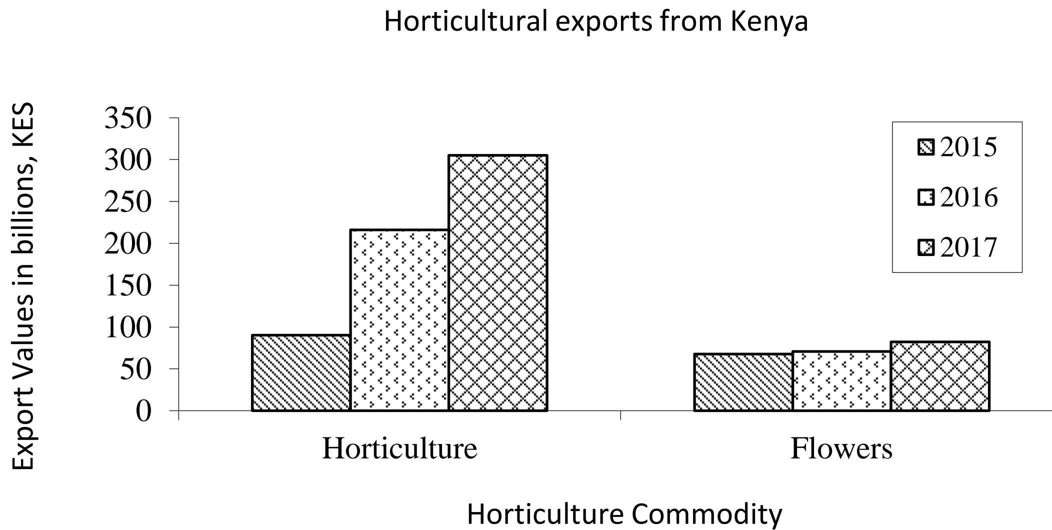


Figure 1: Total export earnings for three years from horticulture and cut flowers in Kenya

Source: HCD, 2015, 2016 and 2017 validated reports

Despite the enormous contribution, the sector is faced with several challenges ranging from social, economic, environmental, preharvest and post- harvest. These factors range from proper choice of greenhouse poly film covers, suitable cultivars and nutrition imbalance. The quality and vase life of rose cut flower is significantly affected if these preharvest factors are not properly monitored and controlled (Amal, 2013). Cut flower quality and extended vase life guarantee customer satisfaction which translates to market sustainability for the grower (Zhao *et al*., 2016). An evaluation of cut flower consumer behaviour showed that 49 % of cut flowers are purchased by guarantee seekers, 31 % by value conscious and 20 % by consumers who are just spenders (Rihn *et al*., 2014). The use of cut flower longevity guarantee can therefore increase consumer confidence and purchase of cut flowers as reported in the study.

Cut flower quality to a significant extent depend on the growth conditions which are greatly affected by the type of poly film used and management practices during growth and development. Greenhouse growth and development conditions are continuously changing especially with the current unpredictable changes in weather. Besides, the greenhouse providing protection from the external hazards, it is desirable that the type of cover used also provides favourable growth conditions for plant growth and development and hastens plant maturity (Gruda and Tanny, 2014) for more harvests (yield) and better quality to be achieved and hence profit. The choice of covers by growers in most cases is based on economic capability of the growers or alternatively they unconsciously purchase what is available because of little or no technical information on their effect on rose plant growth and development (Castellano *et al*., 2008; Al-Helal and Abdel-Ghany, 2011). This therefore leads to high costs of production specially in monitoring and maintaining greenhouse air temperature and relative humidity. Further, the growers have challenges to produce quality flowers under such conditions in order to compete effectively with already established growers on the market.

Currently there are poly films on the market with diverse characteristics in terms of colour, thickness and spectra properties. They have been developed to match certain requirements which include: High transmittance for visible light, such as photosynthetically active radiation (PAR) with wavelengths between 400 nm to 700 nm, low transmittance for long-wave radiation, in the range of wave lengths from 700 nm 2,200 nm, high reflection of near infra-red light (NIR), anti-drip, resistance to dust accumulation and sstrength against wind. Some poly film covers are also designed to reduce day air temperatures within the structure while other increase night air

temperatures accelerating plant growth and development. These poly films therefore differ in terms of light transmission, reflection and absorbance, the resultant effect being changes in greenhouse microclimate which impact on plant growth and development (Dehbi *et al.,* 2017).

Generally, poly films have the capacity to significantly modify the greenhouse air temperature to high levels above the prevailing natural conditions. The rise in air temperature may positively or negatively affect plant growth as the optimal air temperature required varies from plant to plant. In areas with high light intensity, growers have adopted application of white wash on greenhouse structures and poly film covers. In some instances, use of shading nets is commonly being adopted in case of extreme high air temperature and their positive effect has been reported on air temperature reduction (Lorenzo *et al*., 2006). Plant's ability to control growth and development is dependent on photosynthesis, a process that entirely depends on light intensity, quality and duration which in totality affect the surrounding air temperature.

Photosynthesis is the process that enables a plant to convert solar energy to chemical energy that it can use to fuel its activities. Efficiency of this process depends on how well the established the roots are to absorb water, then xylem carries it to the leaves and chlorophyll. It is well known that carbon dioxide is absorbed through stomata and goes to the cells containing chlorophyll. After that, chlorophyll absorbs light energy and lets photosynthesis happen. Interference with light absorption will in turn affect the operation of the stomates and consequently the entire photosynthesis process. Plants physological responses such as photosynthesis vary depending on alterations in the growth environment to the morphological changes therefore, the unconscious use of poly film materials could lead to photo morphogenic changes that may impact on plant growth, deveopment and quality (Eckardt, 2001).

The extent to which greenhouse environment is altered may in turn affect other plant pigments such as carotenoids and anthocyanins, that are predominantly found in the outer layers of the plant organs. In addition to their well-documented beneficial effects on plant physiological processes, anthocyanins have also been proposed to function in a diverse array of plant/animal interactions. These include the attraction of pollinators as well as the repellence of herbivores and pests. The optical properties of anthocyanins may serve as visual signals to potential herbivores, indicating a strong metabolic investment in toxic or unpalatable chemicals. Anthocyanins and carotenoids have also been implicated in the camouflage of plant parts against their backgrounds, in the undermining of insect crypsis, and in the mimicry of defensive structures. Naturally, there

is a wide range of anthocyanins, the most common examples being the glycosides of cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin. The colour and stability of anthocyanins depends on physiochemical processes namely, molecular structure, fluctuation in pH, air temperature, light, oxygen and metal ions (Andersen and Jordheim, 2008). Anthocyanins contribute to quality of rose flowers and they are very sensitive to light quantity and quality including air temperature fluctuations. Elevated air temperature for example inhibits anthocyanin biosynthesis (Yamane *et al.,* 2006) by suppressing the activities of the phenylpropanoid pathway that is responsible for anthocyanin biosynthesis.

Cut flower quality is also affected by the plant nutrients both macro and micro. Every single nutrient plays a specific role in plant growth and development and is required by plants in optimal quantities. Calcium is essential for many plant functions including starch metabolism, enzyme activity, nitrate uptake and metabolism, strengthening of the plant cell wall development and improving its structural integrity and cell division and elongation (Wilkins *et al*., 2016). Despite the myriad uses, calcium redistribution through the phloem vessels is very limited in plant tissues (Gilliham *et al*., 2011; Wilkins *et al*., 2016) warranting its continuous application during crop production. Calcium uptake is not as efficient as other plant nutrients especially when applied to the soil because anything that affects new root growth may prevent calcium uptake and induce a deficiency (Hetherington and Brownlee, 2004). Technologies should be adopted that will ensure availability of this nutrient to plants throughout the growth phase. Advances in plant nutrition have shown that foliar application of nutrients may partially or fully compensate for insufficient uptake of soil nutrients by the roots (Chwil, 2014). Application of plant nutrients through the leaves has been developed which guarantee availability of nutrients to the plants. Foliar application of nutrients has also been adopted for large scale use especially in application of aerial fertilizers.

The efficacy of the foliar feed is however, limited by both environmental and physiological factors. The key environmental factor is air temperature and relative humidity during the time of application. It is not known to what extent the environmental factors affect uptake of foliar based nutrients. Although air temperature and relative humidity must be correct to allow for maximum absorption. High relative humidity dilutes the fertilizer while higher temperature rapidly dries the leaves limiting the translocation. Pre - or post-calcium application is crucial in cut flower production since it plays a vital role in cut flower vase life (Rubistein, 2000; Capdeville *et al*., 2005). Calcium plays a significant role in cut flower post- harvest by creating water balance inside

and outside the plant cell that maintains turgidity leading to extended vase life. However, the right concentration of calcium foliar feed that will trigger production of high quality rose cut flower has not been tested. This research therefore, evaluated suitable combination of greenhouse poly film covers, cultivars and different concentrations of calcium foliar feed for improvement of growth and quality of rose flower.

1.2. Statement of the problem

Rose flower industry is faced with multiple production challenges in the developing countries. The major constraints are: limited information on the choice of suitable greenhouse covering material, suitable cultivar and proper supplemental nutrition for production of high quality flowers. The available greenhouse covers vary in their light transmission properties and are highly photodegradable, yet most cut flower growers are not aware of its effects on growth and quality of rose flowers. The pre - harvest conditions also affect cut flower post- harvest impacting on quality. Calcium, one of the most essential elements of the cell wall that plays a significant role in flower's vase life is not readily available to plants because of its low mobility and translocation. Furthermore, calcium in the old tissues cannot be remobilized and utilized by young tissues in the plant when deficient. However, there are many rose cultivars on the market that differ in their response to different growth conditions which in turn affects growth, quality and vase life. There is need therefore, to quantitatively examine the effects of poly film covers, cultivars and calcium foliar feed on growth and quality of rose cut flower.

1.3. Objectives of the study

1.3.1. General objective

The study was aimed at establishing sustainable ways to improve growth and quality of rose cut flowers through integration of greenhouse growth conditions and calcium foliar feed.

1.3.2. Specific Objectives

The specific objectives of the research were to:

1) Determine the effect of poly film covers on photosynthetic active radiation and greenhouse microclimate.
2) Determine the effect of the poly film covers on growth and quality of rose flowers.
3) Establish the effect of different concentrations of calcium foliar feed on growth and quality of rose flower.

4) Determine the effect of poly film covers, rose cultivars and different concentrations of calcium foliar feed on occurrence of physiological disorders
5) Determine the effect of poly film covers, rose cultivars and different concentrations of calcium foliar feed on the vase life of rose cut flower.

1.4. Hypotheses of the study

The hypotheses for the research were:

1) H_0: Poly film covers have no effect on photosynthetic active radiation and greenhouse microclimate
2) H_0: Growth and quality of rose flower is not affected by the poly film covers
3) H_0: Growth and quality of rose flower is not significantly affected by different concentrations of calcium foliar feed.
4) H_0: Occurrence of physiological disorders is not affected by poly film covers, rose cultivar and different concentrations of calcium foliar feed
5) H_0: Poly film covers, rose cultivar and different concentrations of calcium foliar feed does not significantly affect rose cut flower vase life.

1.5. Research Justification

The choice of suitable greenhouse covering material is a challenge to many rose flower growers. Most growers have therefore missed out on the benefits of greenhouse usage. The result has been production of low quality cut flowers. In addition, proper choice of greenhouse covering material creates suitable micro climate which favours production of high quality rose flower resulting to a reduction in production costs and post-harvest losses. Right choice of rose cultivar which can withstand rapid changes in the environment and maintain quality is also beneficial for the rose cut flower growers. Consumers are so particular on the attractiveness of the cut flowers and vase life longevity. Consequently, every consignment for export must be accompanied by a vase life guarantee. In practice, rose growers use commercial flower preservatives such as silver thiosulphate (STS), Florissant, 8 - hydroxyquinoline citrate (8 - HQC) among others to enhance vase life some of which contain heavy metals making them unsustainable and environmentally unsafe. Manipulation of pre- harvest growth conditions to enhance growth and quality of rose flowers may be a viable, inexpensive and safe alternative to silver-based compounds that are currently being used for vase life extension. In addition, the livelihoods of about 2 million Kenyans

directly or indirectly depend on the rose flower industry. It is therefore imperative to come up with sustainable ways that will assist growers produce high quality cut flower that meet export standards and protect their share on the international markets.

CHAPTER TWO
LITERATURE REVIEW

2.1. Spectra properties of poly film covers

Poly films are popularly known as polyethylene or agricultural plastic films. They are made from materials that are synthetic in origin. Poly film covers are mainly used in agriculture to provide translucent separation between the outdoor and greenhouse environments in protected farming. The main aim being to maintain production all year round, minimise on pest and disease incidences, enhace quality of produce and timing of peak production periods. As a result, the capability of the poly film to allow maximum possible light for better plant growth and development is of primary concern to most greenhouse growers. However, the intensity of solar radiation that penetrates the covering material to reach the plants is considerably affected by atmospheric water vapour, carbon dioxide concentrations, greenhouse structure and other environmental contaminants (Krizek *et al*., 2005). Light transmission is further affected by water condensate on the poly film cover and thickness of the cover. The effect of condensation is more on untreated poly film, the condensate occurs in small droplets which reduces light transmission from solar radiation through multiple reflections.

Reduction in light transmission may occur further due to dirt deposition which accumulate during production that can be approximated around 9 % to 15 % (Cerek and Demir, 2005) and some light is also lost through reflection and absorption by greenhouse construction material. In addition, the degree of poly film degradation and shape of the structure being covered affect light transmission considerably. Poly films degrade with extended exposure to solar radiation. They begin to transmit more light than the initial desired percentage which ncreases the surrounding air temperature. The biggest challenge in greenhouse usage is how to enhance light transmission while keeping greenhouse air temperature at optimal level. Efforts to reduce greenhouse air temperature has equally result to reduction in light transmission. Greenhouse growers therefore must select appropriate poly film covers to avoid negative impact on the plants.

Recently greenhouse production has embraced the use of cladded poly films which comprise of many additives. The technology involves addition of different dyes and other additives during manufacture giving poly films of different colours and consequently spectra properties. Some poly films have infra - red (IR) additives which assist in maintaining a stable greenhouse

working environment especially in areas with strong solar radiation. The modification has several advantages; it creates conducive environment for workers, reduces costs of greenhouse cooling and humidification, reduce irrigation frequency and plant heat stress. Besides controlling day time air temperature, the infra-red treated poly film can maintain higher night air temperature leading to increased plant growth and development. UV - blocking poly film is another type, it is stabilized to minimize the photo degradation and enhance poly film life span. Others poly films have anti-drip properties to allow for maximum light transmission and keep plant foliage dry with minimum diseases occurrence. The colour of the poly film causes transmitted light to diffuse within the greenhouse which is advantageous to plants in areas with high solar radiation. However, this property may not be so beneficial in areas with limited spectra since it reduces the amount of light transmitted.

Light spectra have different wavelengths, of which the most important for plant growth falls within a range of 400 nm to 700 nm i.e. photosynthetically active radiation (PAR) (Kempkes *et al*., 2008) which accounts for 45 % of the solar radiation. Other wave bands include: UV - B (290 nm to 320 nm) and UV - A (320 nm to 380 nm) that constitute 3 % and 52 % is the near infrared light (700 nm to 2200 nm) respectively. Plants response to different wave length differs and any slight change can trigger noticeable changes in growth and development. Within the narrow waveband of PAR, plants perform better photosynthetically at 525 nm (yellow region of the visible light) therefore interrupting any colour of the waveband will cause considerable influence on plant growth and development.

Currently manufacturers modify the poly film colour which has direct effect on light transmission that reaches plants growing under a specific structure. Several studies have demonstrated that poly film optical characteristics can be altered by their colour (El nono *et al.,* 2008). Ngouajio and Ernest (2004) reported variation in light transmission in the PAR wave band of 0 % to 37 % for black and white poly film mulches respectively. Baiyeri (2005) observed light intensity reduction (3.6 %) by the colourless polyethylene compared to 30 % and 35 % reduction by blue and yellow polyethylenes respectively. Similar observation was made by Blanke (2009) where white translucent shade net reduced visible light (PAR 400 nm to 700 nm) by 8.4 % compared to 13 % by red-black and 16.1 % by black.

The range of additives used, alter the spectra properties in terms of their effect on light transmission, absorbance and reflection. Some poly films have been modified to be highly photo

selective at different wavelengths (Li *et al.,* 2000). This in turn creates a dynamic nature of greenhouse environmental variables that alter the greenhouse microclimate (Lefsrud and Kopsell, 2005). Air temperature, photosynthetic active radiation and relative humidity are among the essential affected microclimatic elements (Peetz *et al.,* 2009). Depending on the properties of the poly film used, greenhouse relative humidity builds-up during the coldest part of the day resulting to condensation. The condensate reduces light transmittance through a wet polyethylene film by 15 % compared to dry polyethylene film causing modifications in plant growth and development that could interfere with yield and quality (Monk, 2016; Peetz et al., 2009). The percentage of light transmitted through the poly films is also highly depend on the concentration of the dye added during manufacture. Transmission in the visible spectrum decrease due to dye increment in the film structure and increase in absorption coefficient limiting the amount of light that pass through (Esfahani *et al*., 2014).

During the hottest part of the year, greenhouse farmers adopt whitening of the poly film to mitigate the hot air temperatures. This practice though common in managing high solar radiation, in the tropics it may result to reduced transmission coefficient from 0.62 to 0.31 causing drastic changes in relative humidity, canopy and air temperature (Baille *et al.,* 2001) which may impact on growth and development of plants. In principle the practice imitates the use of coloured poly films that are known to diffuse light within the growth structure and minimise the direct effect of solar radiation on plants. Depending on the choice of the poly film some growers incur added costs to mitigate the effects of elevated air temperature. It has been reported that white poly films reduce air temperature within the structure while the use of blue type of poly film accumulate air temperature within the greenhouse structure compared to green, yellow and black colours (Khandaker *et al.,* 2010). The variation in air temperature and light transmission cause differences in plant growth namely; plant height, stem length, leaf number as well as fresh weight and dry weight (Li *et al.,* 2000; Hemming *et al.,* 2004).

Poly films tend to degrade with repeated usage over time (Shen *et al.,* 2000) making them permissive to transmission of high light intensity. Such poly films allow transmission of wavelength that may not be suitable for plant growth. Environmental conditions such as air temperature, solar irradiance, relative humidity and wind influence ageing and mechanical properties of low density polyethylene (LDPE) greenhouse covers. UV radiation (290 nm to 400 nm) part of the solar spectra, when absorbed by the polyethylene leads to bond

cleavage and polymerization, causing photo-degradation (Shen *et al.,* 2000). Conventionally, greenhouse covers are meant to serve the function of creating an internal environment that is conducive to plant growth compared to the external environment. This has not always been the case since light transmission is influenced equally by structural and environmental conditions (Kittas *et al*. 2006) altering the quantities received by plants. Prolonged usage of the poly film will therefore alter spectral properties causing conditions that are unfavourable for production of quality plants.

2.2. Light quantity and quality effect on plant growth and development

Plants use various mechanisms to detect information in the environment regarding light quantity, quality, direction, periodicity and the degree of polarization. Plants will modify their morphology and physiology, and eventually their growth and reproduction, once one of these components of light changes. Light quantity also referred to as light intensity is the total amount of solar radiation that the plant receives. Light intensity describes the number of photons of light within the photosynthetic wavelength that is received within a second in an area of one square metre. Light is important for photosynthesis a process that supports plant growth. Photosynthesis rate is higher as the photosynthetic active radiation increases until it reaches a light saturation point.

Light quality and quantity affect productivity and quality of ornamental plants besides growth and development. Plant response to light is influenced by the fluctuating environmental conditions and more so light properties like duration, intensity and quality (Kittas *et al*., 2011). Moreover, environmental conditions tend to vary with geographical location, time of the day and season. In recent times, plant growth and development can be regulated by modifying the wavelength of light reaching the plants (Kittas *et al*., 2011; Cerny *et al*., 2004). Manufacturers of greenhouse covers have produced poly films with low far – red light transmission which have been used as alternatives in many plant species to manipulate height (Rajapakse and Li, 2004; Fletcher *et al*., 2005).

Photo selective covers have been developed that regulates the phytochrome photo-equilibrium ratio (Pfr: P total ratio) to an estimated value of 0.72 to 0.82 by incorporating various amounts of far red light absorbing dye into greenhouse covering material (Kubota *et al.,* 2000). Such modifications result in morphological changes altering processes such as; internode elongation, chlorophyll development, flowering, lateral bud outgrowth, root and shoot

development. Through cladding the photo selective covers also regulate UV - radiation that is regarded as a stress factor because of its effect on plant growth. Plant growth attributes such as height, leaf area and leaf length decrease in response to ultraviolet B radiation (Cerny *et al*., 2004). Red and far red wave band influence the phytochrome pigment which initiates photomorphogenic changes in plants (Eckardt, 2001). Light quality is therefore very important since the highest energy corresponds with the lowest wavelength.

Different wavelength impact on plants differently, for example the ultraviolet light when transmitted in the greenhouse may cause damage in the DNA, affect flowering, seed formation and cause reduction in the rate of photosynthesis. Red light of the spectrum increases stem diameter which is a key quality attribute in cut flower production. Thicker stems are not so desirable because of increase in freight charges. The far-red light cause plants to elongate a phenomenon that occurs when plants cast shadows on each other during growth. Poly films cause total variation in the quality of light received as such their use trigger various responses in plants. The wave band varies from one poly film to the other depending on colour and gauge.

The red: far – red ratio also affect bud sprouting, while photon flux density affects flower development. These physiological processes and many more differ with the stage of plant growth (Celik and Odabas, 2009; Omer *et al*., 2010), a fact that should be observed in providing ideal growth conditions to plants. Previous studies have shown that absorption of the far – red light of the poly films increase with the concentration of the dye (Cerny *et al*., 2004; Esfahani *et al*., 2014; Omer *et al*., 2010). Intensity of light in the far – red region affects morphological plant responses (Sultan *et al.,* 2002) which may have positive or negative impact on the plants. The red and far - red light inhibits and promotes stem elongation of seedlings respectively (Takaichi *et al.,* 2000; Cerny *et al.,* 2004). The higher the R to FR ratio or (P_{fr} / P_{total}) the earlier and greater the inhibition of stem elongation (Shumin and Nihal, 2003). Depending on the quality of light transmitted plant quality may be jeopardized.

Different components of productivity and quality such as bud breaking, rate of flower abortion, formation of renewed shoots, duration between harvests, length, weight and diameter of stem and flower bud, leaf area and pigmentation of petals are affected by light. There is a direct relationship between light intensities and growth rate. Decreased illumination results in reduced plant growth and development (Hendrickson *et al*., 2004) because of its effect on photosynthesis. Growth increase with gradual increase in light intensity (Dieleman and Meinen, 2007), up to a

certain limit and decreased illumination decreases the total shoot production (Atsushi *et al*., 2005). Total dry matter production decrease with decreasing light intensity (Akhter *et al.,* 2009). Likewise, increase in light intensity, during the time of flower development, produce cut flower with optimal carbohydrate level hence quality flowers with longer vase life are obtained if the optimal requirement is not exceeded (Akhter *et al.,* 2009; Petridou *et al.,* 2001).

Greenhouse air temperature at any specific time is directly linked to light intensity, the lower the intensity the lower the air temperature and vice versa. Air temperature as such is a critical component of microclimate that affects plant growth and development (Usami *et al.,* 2001; Pettersen *et al.,* 2007). Slight change in air temperature above the ambient conditions, cause increase in stem diameter, biomass and leaf area although extreme air temperatures negates these positive changes (Pettersen *et al.,* 2007). Additionally, increase in average daily air temperature, reduce days to flowering and mean growth rate accelerating flower maturity (Usami *et al.,* 2001; Pettersen *et al.,* 2007). Excess light intensity on the contrary is associated with increase in air temperature that cause reduction in photosynthesis activities while respiration continues. The resultant effect is low availability of carbohydrates required to support plant growth and vase life extension.

Light manipulation leads to modification of growth conditions which contributes to changes in plant photo-morphogenesis. Such alterations also have resulted in physiological disorders in rose plants the most common being blind shoots, i.e. failure of the shoot to develop a flower bud. The occurrence of blind shoot is attributed to low photosynthates accumulation (Dieleman and Meinen, 2007) especially under high air temperature conditions (Naftaly and Mor, 2003). Under such condition reduction in chlorophyll content and light absorption for photosynthesis occur. High air temperature further triggers several physiological processes within the plant; including loss of plant turgor, closure of stomate and slow rate of carbon dioxide diffusion in the leaves. Low air temperatures on the other hand cause petal blackening phenomena that affects colour especially in red roses due to accumulation of anthocyanins in the petals causing massive post- harvest losses to rose growers.

2.2.1. Light and air temperature influence on plant quality and physiological disorders

Light is a major environmental factor that affects plant growth, since plants response depends on the way they perceive light. Extreme air temperatures have been reported to be

detrimental at all stages of plant growth and development (Allen and Ort, 2001; Hilal *et al.,* 2004). Naturally, most plants evolve mechanisms to cope with environmental stresses at various stages of growth and development. It is common for plants to accumulate the ultraviolet light absorbing compounds mostly flavonoids and other phenolic compounds such as phenolic acid within the walls which influence the reflective properties of the leaf by acting as a photo protective layer (Hilal *et al.,* 2004). These self-mitigating mechanisms are very useful in plants, for example elevated air temperature induce stomatal closure, reducing carbon dioxide uptake that could enhance photosynthesis a factor very important in plant quality (Allen and Ort, 2001).

Plants grown under low light levels exhibit enhanced light interception by means of increased biomass allocation to leaves (Wang and Feng, 2004). In addition, low light intensity results in low photosynthates accumulation leading to poor vase life of the cut flower. Low light intensity has been implicated for high percentage of blind shoots (Jens *et al.,* 2005) a physiological disorder that lowers the number of harvestable stems in roses and other flowers like Alstroemeria. High light levels on the contrary, exhibit reduced transpiration losses and increased carbon gain by making small-sized, thick leaves with a low leaf area (Wang and Feng, 2004).

The effect of light on plant quality is not just limited to the physical attributes as mentioned above, some responses are physiological or occur at biochemical level which is not easily noticeable. One of the most affected quality features in rose flower by light is colour development which is linked to the anthocyanin content in the plants (Ubi *et al.,* 2006). Anthocyanin is well known to be the largest group of water soluble pigments that are widely found in cut flowers (Clifford, 2000). Anthocyanins are derivatives of anthocyanidins (sugar free molecules) and they are water soluble vacuolar pigments. Anthocyanins act as light attenuators, antioxidants, give colour to plants and flowers and may be used as pH indicators among others.

Cut flowers and other flowering plants contain high percentage of anthocyanin predominantly in their flower petals. Colour is the major attribute of cut flower quality and it impacts on the value of the produce and if the attractiveness is lost it may affect the market preference. Anthocyanin content and composition is correlated with air temperature and further implicated to a variety of developmental and environmental stimuli (Liu *et al*., 2018). It is mainly dependent on the concentration of anthocyanin in flower petals whose quantity and distribution further depends on substrate pH, light, oxygen concentration, metallic ion and air temperature among others. Environmental factors are imperative in causing noticeable changes, making light

and air temperature the main external factors affecting anthocyanin accumulation in plant tissues (Liu *et al*., 2018; Tian *et al*., 2015).

Anthocyanins biosynthesis is quite dynamic with changes in air temperature. High air temperature for example decreases anthocyanin concentration while low air temperature increases the concentration in plant tissues (Riyuan *et al.,* 2008). However, the effect of air temperature on the anthocyanin biosynthesis tends to differ from one plant species to the other (Mori *et al.,* 2007). The natural variation in quality and quantity of light modifies plant morphology and concentration of biochemical compounds constituted within their cells (Tegelberg *et al*., 2004). In addition, several factors influence plant pigmentation causing major variations in their stability. Among them we have inherent traits within a cultivar, plus environmental factors like light intensity and relative humidity which may have influence singly or synergistically on plant quality (Kleinhenz *et al*., 2003).

In red rose cultivars, physiological, biochemical and genetic changes occur during flower development as a response to environmental stimuli. Visual changes in colour intensity and other hues like bluing and blackening of petals have been observed to occur with changes in environmental stimuli (Sood *et al.,* 2006). The type of anthocyanidin, its glycosides of anthocyanidin, its glycosylation pattern, and its acylation with aromatic and/or aliphatic acids influence anthocyanin stability (Stintzing *et al.,* 2002). In addition, anthocyanin concentration is affected by factors related to media quality such as acidity that negatively correlates with the light intensity (Riyuan *et al.,* 2008). The most frequent occurring anthocyanins are the glycosides of cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin. However, the major export cut flowers; roses, carnations and chrysanthemum do not accumulate delphinidin-based anthocyanins. This is attributed to their deficiency of flavonoid 30, 50 - hydroxylase (F3050H), a key enzyme in the synthesis of delphinidin.

Pigmentation of leaves, fruits and flower differ in plants at various stages of growth. The concentration is further affected by various biotic and a biotic factor. The concentration of chlorophyll and carotenoids is controlled by light via photoreceptors and tissue specific signals and responses (Li *et al.,* 2008). Air temperature is a strong environmental determinant of anthocyanin accumulation and its constant daily fluctuation is in inverse proportionality with the anthocyanin content within the plant tissues (Liu *et al*., 2018). Anthocyanins are further affected

by preharvest management practices which involve nutrition during the production process and the canopy microclimate (Liu *et al*., 2018; Ubi *et al.,* 2006).

Besides colour development, quality of rose flower is defined in terms of sound stems that are strong, free of physiological disorders and any deformities. Light quality and air temperature are implicated in the occurrence of the main physiological disorders in rose flower that reduce quality and productivity. Shoot blindness, petal blackening and bent peduncle all occur as result of fluctuations in environmental factors (Kim and Lee, 2002). Blind shoot occurrence escalates under low night air temperature and low PAR (Kim and Lee, 2002). High air temperature and low relative humidity that affect photosynthesis result in poor quality stems with more blind shoots (Dayan *et al.,* 2000) due to their effect on photosynthesis and other physiological processes.

Management of greenhouse environment is therefore imperative for every farmer to grow quality produce. Greenhouse microclimate control is an essential consideration regarding maintaining photosynthesis at optimal levels. The rate of transpiration varies by plant age and season, warranting continuous control of greenhouse environment throughout the year to attain ideal growth conditions. Increase in relative humidity increase plant growth by causing opening of stomates that enhance carbon dioxide uptake that is essential for photosynthesis. On the contrary, excessive relative humidity favour development of fungal diseases that may not support quality flower production.

2.3. Effect of calcium fertilizer on plant growth and development

Calcium is an important plant nutrient as it forms calcium pectate a major constituent of the plant cell wall. Calcium is highly immobile, it is translocated through the transpiration stream. Factors that affect transpiration such as high relative humidity and low air temperature obviously affect calcium uptake and availability. Calcium contributes significantly to stem strength and minimise cases of cut flower breakages during post - harvest handling. Conventionally, plants take up nutrients from the soil through the roots. However, calcium is mostly unavailable to plants while it is available in adequate quantities in the soil solution due to poor distribution and difficulty in remobilization from old tissue to new tissues.

Advances in fertilizer application has led to development of fertilizer supplements and nutrients that can now be applied directly to the leaves (Baloch *et al.,* 2008) with guaranteed availability of nutrients such as calcium that are hardly translocated in the plant system. One of

the benefits of foliar fertilization is the increased uptake of the nutrients as opposed to soil application. This is because foliar fertilization causes the plant to pump more sugars and other exudates from its roots into the rhizosphere (Nadlhoffer, 2000). Secondly, beneficial microbial populations in the root zone are stimulated by the increased availability of these exudates (Nadlhoffer, 2000) accelerating plant growth. Foliar application of plant nutrients is ideal in immediate correction of plant deficiencies and contribution to proper plant growth and development.

Tests conducted in separate locations, under different environmental conditions, have revealed that when fertilizers are applied as foliar, more significant amount of the fertilizer is utilized by the plant than when a similar amount is applied to the soil (Mengel and Kirkby, 2001). The quantities of fertilizer used as foliar are low as opposed to soil application. The amount of fertilizer applied to the soil, is lost through various processes and only 10 % of it is utilized (Mengel and Kirkby, 2001). Deviation of soil air temperature from normal and soil pH from the neutral, affects availability of nutrients to plants (Jing *et al.,* 2011). Foliar fertilizer application of plant nutrients that are slowly translocated may be the viable mitigation to ensure adequate plant nutrient availability and increased productivity (Yildirim *et al.,* 2007).

Calcium is an important plant element and plays key role in plant cell expansion, physiology and cellular responses to the environment (Antony *et al.,* 2010). Frequent disorders related to calcium deficiency may occur in plants because of poor calcium translocation (Camberato and Pan, 2000). It has been established that calcium deficiency may occur when the concentration of calcium in the irrigation water is lower than 1.0 mmol l^{-1} (Bar-Tal et *al*., 2001). The mode of application therefore determines calcium availability (Naoya *et al.,* 2007) making foliar application of this plant nutrient more viable to soil application. To combat the post- harvest physiological disorders related to this plant nutrient, calcium is applied just before harvest. The amount of calcium ions assimilated in the leaves is proportional to the concentration supplied in nutrient solution (Garduno *et al*., 2008, Amir *et al.,* 2009). Nevertheless, calcium deficiencies may still be exhibited even after foliar spray. Calcium distribution in the plant tissue is not uniform, inferior quantities are found in reproductive tissues as opposed to vegetative (Bonomelli and Ruiz, 2010) necessitating deficiencies.

The effectiveness of foliar feeds is affected by temperature, pH, addition of surfactants and humid moist conditions (Bradley and Brayan, 2002). The efficacy of the fertilizer depends on

the type of salt applied for example calcium chloride, calcium nitrate, and potassium phosphate (Ehret *et al.,* 2002). In most cases calcium, should not be evaluated singly because while in the plant there are antagonistic reactions that occur with other nutrients such as potassium, magnesium and other cations (Amir *et al*., 2009). The effect is referred to as dilution effect which relates to different roles played by the plant nutrients concurrently in the plant. Nitrogen in this case enhances growth of plant tissues while calcium accelerates the dry matter accumulation masking the effect of calcium on plant growth and development.

Calcium uptake is proportional to the rate of transpiration and declines under high humid and low light conditions that limit transpiration rate (Marchner, 2002). Transpiration is affected more by environmental factors under warm conditions where water movement outside the plant increases and vice versa. Warmer environment also triggers opening of the stomates accelerating the transpiration rate. Other factors include; light intensity, wind and more importantly soil water status which is the main source of water for transpiration. Timing of foliar application is therefore very important, for better absorption in fruits and flowers. Best results have been achieved when the foliar feed is applied at the time when the plant presents its greater sink capacity (Bonomelli and Ruiz, 2010) probably at the initial flower bud formation. Faster growth and development of buds on the rose plants is accelerated by calcium application (Asfanani *et al.,* 2008). Calcium therefore, plays vital role in nearly all aspects of plant growth and development.

2.3.1. Effect of calcium on flower quality

Quality is what defines the distinctiveness of an attribute possessed by one produce over the other. Cut flower quality may be described by several factors such as freshness which can be identified from consistency of colour and petal presentation. Quality cut flowers also should have shiny leaves with no pest damage and strong stems that are not likely to break easily. Post- harvest quality of both fruits and flowers is enhanced by calcium application, although results of different authors are contradictory on this issue. While some authors have observed positive results with calcium in fruit quality (Amir *et al*., 2009, Luo *et al.,* 2009), in another study no significance difference with the control treatment was reported (Bonomelli and Ruiz, 2010). In 2003, Conway *et al.* carried out a study on pre-and post- harvest calcium treatment in apples and established that direct application of calcium to the fruit either by dipping or spraying was the most effective method for increasing fruit calcium content. It is important to note that complex processes occur

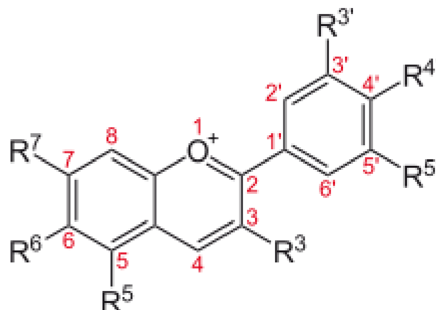
in the soil and one nutrient can influence availability of the other for example effect of excess calcium on potassium is well documented (Dilmaghani *et al.,* 2004).

Foliar feeding is used as a means of supplying supplemental doses of minor and major nutrients, plant hormones, stimulants, and other beneficial substances. Increasing calcium concentration in the irrigation solution, increased calcium concentration in the flower organs (Bar-Tal *et al*., 2001) which pose considerable risk of toxicity. In addition to the role of calcium in cell integrity (Fallahi *et al*., 2006), it also plays a significant role in the sugar transduction pathway regulating anthocyanin accumulation that mostly affect the quality of cut flowers (Xavier *et al*., 2000). The efficacy of calcium either as a foliar or soil applied fertilizer depends on its interaction with other nutrients. Calcium is known to increase the rate of biomass production in plants although negative relationship has been reported whereby nitrogen, potassium and magnesium may have antagonistic effect with calcium depending on the concentration available in the media (Fallahi *et al*., 2006; Favaro *et al*., 2007).

Response of plants to pre- harvest application of calcium, is dependent on species, fertilizer form, concentration, and frequency of application, as well as the stage of plant growth (Kuepper, 2003, Amiri *et al*., 2009). Late season application is more effective at increasing calcium content in the plant tissues than early season applications (Benavides *et al*., 2001). Increasing the calcium concentration in the irrigation solution from 1.25 mmol l^{-1} to 3.75 mmol l^{-1} significantly increased the calcium concentration in the leaf and the petals of rose cv. Jaguar (Bar-Tal *et al*., 2001). Other plant attributes such as stem strength increased under conditions of low nitrogen, phosphorus, potassium and magnesium (Kadir, 2004). During application, there must be a balance with other soil nutrients, since tissue uptake and utilisation depends on other nutrients too.

Colour is an important quality determinant in cut flower production. Marketability and commercial value of cut flowers is directly influenced by it. Anthocyanin is what determine the attractiveness of flowering plants and it is affected by both physical and chemical factors. Anthocyanin content in cut flowers is affected more by pre- harvest conditions to which the plant is subjected during growth and development. Anthocyanins therefore vary considerably with changes in the environment, for example anthocyanin content decreases with increase in air temperature and increase with a decrease in air temperature. The phenomena occur due to reduction in anthocyanin biosynthesis rate that suppress expression of genes relevant in anthocyanin biosynthesis (Lai *et al*, 2011). According to Schiber *et al*. (2005) study, they reported

that a wide range of colours is not only dependent on substrates accumulation but also on other factors such as co-pigments, vacuole pH and cell shape. Anthocyanins, whose basic molecule is shown below is affected by factors that are both pre- or post-production.



Basic anthocyanin molecule

Environmental factors such as air temperature received during growth reduce anthocyanin content of flower petals (Dela *et al.*, 2003). In other studies, it has been argued that plant growth and development is determined by the genomic characteristics of the plant that affect fundamental components like the flower colour (Gudin, 2000). Methylation of anthocyanins from the molecular perspective is catalysed by S-adenosylmethionine an anthocyanin methyltransferase (AOMT) enzyme. Depending on the changes that occur during biosynthesis different molecules are formed resulting to diverse types of anthocyanins. Peonidin type of anthocyanins is biosynthesized from cyanidin, while petunidin and malvidin are biosynthesized from delphinidin type of anthocyanins (Tanaka *et al.,* 2008). The type of anthocyanin produced in plants varies from one species to another depending on their genetic constitution. Rose and carnation the major cut flowers for example are only able to produce anthocyanin based on pelargonidin and cyanidin. Cut flower consumers may prefer rose stem in which the leaves have deep green colour that blends well with other shades of the bloom. The attractiveness of these shades is to a large extent influenced by anthocyanins content in the flower petals.

2.3.2. Effect of light, cultivar and calcium on cut flower vase life

Cut flowers are highly perishable and require utmost care immediately after harvesting for extended longevity. They undergo very high respiration rates, rapid deterioration and are highly susceptible to damage. Cut flower post - harvest is greatly affected by pre- harvest conditions namely the biotic and a biotic factor (Ichimura *et al.,* 2003; Shirzadeh *et al*., 2011). Depending on

the extent of the pre - harvest factors whether they are balanced or there is a deficiency, the cut flowers are pre - disposed to some disorders that may fully express themselves during post-harvest. The common ones include; bend necks, petal blackening like in red rose flowers, wilting of the leaves, early leaf senescence and leaf defoliation. Calcium pre - harvest treatment may defer senescence in cut flowers (Rubistein, 2000; Hussain *et al*., 2012; Bagheri *et al*., 2015) and in fruits and vegetables. Plants grown with enough calcium nutrition are less susceptible to a wide range of fungal infestation (Shirzadeh *et al*., 2011) hence enhanced vase life.

Calcium salts have been used in post- harvest handling of fruits and flowers with a target of extending holding time. Different calcium salts namely the sulphates, nitrates, silicates and chlorides have been tested and established to differ in their solubility and mobility affecting their distribution in the plant organs. Phase of plant growth during application enhance uptake for example during periods of vigorous growth and in leaves because of their larger surface area they have high transpiration rate and are more effective to divert calcium from the fruits and flowers. It is known that greenhouse covers modify the microclimate surrounding plants (Khattak *et al*., 2011) a factor that impacts on cut flower post- harvest. This in turn affects plant characteristics that are important in transpiration rate, number and size of stomata, and closing behaviour of stomata (Uulke *et al*., 2005). With stomata closure the accumulation of photosynthates essential for cut flower growth and sustainability are limited. The relative humidity level in the greenhouse that is partly influenced by microclimate is the most important variable determining differences in vase life (Uulke *et al*., 2005). Relative humidity greater than 70 % for example predisposes flowers to fungal attack. Pathogens infestation at this level affect plant quality at cellular level or by general reduction in yield.

Vase life enhancement requires adequate quantities of carbohydrate reserve (Pompodakis *et al*., 2005). Decreased carbohydrate reserve coinciding with a low photosynthetic capacity may cause post production carbohydrate deficiency. Vase life of roses grown during low air temperature period was reported to be significantly shorter compared to roses grown during warm season. This is correlated to reduced photosynthesis and smaller carbohydrate pools during low air temperature season (Pompodakis *et al*., 2005). Flowers grown at high light intensity had prolonged cut flower post- harvest being associated with presence of high carbohydrate concentration in the flowers. Carbohydrate concentration plays a significant role in flower senescence as a substrate for respiration, structural materials and osmoticum (Ichumura *et al*., 2000). Sugars elongate vase

life by suppression of ethylene biosynthesis and decrease in sensitivity to ethylene (Pompodakis *et al.,* 2005).

Quality of fresh cut flowers depend on many factors amongst them being; maturity at the time of harvest, depletion of food reserve, air temperature fluctuations and suboptimal cultural conditions. Pre - harvest factors in the greenhouse or field are equally very crucial determinants of the quality and vase life of cut flowers. Pre- harvest calcium treatment has been shown to extend rose cut flower vase life (Mehran *et al*., 2007; Asfanani *et al*., 2008). The highest percentage of calcium applied to plants is channeled to the leaves nevertheless, high concentration of the quantities applied is found in the cell wall. The concentration in the leaves is inversely proportional to the application rate (Mortazavi *et al*., 2007). Calcium contributes immensely to the strength of the stem (Luiz *et al*., 2005) which supports cut flowers at post- harvest by minimizing occurrence of bent necks. Although, the mechanical strength of the stems is highly correlated with the content of the secondary cell wall components such as cellulose, hemicellulose and lignin (Liao *et al*., 2003).

The duration between harvest and application of calcium salts dictates its efficacy at post-harvest. Application of calcium sulphate on roses 24 hours before harvest reduced the progress and severity of grey mould and increased vase life of cut flowers (Luiz *et al*., 2005). Pre- harvest calcium sulphate application was found to enhance rose flower vase life by enhancing turgidity of treated flowers for longer period compared to the control. In addition to turgidity enhancement, calcium suppressed ethylene production and ion leakage with time (Nabigol, 2012). A holding solution with calcium chloride enhanced water consumption causing a delay in fresh weight loss by the cut flowers (Cortes *et al*., 2011) and guaranteeing extended vase life. The role of calcium in enhancing water uptake enables the cut flowers to avert water stress at post- harvest that could otherwise accelerate senescence symptoms (Martinez-Noel *et al*., 2006). Besides water stress, senescence is associated with rapid increase in aabscisic acid and ethylene concentration. Carbohydrate depletion hat is directly linked to production processes seriously reduced vase life as observed in *Protea spp.* due to premature leaf blackening. Meulen-Muisers *et al*. (2001) found that carbohydrate redistribution also played a key role in the post- harvest performance

CHAPTER THREE
MATERIALS AND METHODS

3.1. Experimental site

The study was conducted at the Horticulture Research and Teaching Field, Egerton University, Njoro. The field lies at a latitude of 0º 23' South, longitudes 35º 35' East in the Lower Highland III Agro Ecological Zone (LH3) at an altitude of approximately 2,238 meters above sea level. Average maximum and minimum air temperatures range from 19 ºC to 22 ºC and 5 ºC to 8 ºC respectively, with a total annual rainfall ranging from 1200 nm to 1400 mm. The soils are predominantly vitric mollic andosols (Jaetzold *et al.*, 2006).

3.2. Planting material

Two red rose cultivars Red Calypso and Furiosa were selected for the study. The cultivars have medium sized flowers with beautiful bright red petals and no discoloration hence good for a study on petal blackening. On average the cultivars have vase life less than 14 days which makes them good for a study on vase life enhancement. The top grafted plants of the two cultivars were obtained from a commercial propagator Stokman Rozen limited in Naivasha. The plants were obtained at 21 days after grafting for field establishment (Plate 1). They were planted in the field with pegs, which were removed 30 days later after healing of the graft union.

The scions of different cultivars were established on the root stock natal briar. Natal briar is the most preferred rootstock because the excellent compatibility of the graft union with different cultivars. The rootstock has good union and rooting capacity compared to other rootstocks such as *Rosa multiflora* Thunb. and *Canina inermis* L. Rootstocks play a significant role in cut flower production by conferring resistance to soil borne pests and diseases. Natal briar is also good in absorption of nutrients from the soil which is implicated to its ability to enhance production of longer cut flower stems.

Plate 1: Top grafted rose plants in pots before field establishment

3.3. Poly film specifications

Three poly films UV-A clear, UV-A 205/N and IR 504 were used in the study. Two were UV stabilized i.e. they offered protection against UV- radiation at wavelength below 400 nm with a life span of 4 years. The spectra properties of UV-A clear poly film were; 90 % light transmission, 20 % light diffusion, anti-dust protection, low density polyethylene (LDPE). The IR 504 poly film was designed to block part of the infrared spectrum. The cover could transmit 82 % to 87 % light and 35 % light diffusion. The poly film thickness was 200 microns and density of 0.96 g / cm^3. The physical appearance of the poly films was assessed at different times (Plate 2 and 3).

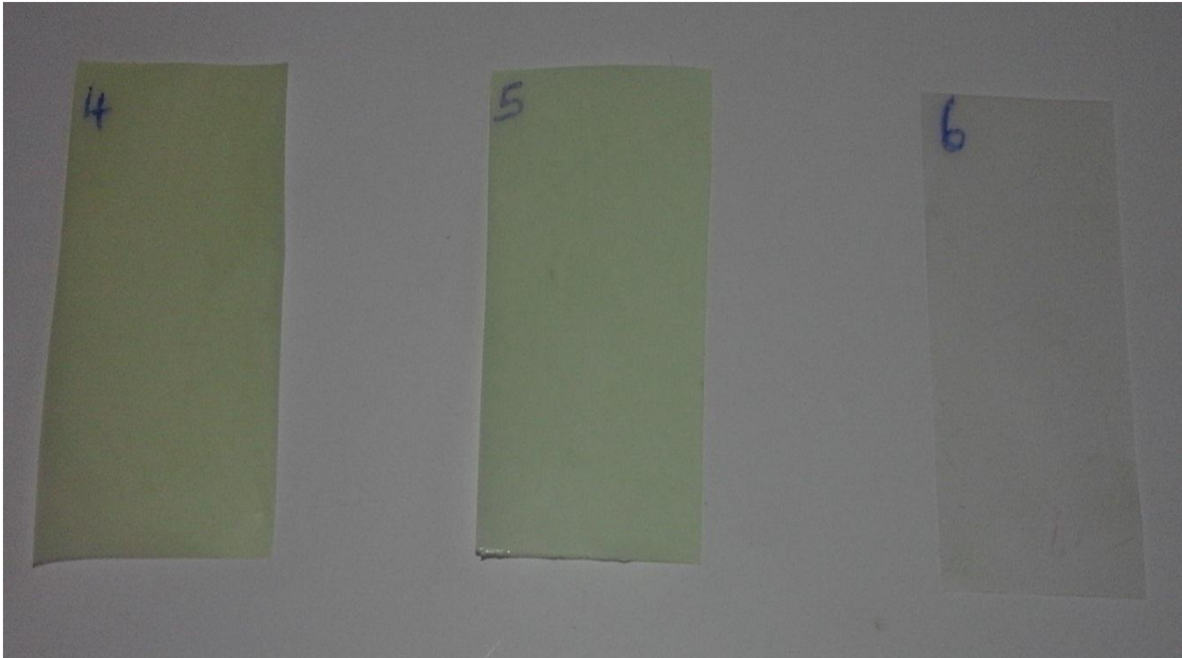


Plate 2: Samples of selected poly films before installation on the greenhouse. Number 4, 5 and 6 represent IR 504 (green), UV – A 205/N (yellow) and UV-A clear poly films respectively.

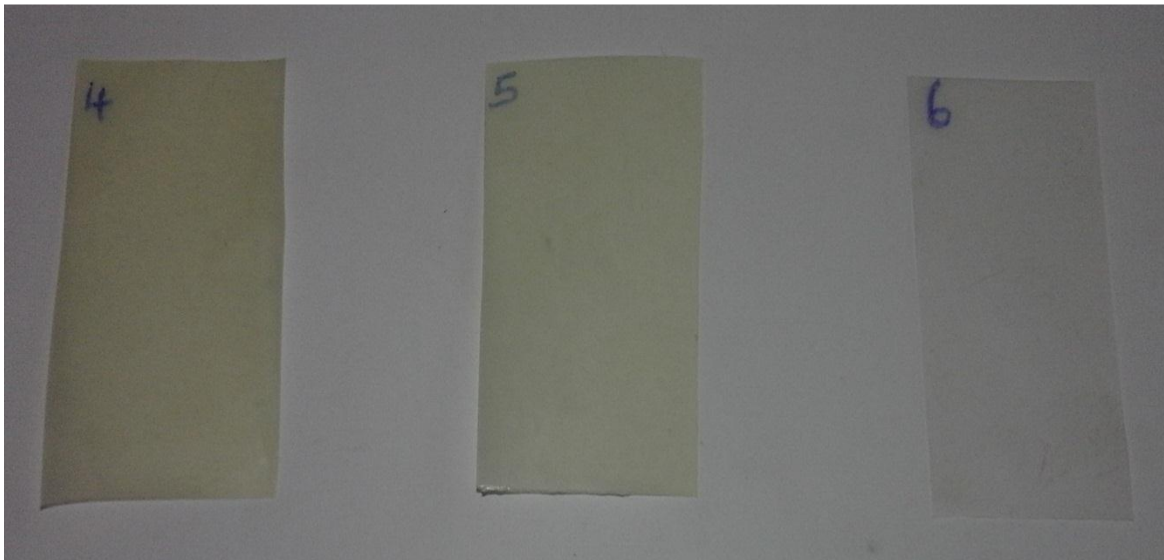


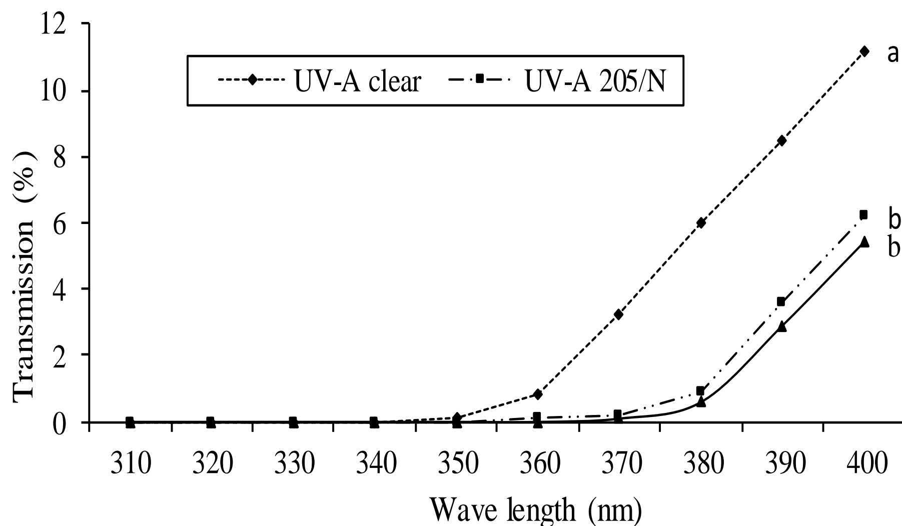
Plate 3: Samples of selected poly films 24 months after installation on the greenhouse. Number 4, 5 and 6 represent IR 504 (green), UV – A 205/N (yellow) and UV-A clear poly films repectively.

3.4. Light spectra: Poly film transmission properties

The transmission and absorbance properties of the poly films were tested in the laboratory. Measurements were carried out on samples taken before the installation of the poly films on the greenhouse structure and subsequent year during the production period. A strip of the poly film was cut from the extra ends of greenhouse. The strip was carefully cut in a rectangular shape and locked in a jig before insertion in the cuvette holder to ensure an upright position is maintained perpendicular to the source of light. The poly films were then scanned at wavelengths (190 nm to 1000 nm) using UV - 1800 Shimadzu spectrophotometer). Care was taken to ensure that the light beam from the spectrophotometer entered through the outer surface of the poly film and left through the inner surface. The effect of the natural conditions on the deterioration of the poly films was examined for a period of 12 months.

The physical properties of the poly films were monitored by assessment of the percent light transmission over time. All the 3 films blocked transmission of UV - B and UV - C light (Figure 2 and 3). The transmission rate of UV-A clear was significantly higher than the IR 504 and UV – A 205/N before installation and 12 months after installation. IR 504 and UV – A 205/N was not significantly different in UV - A transmission before installation, but after being used as cover for over 12 months, the UV - A transmission rate of UV – A 205/N became significantly higher than that of IR 504.

Figure 2: UV



Transmission spectra of the three tested covering poly films before installation onthe greenhouse

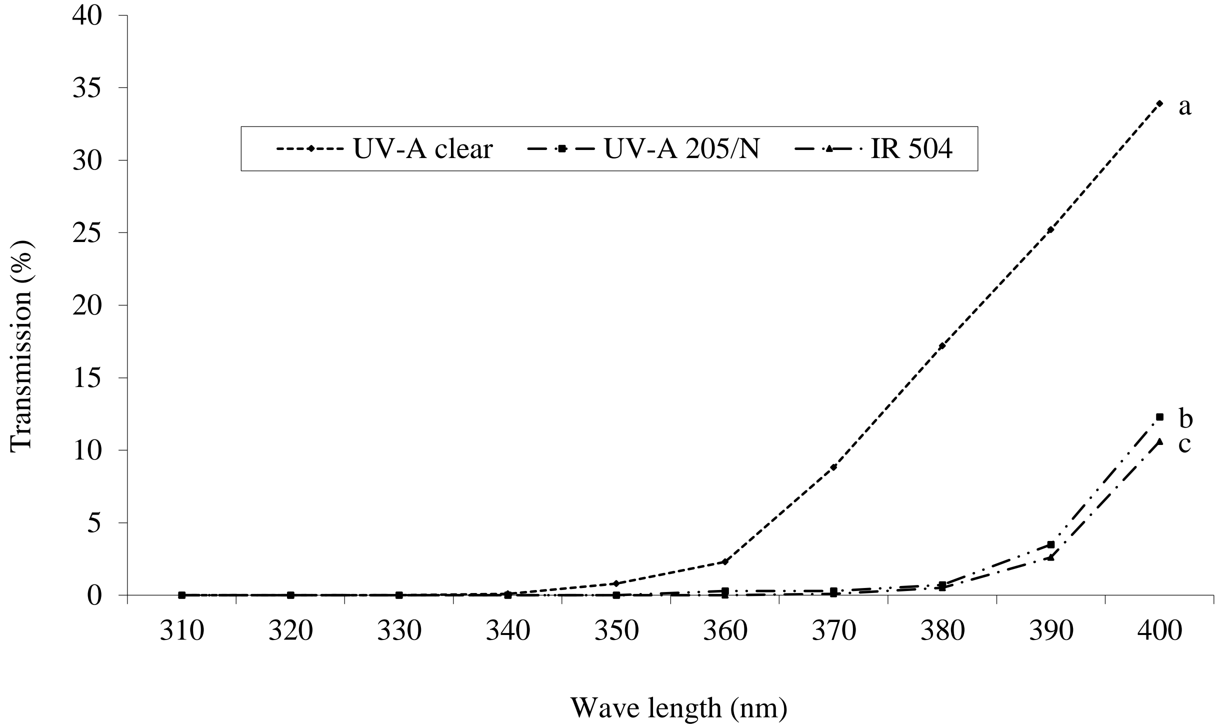


Figure 3: UV Transmission spectra of the three tested covering poly films 12 months after installation on the greenhouse

3.5. Greenhouse construction

Growth tunnel with 17 bays each measuring 8 m × 6 m was used for the experiment. The different poly film sheets were joined according to the colours as per the experimental layout in figure 1, such that the sheets overlapped in the next bay that lay fallow as a buffer between two poly film treatments. The joined sheet was then used to cover the structure. The sides of the bay were covered with Poly film sheet similar to the one at the top. This was repeated for all the 9 greenhouse sections. In terms of transmission rate to visible light and infrared, the three tested films are significantly different (Figure 4 and 5). UV-A clear had the highest transmission rate. IR 504 was higher than UV – A 205 N.

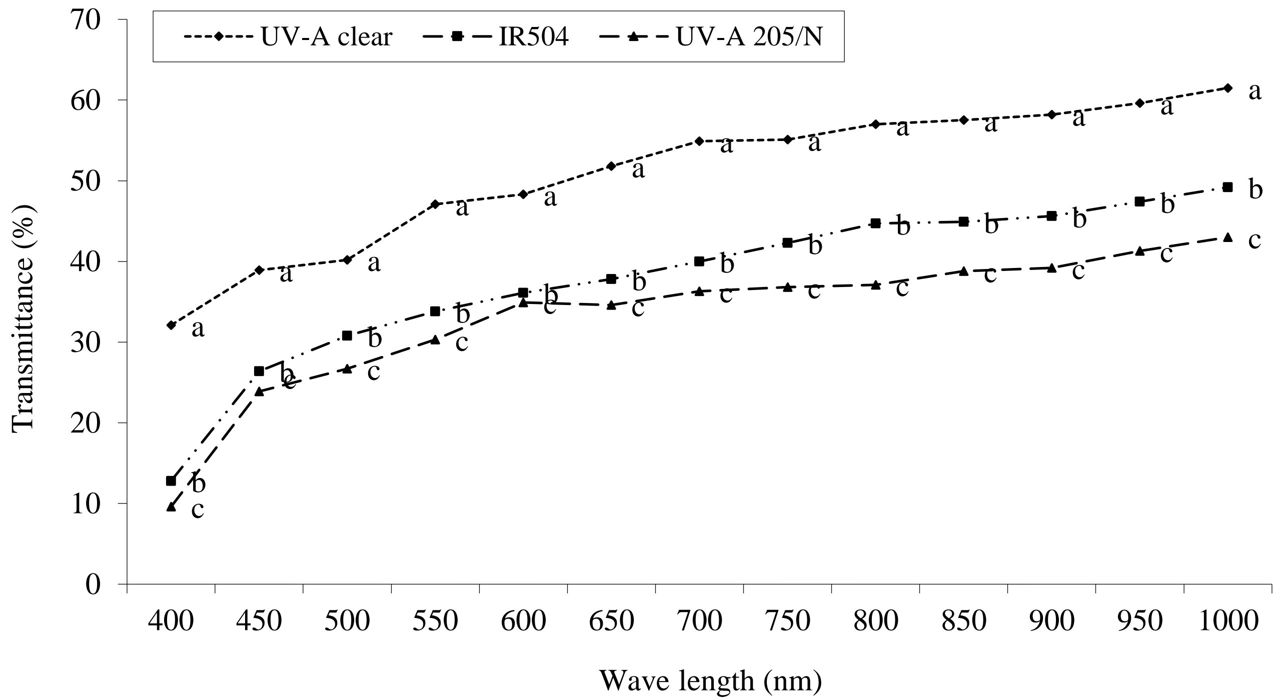


Figure 4: 400 nm to 1000 nm transmission spectra of the tested 3 covering poly films before installation on the greenhouse

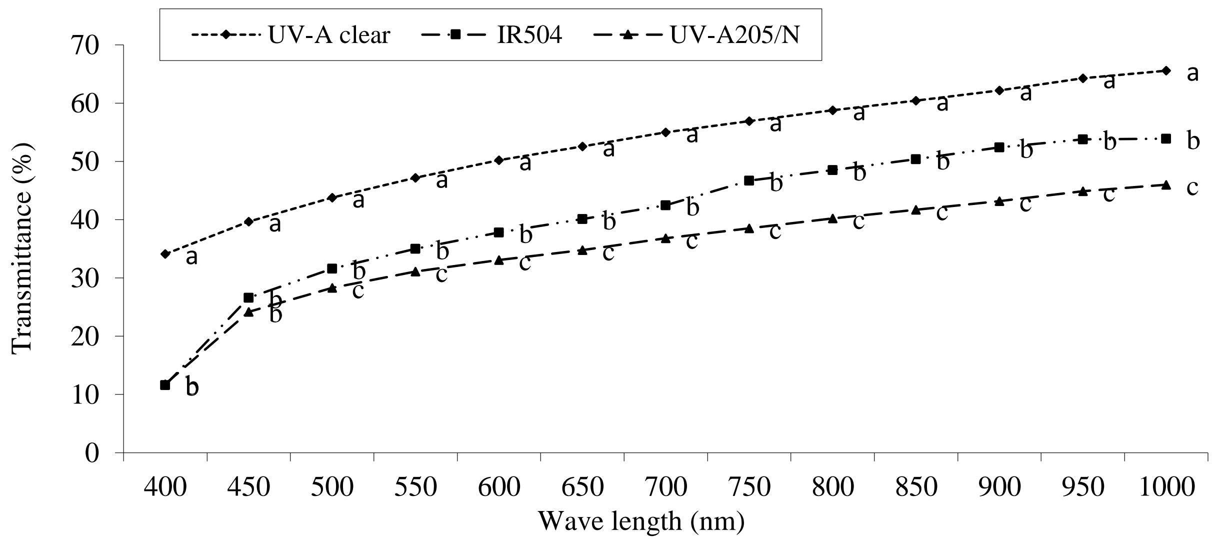


Figure 5: 400 nm to 1000 nm transmission spectra of the tested 3 covering poly films 12 months after installation on the greenhouse

3.6. Experimental design and treatment application

The research was carried out under a split - split plot experiment laid down in a Randomized complete block design (RCBD) with three replications.

3.6.1. Treatments

The main treatments involved three poly film covers applied as a single sheet. The poly films were denoted as follows: G_0 = UV – A clear (Clear transparent polyethylene), G_1 = IR 504 (green tint) and G_2 = UV – A 205/N (Yellow tint). The greenhouse was divided into nine sections of 48 m^2 (0.0048 ha) in size. Each section was covered with a different poly film cover as described above and separated by 1.5 m wide buffer zone. The sub - plot treatment included two rose cultivars Red Calypso denoted as C1 and Furiosa denoted as C2. Calcium was applied in form of foliar feed commercially known as CalMax®. CalMax constituted 15 % calcium and was applied to the sub - sub plots at five levels. The application rate was done according to the manufacturer's specification, half the recommended rate, 50 % higher than the recommended rate and double the recommended rate as shown: the blank T_0 = Distilled water, T_1 = CalMax® 1.25 ml/L, T_2 = CalMax® 2.5 ml/L, T_3 = CalMax® at the rate of 3.75 ml/L and T_4= CalMax® at the rate of 5.00 ml/L. Laboratory salts were used to standardize the concentration of other nutrients mainly Magnesium and zinc while varying only calcium concentrations (Appendix 1).

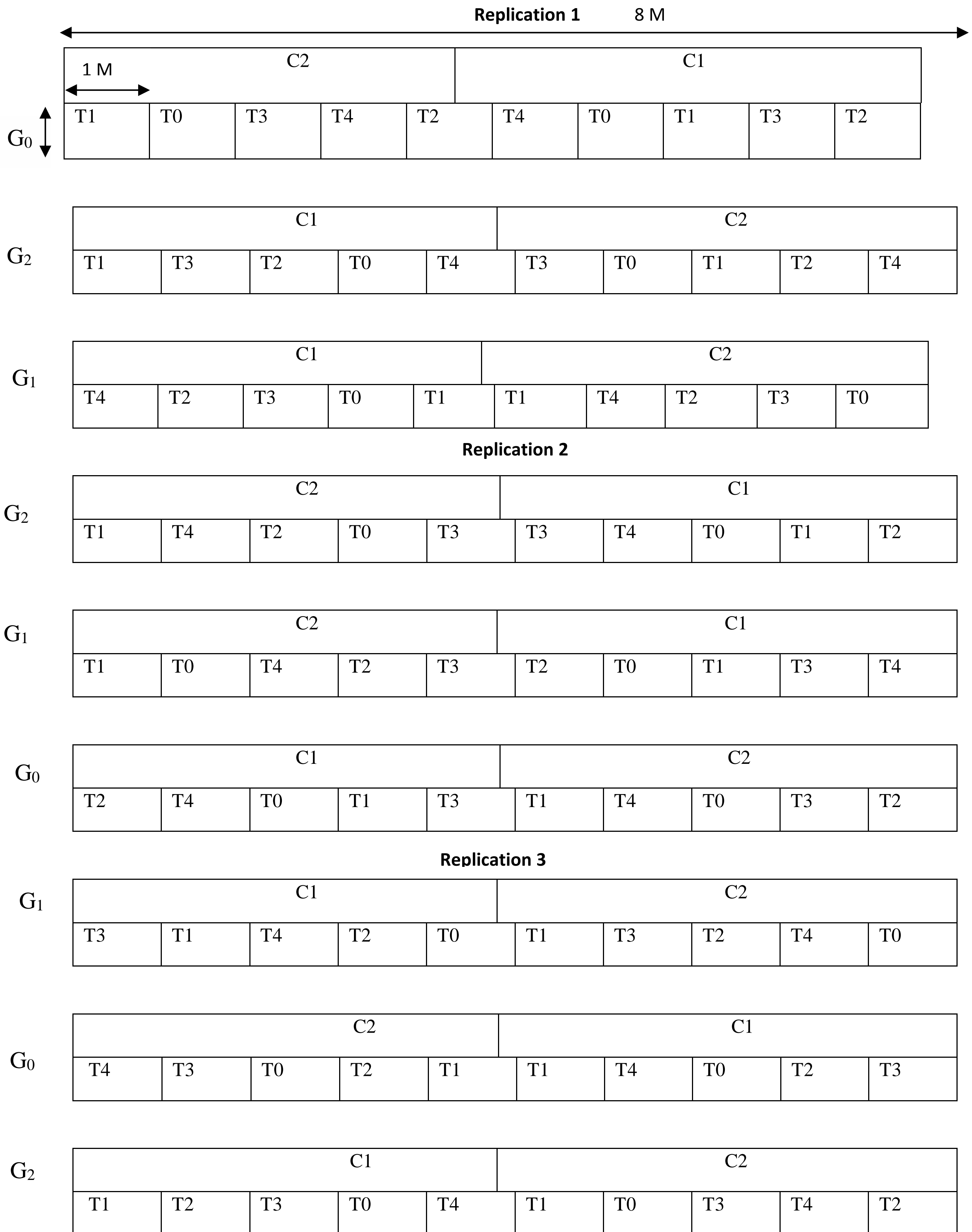


Figure 6: Field layout, split split plot experiment laid in a randomized complete block design

3.7. Crop establishment and maintenance

Land preparation was done by double digging to achieve deeper depth and fine tilth. Decomposed farmyard manure was incorporated in the experimental plots and mixed well with the soil at the rate of 20 kg per m^2. Levelling was done by raking. The field was thoroughly watered by sprinkler irrigation and left undisturbed for 7 days to allow breaking of dormant fungal spores, weeds and other soil borne pests and pathogens. Drip lines were laid on the experimental plots and covered with clear polyethylene of 0.14 mm thickness. The polyethylene edges were buried 15 cm into the soil to ensure airtight conditions. Metham sodium® at the rate of 0.12 ml per m^2 (application rates as per the product specification) was applied through drip lines and plots were left undisturbed for 21 days.

The plots were uncovered for aeration after 21 days. The soil was dug through and left for 7 more days for proper aeration before planting. Germination test was carried out using cucumber seeds to ascertain the depletion of chemical residues from the soil. A bioassay was done by taking soil samples from several points of the treated area. The soil was bulked and divided into equal portions which were later filled into polyethylene tubes. Additional poly tube was filled with untreated soil to act as a control. Equal numbers of seeds were planted in each polyethylene tube. Observations were made to assess germination suppression and discoloration of sprouting seeds which could be an indication of chemical toxins in the soil. Planting was done 7 days later after achieving 95 % germination in all polyethylene tubes.

Two cultivars of top - grafted rose plants were established and the plants grown in double rows spaced at 30 cm × 20 cm to accommodate 10 plants per square metre. Planting was carefully done by ensuring the graft unions were 2 cm to 3 cm above the soil level. The experimental plots were watered thoroughly using a hose pipe to maintain high relative humidity of ≥ 90 % during the first week of planting. Watering to humidify the greenhouse was gradually reduced during the third and fourth weeks. Feeding started after the first 7 days of planting immediately the plants started showing new growth. Balanced nutrient application was done through a fertigation and drip line irrigation system. Plants were raised and allowed to build foliage then bending of primary shoots was done 30 days from the date of planting to allow the outgrowth of water shoots/bottom breaks on which subsequent production was based as shown in Plate 4 and Plate 5. Thereafter plants were managed following the crop agronomic and cultural requirements.

Plate 4: Top grafted plants ready for mother shoot bending

Plate 5: Beds of rose flower showing bending and new shoot sprouting

Feeding programme involved preparation of fertilizer solution in two tanks (A & B). The fertilizer types and quantities used were as shown in Table 1.

Table 1: Fertigation mixes for rose plants during production

Feeding tank A		Feeding tank B	
Fertilizer type	Rate	Fertilizer type	Rate
$Ca(NO_3)_2$	600 mg / M^3	MAP	100 mg / M^3
KNO_3	400 mg / M^3	Urea	75 g / M^3
$Mg(NO_3)_2$	50 mg / M^3	$MgSO_4$	300 mg / M^3

3.8. Data collection

3.8.1. Tunnel microclimate

Relative humidity, air temperature and photosynthetically active radiation (PAR) were monitored using watchdog mini data logger and weather station, spectrum technologies, Inc. The machine was plugged into software that automatically calculates the PAR received. The PAR sensor was plugged into the port on the watch dog while in the field for data collection under the ambient solar radiation. The machine was fixed firmly on a support, with the sensor head being at the level of the plant heads. Relative humidity, air temperature and the PAR were recorded and stored. The data was downloaded at the end of every flush, using spec ware software. Although data was collected for the entire day, 0800 h, 1200 h and 1600 h sampling times were used as a baseline for comparison of the PAR among the treatments. Air temperature and relative humidity were monitored both day and night and recorded.

3.8.2. Growth variables measured through observation

a) Number of visible bud breaks: three plants were randomly marked per treatment. The number of bottom breaks per plant appearing 7 days from the beginning of the flush identified as visible bud breaks were counted and recorded.
b) Plant height (height from base level to apex) was measured weekly using a tape measure. The harvested stems were graded according to the specification given by Post (1952) with some modifications as follows: Shorts < 40 cm, Mediums 40 cm to 49 cm, Extras 50 cm to 59 cm, Fancy 60 cm to 69 cm, Specials 70 cm to 79 cm and Extra specials > 80 cm.

c) Stem thickness was measured using digital Vernier callipers in millimeters. To attain consistency the stems diameter was taken 1 cm from the base of the harvested stem. Diameter was measured from three sampled stems and averaged.
d) Production per square metre was determined for every treatment by counting the number of water shoots or bottom breaks that emerged after mother shoot bending. This was repeated 7 days after harvesting for the subsequent flush.
e) Days to first flower development was recorded starting from the date of bending for the first flush. Time taken for flower development for subsequent flushes was determined starting from the date of harvesting till the stage at which buds showed colour.
f) Flower diameter was measured using digital Vernier callipers in millimeters. Data was tabulated, and comparison made with the standard market requirement where by high quality flower head had to be ≥ 5 cm in diameter (HCDA and MOA, 2003).

3.8.3. Physiological disorders

The number of stems with bent necks, blind shoots and petal blackening (plate 6) were identified per treatment and recorded whenever they occurred from the beginning to the end of every flush. The stems were then quantified per square meter for each flush. Petal blackening was scored following a rating scale described as: 0 = no blackening; 1= less than ¼ blackening; 2 = ¼ to ½ blackening; 3 = ½ to ¾ blackening; 4= more than ¾ blackening of the flower petals.

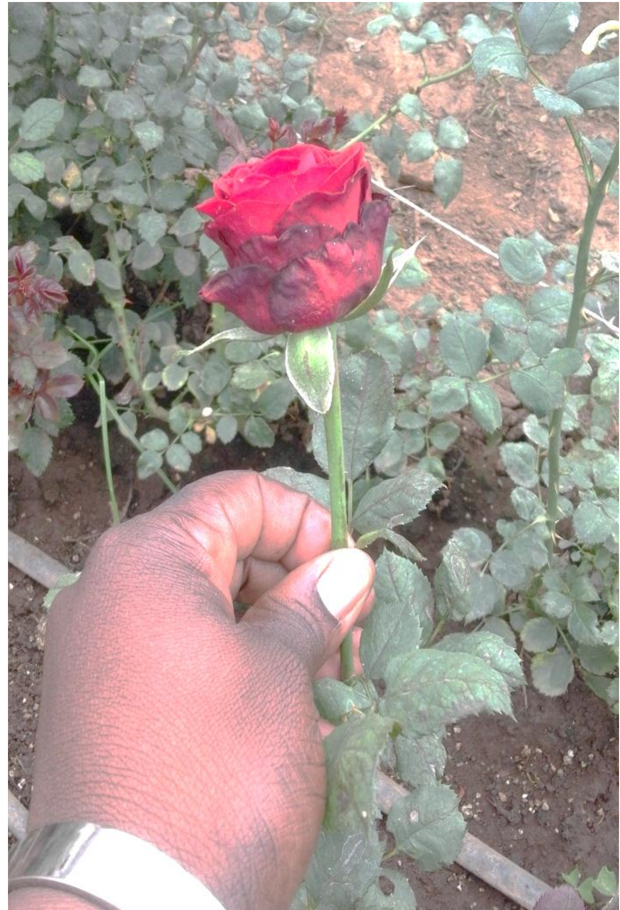


Plate 6: Stems showing some physiological disorders: bent necks (left) and petal blackening (right)

3.8.4. Physiological parameters

a) Leaf chlorophyll content

Leaf chlorophyll content is the quantification of chlorophyll in the leaf. It was done through non-destructive measurement of foliar chlorophyll using chlorophyll content meter (CCM - 200; Opti - Sciences, Tyngsboro, MA) as described by Van den Berg and Perkins (2004). The instrument calculates a unit less chlorophyll content index (CCI) from the ratio of optical absorbance at 655 nm to that of 940 nm. Chlorophyll content measurements were collected by clipping the leaf at the centre while on the plant. Three readings were taken from the 3rd, 4th and 5th fully developed leaves from the top progressing downward and the mean value was determined and recorded.

b) Leaf area

Leaf area is a true estimate of the photosynthetic active area. Determination of leaf area was done based on procedure develop by Rouphael *et al*. (2010). According to this procedure three stems per plant were randomly sampled for leaf area determination. The leaves on each stem were categorized into three i.e. small, medium and large. The area of a leaf in each category was calculated and multiplied by the number of leaves selected from each category. Individual leaf area of rose was done by measurement of length (L) which was measured from the lamina tip to the point of intersection of the lamina and petiole along the midrib and leaf width (W) was measured from end to end between the widest lobes of the lamina perpendicular to the lamina midrib to the nearest 0.1 cm using a ruler. The sum of all the leaves from each category was established and multiplied by the number of stems per plant to find the leaf area per plant to the nearest 0.01 cm^2.

c) Stem firmness

The effect of different treatments on the stem firmness was measured using a penetrometer. The measurement was done approximately one centimeter from the base of the stem after zeroing the penetrometer. A steady pressure was applied downward until the plunger collapsed the stem tissues causing deflection on the penetrometer scale. The plunger was removed, and the force required to collapse the rose stem tissues was recorded. The penetrometer was zeroed, and the process was repeated for subsequent readings, three readings were obtained from different stems averaged and recorded.

d) Dry matter accumulation and partitioning

Dry matter describes the constituents of plant material after water has been evaporated. Dry weight was determined from 3 randomly selected cut flowers in both first and second flushes. Floral and foliage dry weight was determined separately. The floral fresh weight was obtained from flower heads tipped from the stems. Foliage weight involved chopping the stem together with leaves into small pieces. 50 g of the material was weighed separately and placed in khaki papers. Samples were dried in an oven at 60 °C till a constant weight was achieved. The dry weight (DW) was then calculated as; $DW = (B - A) - (C - A)$. Where A = weight of empty sugar paper, B = weight of sugar paper with 50g of fresh material, C = weight of sugar paper with dried material.

e) Leaf stomatal conductance

Stomatal conductance was measured to determine the amount of carbon dioxide entering the leaf. Stomatal conductance was measured using leaf porometer (decagon devices, Inc. model sc - 1). The 4th fully developed leaf from the top of the stem was selected. Stomatal conductance readings were recorded by reading the value on the device after clipping the centre of the leaf. An average of three readings was taken from three leaves on three different plants of the same treatment.

3.8.5. Quantitative and qualitative anthocyanin extraction and analysis

Plants for anthocyanin analysis were harvested when the flower were just showing colour and before the sepals unfolded. The flower heads were sun dried in the greenhouse and packed in khaki papers for further analysis.

Five grams (5 g) of dry ground rose petals were weighed and put into 250 ml conical flasks and covered with aluminium foil paper. The ground petals were mixed with 50 ml methanol (MeOH) and formic acid at a ratio of (99: 1 v / v) and magnetically stirred at 900 rpm for 4 hours at room air temperature. The resultant solution was filtered and evaporated to remove as much methanol as possible using a rotary evaporator (Buchi Rotavapour R - 300, Switzerland) under reduced pressure at 35 °C. The concentrated extract was dissolved in 10 ml distilled water and passed through a membrane filter 0.45 µM and kept in the fridge at -10 °C before analysis.

Anthocyanin purification was then done by passing the extracts through reverse phase (RP) C 18 solid phase extraction (SUPELCO, SPE) (Sigma – Aldrich, USA) cartridge previously activated with 10 % MeOH followed by 0.01 % HCl v / v in distilled water. Anthocyanins were adsorbed onto the column while sugars, acids and other water-soluble compounds were washed out using 0.01 % HCl in distilled water plate 7. Anthocyanins were then recovered using acidified methanol (10 % Formic acid v / v). The cartridges were washed with ethyl acetate (Fischer Scientific, UK) to remove phenolic compounds other than anthocyanins. The purified extracts were stored at -10 °C awaiting further analysis.

Plate 7: Extraction and purification of anthocyanin using solid phase extraction cartridge

3.8.6. Quantification of anthocyanins

The anthocyanins in the rose petals were characterised by High performance liquid chromatogram (HPLC) machine using a Shimadzu LC 20 AT HPLC system fitted with a SIL 20 A auto sampler and an SPD - 20 UV – Visible detector with a class LC10 chromatography work station. UV detection was set at 520 nm using a Gemini C18 ODS (4.0 mm _ 4.6 mm i.d.) (Phenomenex Inc. Torrance CA, USA) fitted with a Gemini C6 ODS column (4.0 mm _ 3.0 mm i.d.) (PhenomenexInc. Torrance CA, USA) guard. The column air temperature was at 35 ± 0.5 °C. The eluents were mobile phase A water/acetonitrile/formic acid-87 / 3 / 10 v / v / v) and mobile phase B (100 % HPLC grade Acetonitrile). The chromatographic conditions were: 3 % B in A at the time of injection, at 45 min; 25 % B in A, at 46 min; 30 % B in A and at 47 min; 3 % B in A (initial conditions). The flow rate of the mobile phase was1 ml per min and injection volume of 20 µl. The anthocyanin cyanidin chloride and pelargonidin chloride were used as standards for the identification and quantification of anthocyanin fractions in dried rose flower petals. Other types of anthocyanins (Cyanidin 3 - 0 glucoside, peonidin, delphinidin and Cyanidin 3 – 0 galactoside) were quantified using the calibrated standard curves done at Tea Research Institute Laboratories, Kenya.

3.8.7. Vase life of rose cut flowers

Vase life refers to the number of days cut flowers can retain their aesthetic appearance in the vase. The flowers for vase life experiment were harvested in the morning when the air temperatures were low. The flowers were then pre-cooled immediately in the laboratory refrigerator at 4 °C for 24 hours before setting up the experiment. Cut flowers at uniform cut stage and relatively uniform bud sizes were selected for the experiment. Post - harvest experiment was set up at prevailing room conditions. During flush I air temperature fluctuated at 15 ± 3 °C and relative humidity averaged at 63 %. The experiment was done under varying light intensity under a daily light period of 12 h. Flush II was characterized by relatively lower room air temperature of 10 ± 4 °C and higher mean relative humidity of 75 % and varying light intensity under a daily light period of 12 h. Cut flowers were stripped off the lower leaves and cut at 30 cm from the flower head. Three stems from each treatment weighing 8.55 ± 0.05 g were placed in 500 ml vases containing 200 ml of distilled water. Vase life was monitored from day one of the experiment till the time flowers become unattractive in the vase. Different quantitative and qualitative parameters were assessed at three days' interval till the flowers senesced as follows:

a) Cut flower fresh weight

Physiological weight loss was determined by periodic weighing of flowers at three days interval. Stems were carefully removed from the vase and weighed quickly using an analytical balance and then returned to the vase with fresh water. Percent weight loss was calculated as:

$$\text{Percentage weight loss} = \frac{W1 - W2}{W1} \times 100$$

Where;

W1 = the initial sample weight

W2 = the final sample weight.

b) Change of petal colour

Flowers held for vase life determination were monitored on every third day and the first sign of petal wilting, yellowing, blackening or blueing were categorized as described by Wang *et al*. (2005) with few modifications. Comparison from different treatments was done using the following visual rating scale: 0 = no discolouration; 1 = less than ¼ discolouration's; 2 = ¼ to ½

discolouration; 3 = ½ to ¾ discoloration; 4 = more than ¾ discolorations. Vase life was recorded as the number of days the flowers were in fresh display condition.

c) Flower bud opening

Flower bud opening was progressively monitored at 3 days interval by measuring the flower diameter using digital Vernier calliper. The diameter measurements were used to assess response of flowers to the treatments while in the vase. Failure to bloom and faster expansion of the petals were assessed as some of the substandard quality attributes.

d) Bent necking

Bent necking refers to bending of the cut flower under the neck region. The flowers were observed while in the vase and curvature of the stem at the neck region was counted as a bent neck. The number was quantified and analysed to assess the performance of different treatments on reduction of bent neck incidences.

3.9. Data Analysis

The collected data was subjected to analysis of variance (ANOVA) using SAS version 9.2 (SAS Inst., Inc., Cary, NC) computer package at $P \leq 5\%$. Further analysis of treatments involving interaction was run using JMP statistical package. Where there were treatment differences, mean separation was done using Tukey's honestly significant difference test. Split-split plot mathematical model was fitted for analysis as shown below:

$$X_{ijk} = \mu ... + B_i + G_j + (BG)_{ij} + C_k + GC_{jk} + (GC)_{jk} + T_l + GT_{jl} + CT_{kl} + GCT_{jkl} + (GCT)_{jkl} + e_{ijkl}$$

Where;

Xijk = Response of rose plants

μ = the overall experiment mean

G_i = the main plot treatment effect

B_j = the block effect

$(BG)_{ij}$ = the main plot error (error a)

C_k = the subplot treatment effect

GC_{ik} = Interaction effect of the jth poly film type and the kth rose cultivar effect

$(GC)_{jk}$ = the sub-plot error (error b)

T_l = calcium foliar feed treatment effect

$(GT)_{jl}$ = the treatment interaction effect of poly film and calcium foliar feed

(CT)kl = the treatment interaction effect of varieties and calcium foliar feed

(GCT)ikl = the treatment interaction effect of jth poly film, the kth rose varieties and l^{th} calcium foliar feed

eijk = the sub subplot error (error c)

i, k, l = a particular treatment

j = a particular block

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1. Effect of poly film covers on greenhouse microclimate and PAR

4.1.1. Light transmitted within the structures covered by the poly films

The quantity of PAR transmitted was dependent on poly film covers, sampling time and the prevailing weather conditions. Average PAR values ranged from; 222 µmol m^{-2} s^{-1} at 0800 hrs, to 1613 µmol m^{-2} s^{-1} at 1200 hrs and 115 µmol m^{-2} s^{-1} at 1600 hrs for UV- A clear poly film (Figure 7). The percentage transmission was significantly higher for UV-A clear poly film compared to IR-504 that transmitted, 245 µmol m^{-2} s^{-1}, 1063 µmol m^{-2} s^{-1} and 40 µmol m^{-2} s^{-1} at 0800 hrs, 1200 hrs and 1600 hrs sampling time respectively. Comparison of the PAR values at 0800 hrs for UV-A clear and IR 504 poly film indicated low PAR values for UV-A clear. Transmission through the clear poly film was consistently higher, followed by UV- A 205/N and IR 504. Average transmission through the clear poly film was 10 % lower than the IR 504 at 0800 hrs. This could have been caused by the water condensate that was observed on the UV-A clear poly film thus hindering maximum transmission in the early morning hours.

The poly films transmitted the highest quantity of light during the fifth week of production in flush I. During the 6th and 7th weeks steady drop was recorded across all the poly films. PAR transmission varied with the prevailing weather conditions. Transmission properties of the poly films were consistent in both flushes I and II (Figure 7 and 8). The least PAR but higher air temperature was recorded under IR 504 cover. Fluctuation in light transmission was high under UV-A clear cover with high peaks being recorded at 1200 hrs. Although transmission through UV-A 205/N poly film was consistently higher than IR 504, during the 2nd and 6th week the values recorded were statistically similar (Figure 8). This observation indicates that the PAR light transmission through the poly films varied with the additives in the specific poly films. This could be the reason why transmission through coloured poly films IR 504 and UV-A 205/N was relatively stable compared to the UV-A clear poly film. The IR 504 and UV-A 205/N poly films enhanced light diffusion within the growth structure, opposed to the UV-A clear that transmitted direct rays.

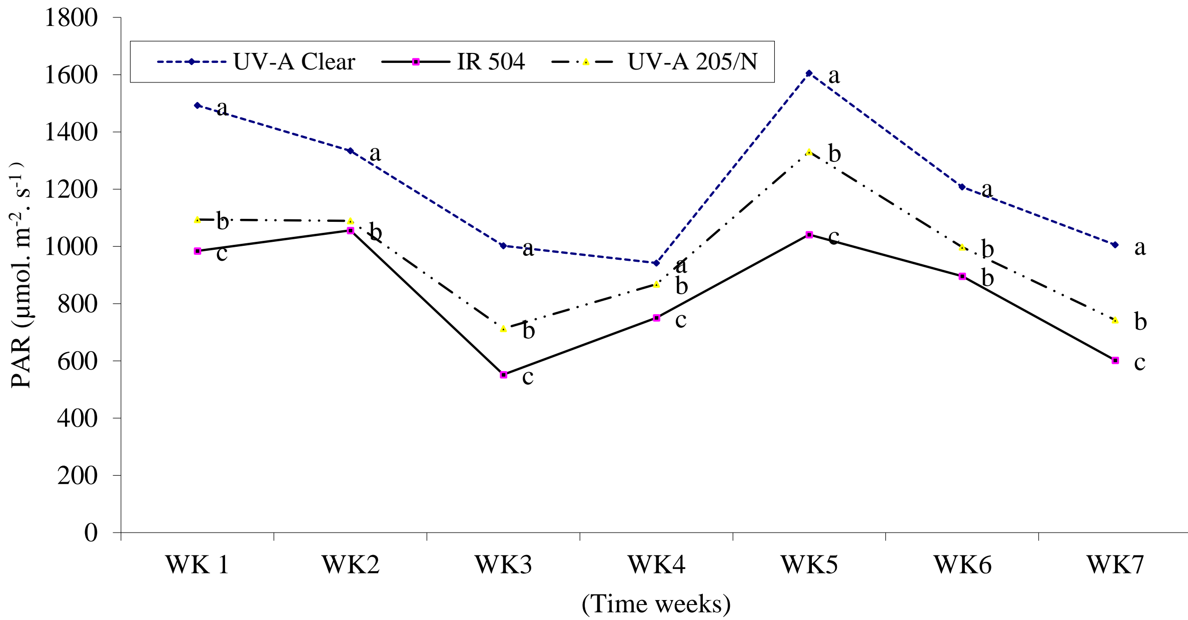


Figure 7: Light transmission under different poly film covers (flush 1),
(Values presented are weekly averages (n = 3) of light measured at 1200 hrs).

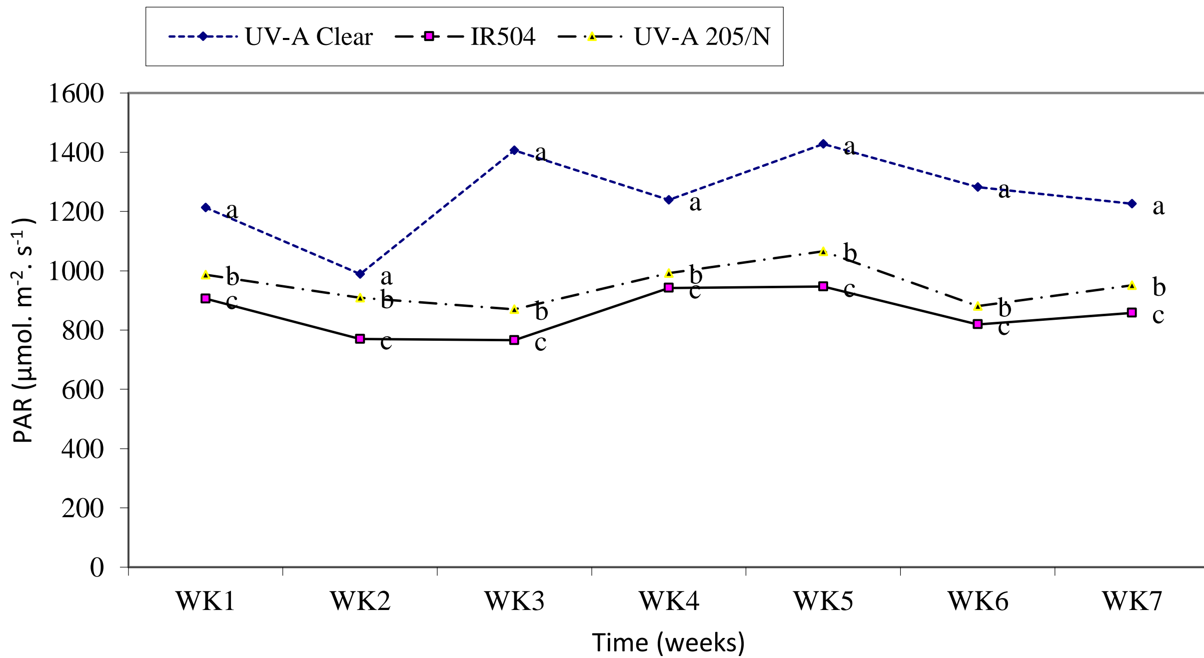


Figure 8: Light transmission under different poly film covers (flush II).
(Values presented are weekly averages (n = 3) of light measured at 1200 hrs).

4.1.2. Variations in greenhouse air temperature and relative humidity under poly film covers

Relative humidity was significantly high under UV-A clear poly film at 0400 hrs while the lowest values were recorded under the same cover at 1200 hrs. Relative humidity was noted to be inversely proportional to the air temperature with the lowest values being recorded at 1200 hrs across all covers. Relative humidity of 69.2 %, 76.1 % and 71 % with air temperatures of 31.2 °C, 31.1 °C and 30.9 °C were recorded under UV-A clear, UV-A 205/N and IR 504 poly films respectively at 1200 hrs. Although UV-A 205/N and UV-A clear poly films were both UV- treated higher relative humidity levels at 1200 hrs of 76.1 % was recorded under UV-A 205/N compared to UV-A clear that recorded 69.2 %. Relative humidity in this case differed by 7 % while there was a narrow gap in air temperature of 0.1 °C.

This trend was consistent at different sampling times, for example higher values of relative humidity 93.1 %, 96.5 % and 93.3 % and low air temperature of 8.5 °C, 8.9 °C and 9.2 °C was recorded at 0400 hrs under the UV- A 205/N, UV-A clear and IR 504 respectively (Figure 9 and 10). The IR 504 poly film recorded the lowest air temperature value at 1200 hrs but highest air temperature at 0400 hrs. Although the UV-A clear recorded the highest air temperature at 1200 hrs, it recorded the least values 0400 hrs. Drop in air temperature was drastic under the UV- A clear poly film, followed by UV-A 205/N compared to IR 504.

Air temperature fluctuation between 1600 hrs and 0400 hrs was statistically similar across all covers. The highest difference in air temperature was noted at 1200 hrs where by the UV-A clear recorded 2.5 °C and 1.3 °C higher than UV - A 205 / N and IR 504 respectively. Conventionally, under conditions of elevated air temperatures farmers have adopted whitening of the greenhouse roof to reflect excess light and reduce air temperature within the structure. Influence of whitening of greenhouse microclimate was studied and observed to reduce the transmission coefficient of solar radiation from 0.62 to 0.31 as a result changing air temperature drastically (Baille *et al*., 2001). Apparently, the UV-A clear poly film that was permissive to high transmission accumulated higher air temperature during the day although it could not retain higher air temperature at night. Holcman and Sentelhas (2012) evaluated microclimate under different shading screens among them being red, blue and black.

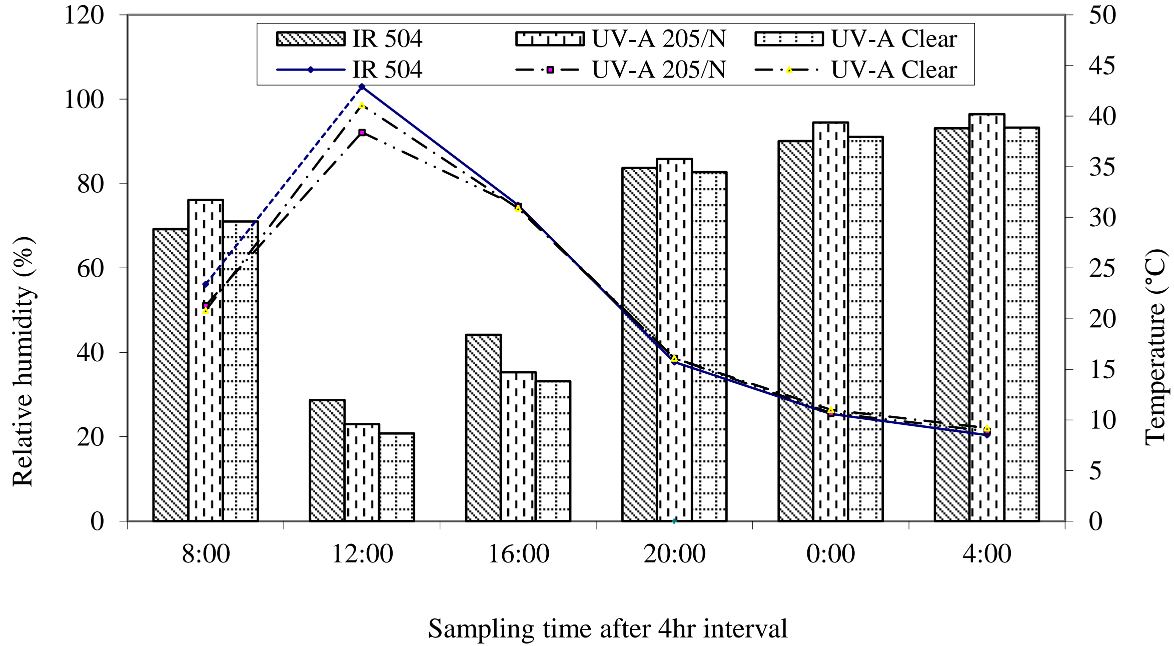


Figure 9: Effect of poly film covers on relative humidity and air temperature (flush 1).

Values presented are means over a growth period of 42 days at different sampling time; 0800 hrs, 1200 hrs and 1600 hr (n = 3)

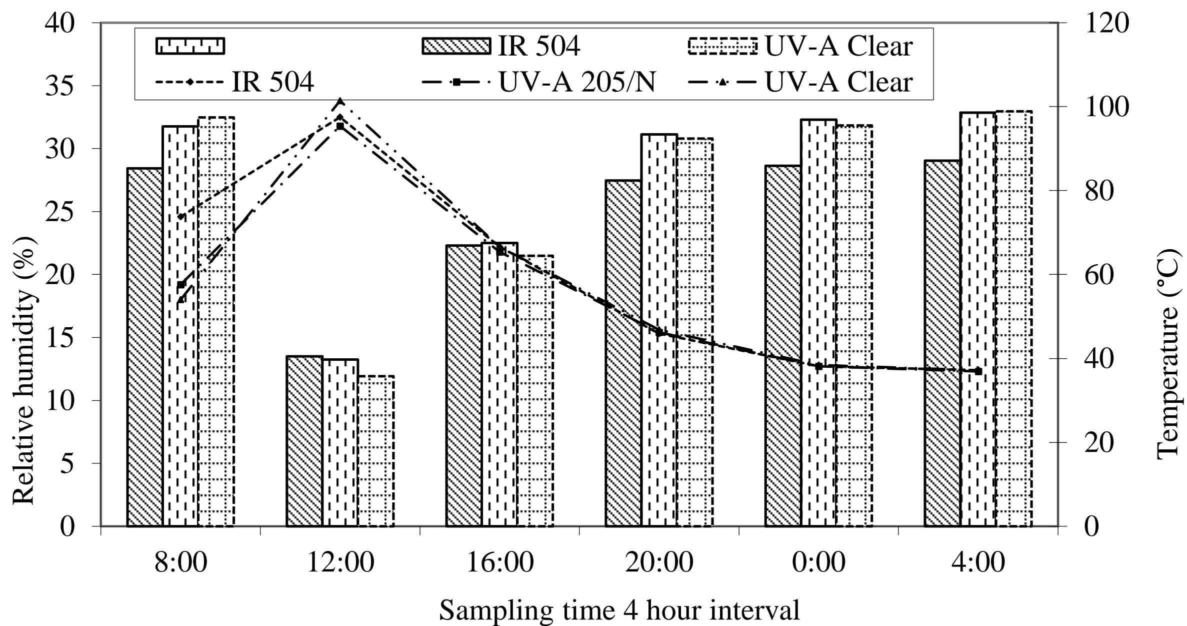


Figure 10: Effect of poly film covers on relative humidity and air temperature (Flush 1I). Values presented are means over a growth period of 42 days at different sampling time; 0800 hrs, 1200 hrs and 1600 hrs (n = 3)

The reflective shade screen used as the control transmitted 56.3 % light followed by the red screen which transmitted 27 %, while black recorded the least transmission of 10.4 %. Air temperature also varied drastically, with the blue screen recording 1.3 °C higher than the external conditions. The colour of the greenhouse cover therefore, affects greenhouse microclimate and the extent varies among different shades as observed in this study. The structures in the current study had higher air temperature than normal outdoor air temperature, which could be attributed to the small size that was comparably lower in terms of width and height. The bigger the greenhouse the better the air flow thus size could greatly affect air temperature and relative humidity within a structure.

According to AlHelal and Abdel-Ghany (2011) they observed that greenhouse air temperature is affected by more than one single factor including: solar radiation, level of greenhouse venting and the size of the greenhouse. Structural units used in this experiment were precisely 48 m^2 and about two-thirds the height of normal greenhouse. The air temperatures recorded in this study could have been relatively higher due to the size of the structure. The smaller the greenhouse size the higher the air temperature and the bigger the greenhouse in width and height the lower the air temperature. Holcman and Sentelhas (2012) established that besides climate, greenhouse design, size and height affect the internal microclimate. Greater height of the greenhouse structure enhanced air circulation minimizing heat build-up within the greenhouse. Conventionally, reduction in air temperature is a key objective of greenhouse use in the tropics and as observed in the current study it may have been limited by height of the structure.

Several modifications have been adopted in the poly film industry, including use of shade screens, cladding of the poly films with assorted colours and treatment with UV and IR additives among others. According to the findings of this study, coloured poly films have the capacity to alter the spectral properties which in turn influence the microclimate. It was observed that the UV-A 205/N and IR 504 poly films that were coloured recorded higher relative humidity compared to the UV-A clear. This observation supports the work done by Shahak (2008) who reported that the darker the colour of the poly film the less the amount of light transmitted. This could explain why the UV-A clear poly film transmitted the highest light quantity and the highest air temperature at 1200 hrs compared to IR 504 and UV-A 205/N.

The poly films had significant effect on relative humidity and corresponding air temperature within the greenhouse structures which also varied with the specific sampling time. Greenhouse relative humidity directly influence the plant water relations and indirectly affect plant growth and development. Plant cell enlargement is enhanced by turgor pressure developed within the cells due to high relative humidity. High air temperature, as was the case under UV-A clear cover translated to low relative humidity at 1200 hrs which demonstrates that relative humidity is highly affected by air temperature. The size of the greenhouse structures used in the study was small and could have contributed to very high air temperatures recorded. Implying that different results may be recorded using similar poly films under structures bigger in size than the ones used in the current study.

During the first flush mean air temperature values of 41 °C, 35.2 °C and 32.8 °C were recorded at 1200 hrs during the hottest part of the day and 12.3 °C, 13.1 °C and 12.1 °C at 0400 hrs during the coolest part of the day under UV-A clear, IR 504 and UV-A 205/N covers respectively. It is evident that during the day UV-A clear recorded the highest air temperature compared to UV-A 205/N, while the mean night air temperatures between the two poly films were not significantly different. However, it is also important to note that, while IR 504 poly film recorded the lowest mean day air temperature than the control it was significantly higher than the UV-A 205/N. IR 504 had the highest mean night air temperature (13.1 °C) being 0.8 °C and 1.0 °C higher than UV-A clear and UV-A 205/N respectively. It is argued that poly film colour is an integral part of greenhouse microclimate. Kittas *et al*. (2011) maintains that microclimate under different shading screens is dependent on the colour of the greenhouse covering material used. They evaluated thermo reflective screens of different colours red, blue, black and the control with 70 % shading and observed high solar transmission under the control 56.3 %, 27 % under red and the lowest value 10.4 % was recorded under the black screen. It is apparent therefore, that colour of the covering material affects the microclimate under the growth structures.

4.2. Effect of poly films, cultivar and calcium on growth and quality of rose flowers.

4.2.1. Poly film covers and cultivars on rose plant bud break

The number of bud break was significantly affected by the interaction of cover and cultivars (Appendix 2). The highest number of bud breaks in flush I was observed under UV-A clear and there was no significant difference in the cultivars used under this cover. The least

number of bud breaks was observed under UV-A 205/N and IR 504 covers for cultivar Red Calypso (Table 2). The number of bud breaks was statistically similar under UV-A clear and IR 504 poly film covers for the cultivar Furiosa. Although the highest number of bud breaks was recorded for the cultivar Red Calypso under the UV-A clear poly film, the lowest number of bud breaks was also recorded for the same cultivar under the IR 504 and UV-A 205/N poly films.

Table 2: Effect of poly film covers and cultivars on rose bud break in flushes I and II.

Treatments	Flush I		Flush II	
Cover/Cultivar	Red Calypso	Furiosa	Red Calypso	Furiosa
UV-A clear	2.20 a*	2.00 ab	3.47 a	2.53 bc
IR 504	1.07 c	2.07 ab	2.07 c	3.20 ab
UV - A 205 / N	1.33 c	1.73 b	2.1 c	2.40 c

*Means followed by different letter (s) along the column for cultivars and a cross the row for poly films per flush are significantly different at 5 % level of significance according to Tukey's honestly significant difference (HSD) test.

In the second flush, the highest number of bud break was recorded under the UV-A clear poly film for cultivar Red Calypso and under IR 504 for cultivar Furiosa. The lowest number of bud break was recorded under the UV- A 205/N cover for both cultivars. Although Furiosa had significantly higher number of bud breaks than Red Calypso under UV-A 205/N in flush I, during the second flush the two cultivars had statistically similar number of bud breaks under this cover. The number of bud breaks was high in flush II compared to flush, this is due to increase in the number of shoots and harvestable surface that presents more potential buds for sprouting.

Comparison between the three poly films show that air temperature is a key factor in bud break initiation. The number of bud breaks was higher under the UV-A clear poly film which recorded the highest air temperature as evidenced by the microclimate results of this study. It is therefore likely that elevated air temperature as was the case under the UV-A clear poly film caused increased root zone air temperature which might have induced changes in sink activities of the plants. Plants store carbohydrates predominantly in roots, thus increase in temperature could have resulted to breakdown of the stored starc. Further, it is possible that there was a shift in assimilate partitioning due to low night air temperatures and high day air temperatures recorded under UV-A clear poly film by promoting carbon translocation to the basal parts of the rose plants that

enhanced bud break. The results of this study support the findings of Andreini *et al*. (2009) who studied the morphological evolution of bud break in eight cultivars of *Vitis vinifera* a plant in Rosaceae family similar to rose flower in heat chambers at 25 °C ± 1 °C and 65 % relative humidity and reported that the intensity of bud break was related to warm air temperature above 10 °C and growth was inhibited at lower air temperature below 10 °C. The UV-A clear was characterized by high light transmission and consequently higher bud breaks compared to UV-A 205/N.

Bud break has been identified to perform well at low light intensity of 200 µmol m^{-2} s^{-1} and 400 µmol m^{-2} s^{-1} compared to high levels of 600 µmol m^{-2} s^{-1} and 800 µmol m^{-2} s^{-1} It is therefore expected that IR 504 poly film could have higher number of bud breaks compared to UV-A clear cover that exhibited higher light intensity. Contrary to this in the current study, UV-A clear that recorded higher transmission and consequently high number of bud breaks. Air temperature seems to have taken the significant role in bud break initiation compared to light intensity as it increased with increase in solar irradiance. Wubs *et al*. (2014) studied the axillary bud break in a cut rose crop as influenced by light intensity and red: far- red ratio at bud level and established that bud break was affected more by light intensity than the red: far- red ratio. However, it is apparent that in addition to light intensity, air temperature also has a role to play in initiating bud breaks.

It is important to point out at this stage that growth is not only affected by environmental factors such as air temperature and relative humidity, but bud break was also observed to be dependent on cultivar. Rose cultivar responded differently under different covers. The least number of bud breaks was recorded under UV-A 205/N for cultivar Furiosa in the second flush while the same cultivar had performed better than Red Calypso under the same cover in flush I. This presents inconsistency in the number of bud breaks and cultivar response. Bud sprouting can be implicated to the cultivar response to the environment which is characterized by different plant physiological and morphological behaviour (Andreini *et al*., 2009). Besides environmental factors the position from which the propagule was obtained could cause differences in bud sprout. The position at which the propagules were obtained determines maturity and level of carbohydrate storage that is important for bud breaks. Differences among cultivars used in the current study could be due to inherent characteristics since the cultivars were top grafted on similar root stock (natal briar). .

Growth cycle of the plants depend on the genotype and the environment. This fact may have contributed to the differences in bud break among the two cultivars in the study. Furthermore, cultivars of the same plant have been established to vary in their maturity period depending on the age of the wood and position at which the cutting was picked from the plant. Bud break may vary among cultivars and more still within the same cultivar depending on the location of the branch on the plant where the propagation material was obtained and even age of the branch. This explains why the number of bud breaks varied for the same cultivar among different covers. Air temperature has significant impact in initiating bud break by causing changes in endo- or eco-dormancy (Shin *et al*., 2001) however, what may not be clear for rose cultivars from this study is that the amount of air temperature that can induce this change was not monitored, although it was apparent that sprouting occurred faster under the poly film that exhibited higher air temperature.

The poly film (UV-A 205/N) which recorded least air temperature (38.4 °C) had the least number of bud breaks compared to the control where the highest air temperature recorded was 41.1 °C in flush I. Air temperature greatly varied under the poly films in the study implying that the greatest influence on the number of bud breaks was not only cultivar dependent but was also affected by air temperature. Response of plants to light and air temperature differ among plants and affect biomass allocation which could be implicated to the differences in bud break. Exceptional performance was observed under the IR 504 poly film, where by unlike the other poly films Furiosa out performed Red Calypso under this cover. During flush I, an average of 2 buds sprouted for the cultivar Furiosa. However, in flush II the number of bud breaks averaged 3. The UV-A 205/N cover recorded the least number of bud breaks for all cultivars which could be attributed to the fact that this cover recorded the least air temperature consistently in both flushes I and II. This observation could suggest difference in the level of dormancy among and within cultivars.

Previous studies have shown that alternating low and warm air temperature have synergistic effect in breaking dormancy compared to low or warm air temperature alone (Zanette *et al*., 2000). It is likely that warm air temperatures increased metabolic activities and other process like photosynthesis resulting in higher levels of carbohydrate required for bud breaking. At low air temperature, the process of cell division reduces or ceases under extreme cases leading to bud dormancy. Conventionally, rose flower performs well under a range of air temperatures 20 °C to 30 °C during the day and 18 °C to 20 °C at night with an ideal air temperature of 19 °C to 27 °C. Maintenance of this air temperature is within the optimal range is a challenge and it is usually a trade off with other factors like relative

humidity in most farming systems. Quality and productivity have therefore not been optimized due to continuous variations in environmental factors.

4.2.2. Effect of poly films and cultivars on the number of rose stems

The number of stems per square meter was significantly dependent on the poly film and cultivar response (Appendix 3). Application of calcium foliar feed had no significant effect on the number of harvestable stems that sprouted. The combined effect of Red Calypso under the poly films UV-A clear and UV-A 205/N significantly increased stem density compared to Red Calypso and IR 504 poly films. The observation made on poly films and cultivars interaction were inconsistent between flushes. In flush I, the combination of UV-A clear poly film and cultivar Red Calypso recorded the highest number of stems while in flush II both cultivars recorded higher number of stems under the UV-A clear poly film. The number of stems recorded as a result of UV-A 205/N and IR 504 interacting with Red Calypso were 31 stems/m^2 and 35 stems per m^2 respectively which was statistically similar compared to similar poly films with Furiosa that produced 25 stems/m^2 and 31 stems/m^2 respectively in flush II.

The interaction between IR 504 poly film with Furiosa produced the lowest number of stems per square metre (10 stems per m^2) in flush I compared to production under UV-A clear and UV-A 205/N covers (Table 3). A steady increase of 98.2 %, 136 % and 34.6 % in the number of stems was recorded under UV-A clear, IR 504 and UV-A 205/N poly films between flush I and II respectively for cultivar Red Calypso. The number of stems increased almost three times for the cultivar Furiosa under UV-A clear and IR 504 cover between flushes I and II while UV-A 205/N recorded 60.7 % increase. Stem production corresponded with the number of bud breaks under the different poly films. More shoots were harvested in flush II compared to flush I since the harvested shoots increase progressively with flushes in rose production. One key factor in rose plants is that productivity is determined by the sprouting of auxiliary buds after harvesting and their potential to develop to harvestable shoots. The more the harvestable shoots, the higher the number of potential buds leading to productivity increase. However, he potential to sprout is triggered by the right environmental conditions. The cultivar Red Calypso recorded higher increase in stem production under the IR 504 cover between flushes I and II. Low day and high night temperatures were recorded under this poly film cover. It is probable that low air temperature is suitable for rose production (Ushio *et al*., 2008). Higher air temperatures above optimal may favour bud sprout but fail to support optimal growth and development.

Table 3: Effect of poly film cover on rose stem production per square metre of different cultivars

Treatments	Flush I		Flush II	
Cover / Cultivar	Red Calypso	Furiosa	Red Calypso	Furiosa
UV-A clear	22.53 a*	14.4 b	44.67 a	43.07 a
IR 504	14.8 b	10.6 c	34.93 b	30.9 b
UV - A 205 / N	22.67 a	15.47 b	30.53 b	24.87 c

*Means followed by different letter (s) within a column for cultivar and within the row for poly film cover per flush were significantly different at 5 % level of significance according to Tukey's honestly significant difference (HSD) test. Values are means (n = 3)

During the second flush the poly films were permissive to transmission of light at lower wavelength and different quantities of UV- B and UV- A. The impact of these short wavelength radiations is likely to have affected plant growth, since under the UV-A treated poly films had recorded higher number of bud breaks that eventually might not have developed to harvestable shoots. Some of the potential buds under IR 504 poly film eventually develop to harvestable shoots causing a drastic increase of 136 % in the number of stems. Under UV-A clear poly film that received high air temperature (42.9 °C) at 1200 hrs compared to 38.4 °C recorded under IR 504 cover. Apparently, high air temperature induces stomata closure which impact on reduction of the photo assimilates that are critical in energy supply for plant development, as such shoot development is likely to fail.

The effect of air temperature on growth as observed in this study supports the findings of Nadeem *et al*. (2011) who screened rose cultivars for adaptability to varying climatic conditions and concluded that of all climatic factors air temperature is the main determinant of growth and yield of roses followed by relative humidity. Their results indicated that there was a decrease in growth and flowering in rose bushes as air temperature rose above 32 °C and relative humidity decline to 29 %. It is strongly evident that rose cut flower production varies with the prevailing weather conditions. Nadeem *et al*. (2011) further observed that as the air temperature increases, and relative humidity decrease and the number of stems per bush increased in quantity. Despite the environmental conditions, comparing the two cultivars it is also evident that productivity varied by cultivar and other ecological factors as well.

Results presented from previous work, argued that variations in the number of flowers per plant are due to the cultivars difference in response to the surrounding (Manjula, 2005). In the current study, however, specific poly films had different impact on the greenhouse microclimate as discussed earlier which could influence difference in stem productivity. Generally, production per square metre was significantly high for Red Calypso compared to Furiosa. Productivity difference between cultivars could be attributed to the environmental changes as influenced by the greenhouse microclimate. In addition, the interaction of a range of factors (environment, genetics and management) may cause changes in the plants in synergism.

4.2.3. Poly films and cultivars effect on rose plant growth

Plant height was significantly affected by the combined effect of poly films and cultivars. Calcium foliar feed had no significant effect on plant growth. Minimum height was recorded during the first 10 days after the sprouting of the visible buds (Appendix 4). Basically, this stage of plant growth is characterized by cell division hence growth was relatively slow across all the treatments. Cell division stage is of primary importance since it influences subsequent stages in growth and development. Thereafter height increased steadily from day 10 to day 17 recording almost 10 times increase in plant height across all treatments (Appendix 5). Between the day 24 and day 31 DAH (Days after harvest) plant growth increased steadily, under UV-A clear growth increased by 82.3 % compared to previous height at day 24 (Appendix 6). Height increased by 86.7 % and 90.5 % for IR 504 and UV-A 205/N poly films respectively being characteristic of the cell enlargement phase.

Faster growth was recorded under the UV-A 205/N poly film compared to UV-A clear since growth increased by 90.5 % under UV-A 205/N compared to UV-A clear where growth increased by 82.3 % within similar time interval for the same rose cultivar. The rate of growth was quite slow during flush II, while in flush I growth between day 10 and day 17 DAH increased by almost 10 times in flush II the increase as slow as 4 times. Plant growth was also noted to be faster during the first flush compared to the second (Appendices 7 and 8). Steady increase in growth was continuously recorded between day 10 and day 38 almost doubling the height at every 7-days sampling interval and thereafter gradually declined was noted (Table 4).

The coloured poly films IR 504 and UV-A 205/N had the potential to diffuse light. This means that light reached the plants after being scattered by particles inco-operated in the cover

during manufacture. The potential of the coloured poly films to diffuse light reduce crop air temperature and encourage even light penetration in the leaf canopy. Better plant growth was therefore achieved under UV- A 205/N poly film due to enhanced photosynthesis. UV-A clear cover on the other hand transmitted large amount of direct solar radiation resulting to heat stress in the greenhouse that led to reduced plant growth.

Plants grown under the UV-A clear poly film were relative shorter, this could be due to the high light transmission that was transmitted through this poly film. There was evidence of transmission of short wavelength UV-light through the UV-A clear cover that could have impacted on plant growth. It is possible that high air temperature and UV radiation particularly UV-B interfered with stomata function which in turn affected photosynthesis leading to reduction in growth (Kittas *et al*., 2006). The results of this study further support the work done by Hatem *et al*. (2007) who showed that the growth of *Diffenbachia amoena, Schefflera species* and *Aglaonema crispum* in shading greenhouse were taller than those grown in un-shaded greenhouse exposed to higher irradiance. Thus, plants under UV-A clear poly film with high light transmission of 66 % were not as tall as those grown under UV-A 205/N and IR 504 poly films that transmitted 49.6 % and 49.1 % light respectively at wavelength 400 µmol m^{-2} s^{-1}.

Table 4: Effect of selected poly film covers on height of rose plants in flushes I and II

	Days after harvesting (DAH)				
	10	17	24	31	38
Type of poly film cover	Flush I				
UV-A clear	1.12 b[*]	4.77 b	14.50 ab	35.20 a	43.79 b
IR 504	1.27 ab	4.50 b	13.50 b	36.22 a	50.68 a
UV - A 205 / N	1.42 b	5.53 a	16.16 a	37.75 a	51.92 a
	Flush II				
UV-A clear	1.20 b[1]	10.55 a	18.65 b	34.01 b	55.56 b
IR 504	1.33 ab	10.69 ab	18.68 ab	34.88 ab	56.9 ab
UV - A 205 / N	1.43 a	10.98 a	19.07 a	36.33 a	59.64 a

[*]Means followed by same letter (s) along the column for number of days after harvesting and across the rows for different poly film types are not significantly different at 5 % level of significance according to Tukey's honestly significant difference test. Values are means of increase in growth for number of days after harvest (DAH).

There was no evidence of transmission of light within the short wavelength of UV-C and UV-B radiation (Figure 2 and 3 above) which could result to decrease in carbon dioxide fixation, impairment of photosystem II, decrease in the Rubisco activity, reduction in dry weight starch and chlorophyll content among others. Further influence on growth could be implicated on microclimate attributed more to variations in relative humidity. Low humidity affects growth of greenhouse crops by impacting on the leaf size rather than directly on photosynthesis by its effect on stomata closure (Mortensen, 2000). The UV-A clear poly film consistently had low relative humidity which inversely correlated with high day air temperature during the growth period that could have led to shorter stems.

The extent of stem elongation was found to vary among the cultivars as well as flushes which conventionally could be represented by production season (Table 5). In flush I Furiosa had higher stems than Red Calypso while in the second flush the results were inconsistent at day 17 and day 24 plant height was similar across the cultivars. A significant increase in plant height of 21.1 % and 12.3 % was recorded during the second flush for Red Calypso and Furiosa respectively.

The increase in height could be due to air temperature differences between flushes I and II. This observation illustrates that decrease in air temperature result to significant increase in growth which supports the findings of Mata and Botto (2011) who studied the effect of photoperiod, light and air temperature on plant architecture and flowering of *Salvia exserta* and reported that high irradiance (870 µmol m^{-2} s^{-1} – 1,040 µmol m^{-2} s^{-1}) reduced plant height by 20 %. They also demonstrated that irradiances above 400 µmol m^{-2} s^{-1}, and air temperatures higher than 20 ºC accelerated flowering. Plant height was reduced by 17 % under environmental condition with high R: FR ratio and the laterial shoots doubled in number.

Table 5: Effect of cultivar on rose plant height in flushes I and II

	Days after harvesting (DAH)				
	10	17	24	31	38
Rose cultivar			Flush I		
Red Calypso	1.16 b[1]	4.80 a	14.04 a	33.46 b	43.30 b
Furiosa	1.38 a	5.07 a	15.46 a	39.32 a	54.29 a
			Flush II		
Red Calypso	1.23 b[1]	9.84 b	17.19 b	32.23 b	52.44 b
Furiosa	1.41 a	11.64 a	20.41 a	37.91 a	60.95 a

[1]Means within a column for rose cultivars followed by different letter (s) are significantly different at 5 % level of significance according to Tukey's honestly significant difference (HSD) test. Values are means (n = 3) for number of days after harvest.

Growth was observed to be generally slow during the second flush and plants took long time to mature compared to flush I. Apparently, it is possible that the quantity of light diffused under the growth structures in flush II that was characterized by overcast was low. This enhanced growth under the UV-A clear cover to attain a plant height similar to plant grown under IR 504. The difference among the two flushes demonstrate that cloud cover reduces direct light transmission which may further have affected growth for plants established under coloured poly covers. Low light intensity increases chlorophyll content within the plant that enhance light interception utilised in photosynthesis implying better growth. This could probably be the reason why plants under diffused light grew taller than those under direct light transmission.

4.2.4. Relationship between height and PAR

A positive relationship was established between height and PAR, increase in PAR caused significant increase in growth. Although an R^2 values ranged between 0.55 and 0.46 in flushes I and II respectively. These values provide an average estimate of the strength of the relationship between PAR and growth. It is possible that plant height was affected by PAR due to variations in the physical environmental factors that kept changing from time to time and could not be easily predicted (Figure 11). There is a very strong relationship between plant height and PAR, which could be influenced by photosynthetic responses of the plant.

Absorption of light for photosynthesis is controlled by the leaf anatomy which is further influenced by the external light environment. Generally, the highest percentage of light that reaches the ground is not fully utilized by the plants or converted into carbohydrates by photosynthesis. The bigger fraction of incident light reaching the earth is of wavelength either too short (less than 400 nm) or too long (> 700 nm to far red and infrared) to be utilized for photosynthesis. Light transmission through the UV-A clear poly film was at higher wavelength with less concentrated within the PAR region in both flushes I and II (Figure 5 and 6). This could explain the reason as to why plant height was shorter under this cover since there is high likelihood of less photosynthetic activities. Additionally, productivity was high under the UV-A clear poly film this implies that there was competition for light and only a small percentage of the leaves could be exposed to full light necessitating photosynthesis and hence better plant growth.

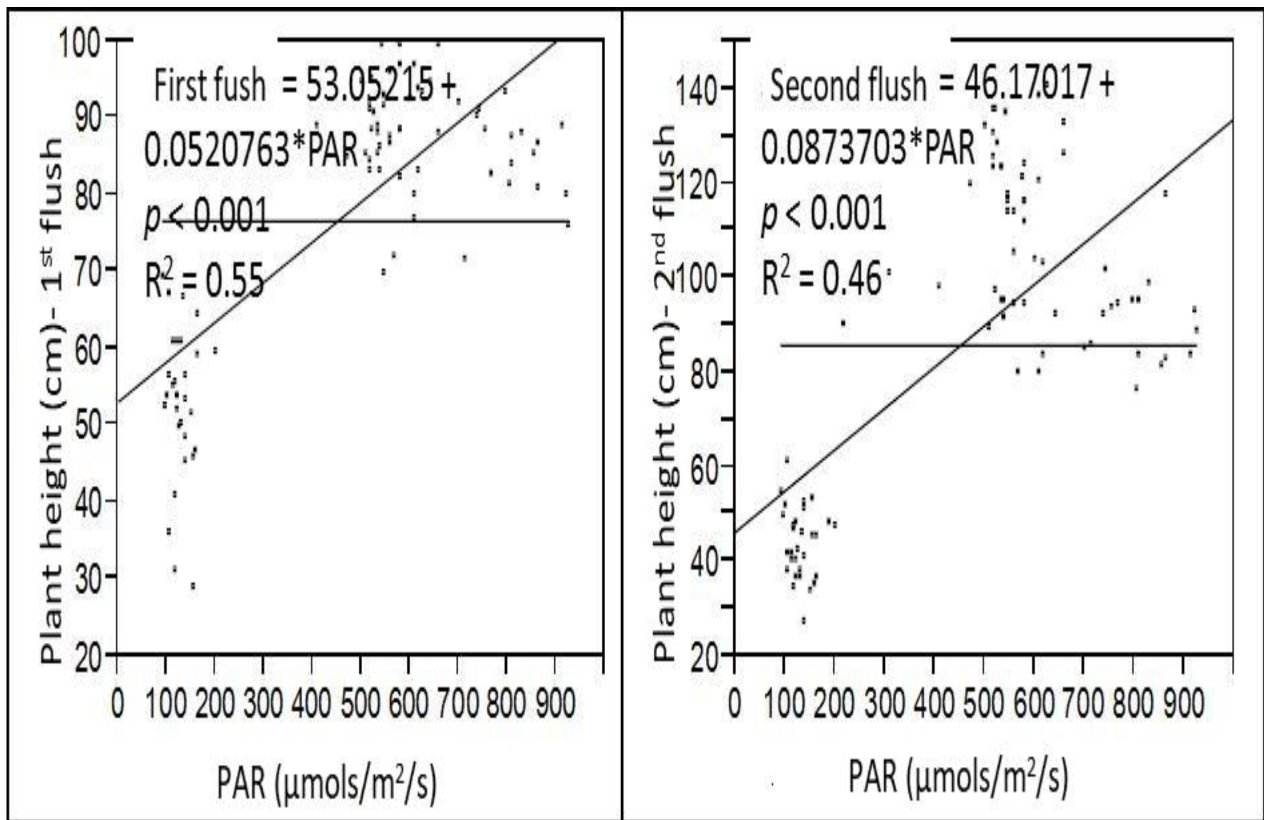


Figure 11: Effect of Photosynthetic active radiation on rose plant growth.

4.2.5. Effect of poly film covers and cultivars on rose stem grades

UV-A clear poly film recorded 12.1 % of its production as short stems (< 40 cm) compared to IR 504 and UV-A 205/N that had 5.0 % and 6.2 % respectively in this category. 41.3 % of the stems produced in flush I under the UV-A clear poly film were in the category of short stems (40 cm to 49 cm) accounting for almost 50 % of the total production per square metre while 35.3 % and 28.6 % stems were recorded under IR 504 and UV-A 205/N poly film covers in similar category respectively. The number of stems in the medium size grade (50 cm to 59 cm) was statistically similar across all treatment and their combined effects of poly films and cultivars (Appendix 9). In first flush, the longest stems harvested were in the category of 70 cm to 79 cm with 3.8 % of the stems being harvested under UV-A 205/N poly film and the least number of 1.7 % under UV-A clear poly film.

Premium grades of rose cut flowers measuring 80 cm to 89 cm and even greater than 90 cm were harvested across all poly film covers in flush II whereas none within this category was harvested in flush I. UV-A clear, IR 504 and UV-A 205/N produced 3.3 %, 4.6 % and 6.6 % stems

respectively in the premium grade. Apparently, the number of stems produced per square metre reduced in flush II by 8.4 % under the UV-A clear poly film, 31.6 % under IR 504 and 6.1 % under UV-A 205/N. The highest number of short stems in the category of < 40 cm and 40 cm to 49 cm were recorded under the cultivar Red Calypso compared to Furiosa. The number of stems in premium grades 80 cm to 89 cm and up to ≥ 90 cm increased in second flush compared to the first for both poly film and cultivar treatments. The number of stems in the middle category (50 cm to 59 cm) was statistically similar under all poly films in flush I, however in flush II Furiosa had more stems in this category compared to Red Calypso. In flush I, Furiosa consistently had longer stems from grade 60 cm to 69 cm and the consecutive grade of 70 cm to 79 cm compared to Red Calypso. The number of stems were higher by 26 % and 8 % for the 60 cm to 69 cm and 70 cm to 79 cm grades respectively across all treatment (Table 6 and 7).

Table 6: Effect of poly film covers on rose cut flower grades (stem length).

	Stem length						
Poly film covers	< 40 cm	40 to 49 cm	50 to 59 cm	60 to 69 cm	70 to 79 cm	80 to 89 cm	> 90 cm
			Flush I				
UV-A clear	3.6 a	12.3 a	11.4 a	2.0 a	0.5 a	0.0 a	0.0 a
IR 504	1.3 b	9.1 b	12.2 a	2.5 a	0.7 a	0.0 a	0.0 a
UV - A205 / N	1.3 b	6.0 c	10.7 a	2.2 a	0.8 a	0.0 a	0.0 a
			Flush II				
UV-A clear	3.0 a	10.1 a	7.2 a	4.7 b	1.4 b	0.9a	0.2 a
IR 504	1.0 b	1.8 b	6.7 a	6.6 a	2.1 a	0.9a	0.5 a
UV - A205 / N	1.0 b	2.1 b	5.2 b	6.9 a	2.9 a	1.3a	0.4 a

[1]Means followed by different letter (s) along the column for poly films are significantly different at 5 % level of significance according to Tukey's honestly significant difference (HSD) test. Values are means n = 3

Table 7: Effect of rose cultivar on cut flower grades (stem length)

			Flush I				
Cultivar	< 40 cm	40 to 49 cm	50 to 59 cm	60 to 69 cm	70 to 79 cm	80 to 89 cm	> 90 cm
Red Calypso	3.3 a	13.2 a	11.9 a	0.9 b	0.1 b	0.0 a	0.0 a
Furiosa	0.9 b	5.1 b	11.0 a	3.5 a	1.2 a	0.0 a	0.0 a
			Flush II				
Cultivar	< 40 cm	40 to 49 cm	50 to 59 cm	60 to 69 cm	70 to 79 cm	80 to 89 cm	> 90 cm
Red Calypso	2.4 a	5.3 a	5.9 b	4.9 b	1.0 b	0.3 b	0.1 b
Furiosa	1.0 b	4.0 b	6.9 a	7.2 a	3.3 a	2.0 a	0.6 a

[1]Means followed by different letter (s) along the column for different cultivars are significantly different at 5 % level of significance according to Tukey's honestly significant difference (HSD) test. Values are means n = 3

Light transmission through the poly films was a key determinant of stem length. Low air temperatures favoured production of longer stems as evidenced in flush II compared to flush I. Rose plants grown under coloured poly films with diffused light had higher percentage of longer stems per square metre compared to those under UV-A clear poly film. Plants cells tend to elongate more under perceived conditions of insufficient light. UV- A clear poly film transmitted more light hence the plant cells thickened more than elongating resulting to shorter stems. Variations in day and night air temperature affect response of plants in terms of growth. Carvalho *et al*. (2002) studied the effect of day and night air temperature (DIF) on Chrysanthemum and reported that internode and stem length was affected by the combination of the independent effects of air temperature during the day and night periods. Therefore, plants elongate more under air temperatures not exceeding optimal due to rapid cell elongation which vary among plants. This could be the reason why plants under coloured poly film were taller since air temperature under the UV-A 205/N and IR 504 were slightly lower than UV-A clear cover. Air temperature above optimal damage the cell division among other physiological processes and result to small plants.

Elevated air temperatures result to high transpiration rate in plants, this triggers water stress in plants and affect the enzyme activities especially the sucrose-6-phosphate synthase (SPS).

This enzyme reduces because of photosynthesis inhibition hence plants subjected to high air temperature remained short due to limited availability of photosynthates to support growth. Additionally, occurrence of shorter stems could also be linked to the consistent low relative humidity. Relative humidity directly influences the water potential and indirectly reduces dry matter production through stomata closure that automatically limits photosynthesis. Nutrient movement within the plant tissue is also dependent on relative humidity such that low humidity limits the process of plant nutrient translocation directly impacting on growth.

4.2.6. Poly film covers and rose cultivars on number of days taken to flowering

The number of days taken to flowering by the rose cultivars was dependent on their interaction with the poly films under which they were grown (Appendix 10). The number of days taken from visible bud to harvesting by Red Calypso and Furiosa under the UV-A clear poly film were 43 and 50 respectively. The combined effect of the cultivars Red Calypso and Furiosa with IR 504 poly film took 47 and 52 days to flowering respectively. These treatments took more days to flowering than other combined treatments. Furiosa under the poly film IR 504 and UV-A 205/N took more days to flower compared to Red Calypso under the same poly films. Less number of days were taken from visible bud to harvestable stem by both cultivars under UV-A clear poly film compared to number of days taken under the IR 504 and UV-A 205/N Poly films by the same cultivars. The results of flushes I and II were consistent in that Red Calypso took less days to flower under the UV-A clear poly film (46 days) and UV-A 205/N (47 days) poly films compared to the same cultivar under IR 504 (49 days) poly film (Table 8).

Changes in greenhouse microclimate that occurred between flushes I and II led to an increase in the number of days taken to flowering under different poly films by 2 to 3 days. These results demonstrate variability in plant response and transition from vegetative stage to reproductive stage as influenced by light quality, air temperature and accessions. Growth under the UV-A clear poly film was faster due to the high transmission of far red light which accelerates flowering. Previous study by Runkle and Heins (2001), established that far red-light deficient environment delayed flower initiation in *Campanula carpatica* jacq while the time to flower development was reduced by supplementing the growth environment with far red light. Rose plant growth is dependent on cultivar and production may also vary from season to season with variations in far red light as demonstrated by variability between flushes I and II in this study.

Table 8: Effect of poly film covers and cultivars on the number of days taken from visible bud to flowering (flushes I and II)

Treatments	Flush I		Flush II	
Cover/cultivar	Red Calypso	Furiosa	Red Calypso	Furiosa
UV-A clear	43 d[1]	50 b	46 c	52 ab
IR 504	47 c	52 a	49 b	54 a
UV - A 205 / N	46 c	51 ab	47 c	54 a

[1]Values in the column followed by different letter (s) are significantly different at 5% level of significance for the same cultivar by the poly film type along the rows according to Tukey's honestly significant difference (HSD) test. Values are means n = 3

Early flowering was noted to occur under the UV-A clear poly film in flush I compared to IR 504 and UV-A 205/N which primarily could be due to the influence of microclimate under the growth structures. Air temperature is known to accelerate the rate of growth and development for plants thus under the UV-A clear poly film duration taken from bud initiation to flowering was shorter. The number of days to flowering increased in flush II as air temperature reduced. This observation supports the findings of Shin *et al*. (2001) who observed that the number of days from bud break to flowering increased from 22 days to 63 days as air temperature decreased from 30 °C to 15 °C. In another study done to investigate the effect of air temperature on orchids by Lopez and Runkle (2004), they reported a decrease in the number of days taken from visible inflorescence to open flower from 73 days at 14 °C to 30 days at 26 °C. It is therefore clear that air temperature plays a vital role in the number of days taken to flower in rose plants.

The number of days taken to flowering was noted to increase across all the poly film covers especially in the second flush. Flush II was characterized by low greenhouse air temperature and high humidity. UV-A 205/N poly film took the greatest number of days to flower in flush II compared to the IR 504 and UV-A clear poly films giving inconsistent results to observations made in flush I where the number of days recorded under UV-A 205/N and IR 504 were statistically similar. Generally, calcium pre- harvest treatment applied had no significant effect on the number of days taken to flowering in both flushes. Although calcium plays several functions in plants ranging from physiological processes, metabolism of lipids, proteins and carbohydrates, cell division and development among others (Tuteja and mahajan, 2007) its effect on growth rate in this study could not be easily quantified.

The effect of calcium in this case could have been masked by the external environmental stimuli (Sanders *et al*., 2002) among other stresses. It is expected that with enough calcium in the plant, growth would have been faster especially under high calcium concentration since plants would accumulate higher biomass (Martinez-Noel *et al*., 2006). On the contrary, previous works have also shown that environmental signals induce changes in the cytosolic Ca^{2+} which is important for cell survival (Tuteja and Mahajan, 2007). Depending on the changes induced, the plant may positively or negatively perceive the environmental stress resulting to earlier or delayed maturity a phenomenon that could be linked to the observation made in the current study.

4.2.7. Effect of cultivar on cut flower stem and bloom diameter

Stem diameter significantly differed from one cultivar to the other. During flush I the mean stem diameter under the cultivar Red Calypso was 6.11 mm and decreased by 0.3 % in flush II to a thickness of 6.09 mm (Appendix 11). Stem diameter for Furiosa increased from 6.58 mm to 6.62 mm translating to 0.61 % increase in diameter (Figure 12). Pre - treatment application of calcium foliar feed had no effect on stem and bloom diameter. Stem thickness for cultivar Furiosa were 7.7 % bigger in size than Red Calypso in flush I and increased further to 8.7 % in flush II. Average bloom thickness of 29.74 mm, 27.85 mm and 27.67 mm were recorded under UV-A 205/N, IR 504 and UV-A clear poly films respectively. Stems harvested from UV-A 205/N poly film had bigger bloom diameter compared to UV-A clear and IR 504 whose thickness was statistically similar (Appendix 12).

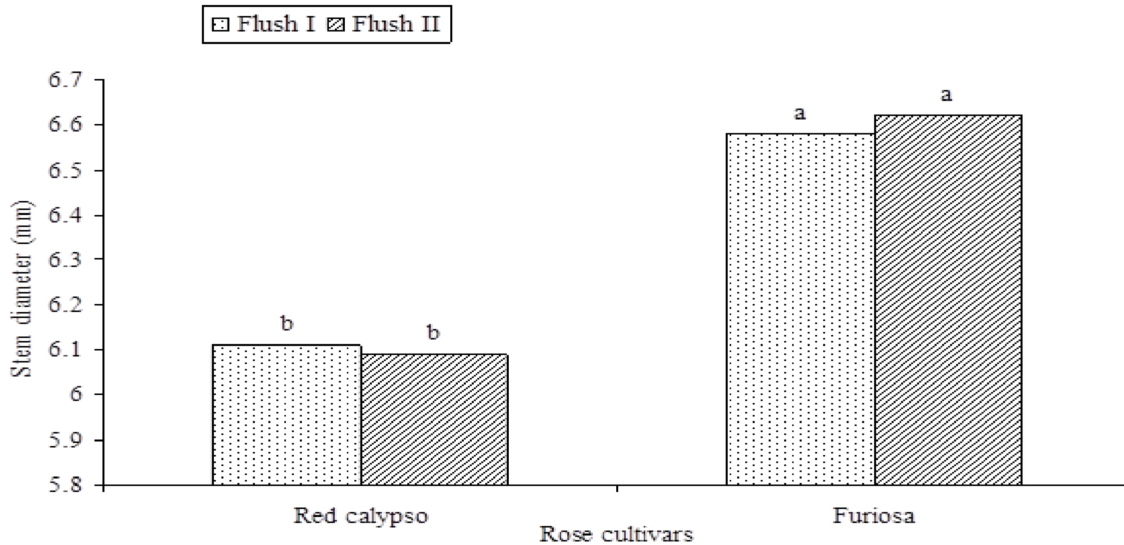


Figure 12 : Effect of cultivar on rose cut flower stem diameter during flushes I and II

Flower diameter was significantly different among the poly film covers in flush I however results of flush II did not indicate any significant difference (Appendix 13). UV-A clear poly film and IR 504 recorded the smallest bloom size of 27.7 mm and 27.85 mm and recorded high air temperatures of 38.9 °C and 37.1 °C respectively than UV-A 205/N whose bloom size was 29.74 mm at air temperature 34.4 °C in flush I. Bloom size was statistically similar among all the poly films in flush II, however compared to flush I the increase in size varied by 32.9 %, 34.6 % and 25.6 % for poly films UV-A clear, IR 504 and UV-A 205/N respectively. Generally, there was a decline in air temperature in flush II compared to flush I. In flush II the different poly films exhibited air temperatures of 32.5 °C, 34.4 °C and 38.9 °C for IR 504, UV-A 205/N and UV-A clear poly films respectively (Figure 13).

Stem and flower diameter were generally affected by the growth conditions in the current study as it was observed by variations in size of flowers harvested from different poly film covers. In a different study, Oren-Shamir *et al*. (2000) demonstrated that flower size in Asters increased by 20 % at the lowest air temperature regime of 17 °C / 9 °C compared to 29 °C / 21 °C. This supports the observation made in the current study where by flower diameter under the UV-A clear poly film increased significantly from 27.67 mm to 36.77 mm indicating 32.9 % increase in size when air temperatures changed from 38.9 °C in flush I to 33.8°C in flush II.

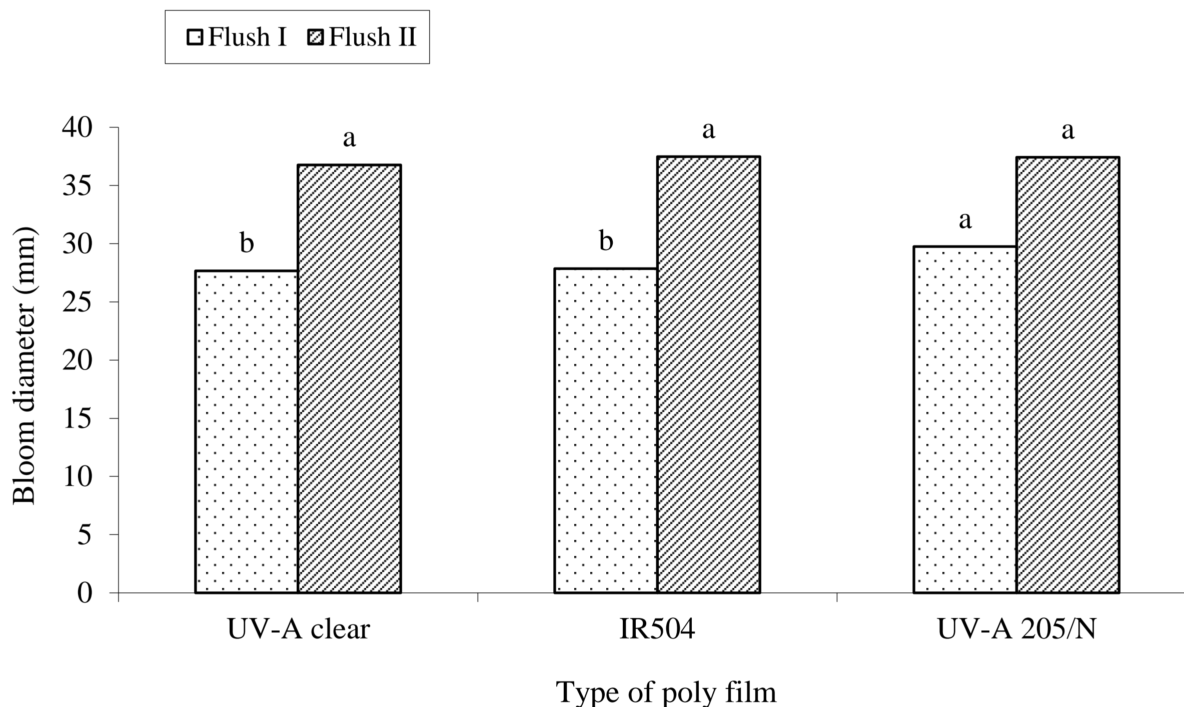


Figure 13: Effect of poly film covers on cut flower bloom diameter in flushes I and II

Low air temperature limits energy utilization in the plant and increase the sugar storage capability. It is likely that the sugar storage increased during the second flush leading to formation of better flower blooms. Low air temperature slows the plant physiological activities, lengthening the time take from bud initiation to flowering. Eventually, this delay necessitates the plant to develop more petals and whorls forming flower blooms that are bigger in size. This could be the probable reason why bloom size increased under poly film UV-A clear between flushes I and II.

4.2.8. Effect of calcium foliar feed and rose cultivar on leaf chlorophyll content

Leaf chlorophyll content was dependent on the cultivars and calcium foliar feed treatment (Appendix 14). Combined treatment of Red Calypso and calcium foliar feed at an application rate of 3.75 ml/L recorded the highest leaf chlorophyll content (56.23 concentration index). Low chlorophyll content (40.18) was recorded under the cultivar Furiosa with the control experiment compared to Red Calypso under similar treatment. Chlorophyll content was high in flush II compared to flush I. Under the control treatment there was remarkable increase of 5.45 % and 11.2 % in chlorophyll content in Red Calypso and Furiosa respectively between flushes I and

II respectively. Under calcium treatment 5.0 ml /L chlorophyll increased by 6.35 % in flush I and 9.75 % in flush II. The increase in chlorophyll content between the control and 5.0 ml/L calcium treatment was 0.9 % and 1.45 % for Red Calypso and Furiosa respectively (Table 9). The results of this study confirm that pre-harvest application of calcium increase the leaf chlorophyll content.

This observation could in part be attributed to the potential of different cultivars to acclimate to environmental factors at specific levels, with every cultivar having its own optimal requirements. Plants acclimate to increased irradiance through enhanced dissipation of absorbed excess energy in the thylakoids through the zeaxanthin quenching process (Demmig-Adams and Adams, 2006). In the process, there is induced chlorosis that result in reduction of this important photosynthetic pigment. High air temperature under the UV-A clear poly film is likely to have initiated stomata closure leading to reduced photosynthetic activities and consequently low chlorophyll content.

Calcium is known to prevent damage from cellular destruction and additionally plays a key role in balancing of osmotic strength in plants. This could explain why higher leaf chlorophyll content was reported where calcium foliar feed was applied than the control. Further, similar results were reported by Xu *et al*. (2013) who established that calcium pre-treatment application could act as a physiological treatment to increase plant environmental stress tolerance.

The difference noted here indicates the genetic variability and reflect responses to environmental changes by the different cultivars. Depending on genotypic differences plant organs respond differently to the environment including photosynthetic traits, light saturation and water use efficiency. High irradiance as was the case in flush I impacted negatively on leaf chlorophyll content. The amount of solar radiation absorbed by a leaf is basically a function of the foliar concentration of photosynthetic pigments (Richardson *et al*., 2002) which may differ from one plant species to the other. This confirms the observation made on chlorophyll content for cultivars under no calcium treatment. Red Calypso had higher chlorophyll content compared to Furiosa by 22.1 % in flush I and 15.7 % in flush II respectively. Indicating that besides other factors plant chlorophyll content could be influenced at genetic level.

Table 9: Effect of calcium and rose cultivar on leaf chlorophyll content

	Flush I				
	Calcium foliar feed concentrations				
Cultivar	Distilled water	1.25 ml/L	2.5 ml/L	3.75 ml/L	5.0 ml/L
Red Calypso	49.81 cd[1]	51.79 bc	55.89 a	56.23 a	54.43 ab
Furiosa	40.81 f	45.56 e	47.51 de	50.12 cd	51.57 bc
	Flush II				
	Distilled water	1.25 ml/L	2.5 ml/L	3.75 ml/L	5.0 ml/L
Red Calypso	52.52 de[1]	54.59 bcd	57.26 ab	60.59 a	57.89 ab
Furiosa	45.39 f	49.56 f	53.32 cd	55.96 bcd	56.6 bc

[1]Means followed by the same letter (s) along the row for different calcium foliar feed levels by cultivars are not significantly different at 5 % level of significance according to Tukey's honestly significant difference test.

4.2.9. Effect of cover and calcium foliar feed on leaf chlorophyll content

Combined treatment of poly film covers, and calcium had significant effect on leaf chlorophyll content. Leaf chlorophyll content was high under the IR 504 poly film and calcium foliar feed at concentration 3.75 ml/L. Under the UV-A clear poly film and control experiment with no calcium foliar feed leaf chlorophyll content was significantly low compared to IR 504 cover. Leaf chlorophyll content was statistically similar in all calcium foliar feed levels under the IR 504 poly film. Under the UV-A clear and UV-A 205/N poly films there was high leaf chlorophyll content under 2.5 ml/L, 3.75 ml/L and 5.0 ml/L while 1.25 ml/L and control treatment were statistically lower (Table 10). Calcium levels 3.75 ml/L and 5.0 ml/L exhibited a similar trend in chlorophyll content in both flushes I and II.

Table 10: Effect of cover and calcium on the leaf chlorophyll content in flushes I and II

	Flush I			Flush II		
	Poly film covers			Poly film covers		
Calcium Foliar	UV-A clear	IR 504	UV - 205 / N	UV-A clear	IR 504	UV - 205 / N
Distilled water	41.68 f[1]	48.38 de	45.87 ef	46.2 f[1]	51.62 de	49.05 ef
1.25 ml/L	45.92 ef	52.00 abcd	48.13 de	49.88 ef	55.43 abcd	50.90 def
2.5 ml/L	49.62 cde	54.90 ab	50.58 bcd	52.08 cde	58.97ab	54.82 bcd
3.75 ml/L	51.20 bcd	55.98 a	52.35 abcd	56.53 abc	59.63 a	58.65 ab
5.0 ml/L	51.37 bcd	53.95 abc	53.68 abd	55.35 abcd	57.67 ab	58.75 ab

[1]Values in the column followed by different letter (s) are significantly different at 5 % level of significance for selected poly film covers and along the rows for calcium foliar feed according to Tukey's honestly significant difference test. Values are the means of the treatments (n = 3)

Under flush II calcium level 3.75 ml/L and 5.0 ml/L had high and statistically similar concentration of chlorophyll to calcium treatments under IR 504 and UV-A 205/N. In flush II the chlorophyll content under combined treatment of 1.25 ml/L and IR 504 was statistically similar to calcium treatments 2.5 ml/L, 3.75 ml/L and 5.0 ml/L under the same cover. Although calcium had influence on leaf chlorophyll content, it is apparent that poly film cover that recorded higher air temperature and light intensity recorded lowest chlorophyll content. The effect of calcium in chlorophyll content was insignificant where air temperature was high however it was noticeable where the air temperature was low. Calcium is known to sustain membrane integrity by deferring senescence and loss of chlorophyll. It plays this role by maintaining proteins and RNA levels within the plant tissues that are central indices of chlorophyll. Sharp decline in chlorophyll content where high air temperatures were recorded suggest that there might be some evidence of tissue damage.

Low and high calcium concentration has been reported to influence plant growth (Mengel and Kirkby, 2001). Further, similar results were reported by Gupta *et al*. (2007) who demonstrated that low and high calcium concentrations decreased plant biomass production and fruit yield of bitter gourd. In this study done by Gupta *et al*. (2007), they looked at the influence of calcium concentration on chlorophyll a and chlorophyll b separately. They established that Chl

a and Chl b were high (1.67 and 0.81 mg g^{-1} fresh weight) at 4.0 mM of calcium than 8.0 mM (1.19 and 0.497 mg g^{-1} fresh weight) respectively. This observation is in consonance with the results of the current study where higher calcium concentration at 3.75 ml/L and 5.0 ml/L had no significant effect on leaf chlorophyll content (53 and 53.18) compared to calcium concentration 2.5 ml/L (51.7 chlorophyll concentration index units). However, lower concentration of 1.25 ml/L and the control had significantly low chlorophyll content of 48.67 and 45.31 respectively. Calcium concentration at 2.5 ml/L recorded the optimal level of chlorophyll content.

UV-A clear poly film recorded higher levels of air temperature averaging 38.9 °C compared to IR 504 poly film that recorded 34.4 °C In both flushes air temperature was consistently high under the UV-A clear poly film which impacted negatively on the total chlorophyll content. Under conditions of high air temperature plant chlorophyllase enzyme initiates the de-greening process of chlorophyll for plant organs development and survival from damage. In addition, when the photosynthetic complexes absorb excess light, reactive oxygen species are generated in the chloroplasts causing damage in the systems of photosynthetic pigments (Hutin *et al*., 2003).

Chlorophyll content also decreased drastically under conditions of high irradiance probably due to failure of chlorophyll accumulation and induced chlorosis. Under the UV-A clear poly film, mean day air temperature of 38.9 °C was recorded which is higher than the optimal for rose production (28 °C). Under such conditions plants develop mitigation strategies to avert destruction of the photosynthetic apparatus. Several changes occur within the plant cell, among them being the closure of the stomata. Once the stomate close the photosynthetic activities are affected and assimilation of carbon dioxide is also affected hence reduction in the chlorophyll content.

Chlorophyll content was also noted to be high in flush II compared to flush I. Generally, the overall mean day air temperature was low in flush II compared to flush I. The relationship between chlorophyll type and the environmental conditions show that Chl b, is lower than Chl a at low air temperature indicating the sensitivity of Chl b to environmental changes (Gilmore and Marilyn, 2000). Leaf chlorophyll content was affected more by high irradiance and air temperature during the day fluctuation in night air temperature had insignificant variation amongst the poly films. Results of the current study show that chlorophyll is more affected by high solar radiation as opposed to air temperature alone.

Different authors present varying arguments about the effect of air temperature on leaf chlorophyll, with some findings describing that chlorophyll increases with increase in air temperature (Gilmore and Marilyn, 2000). Leaf chlorophyll content in the current study was observed to be low under the control poly film cover that exhibited higher solar radiation and air temperature. This could be attributed to the mechanism to survive the excess air temperature by reducing the capabilities of leaf absorbance of solar radiation. Chloroplast avoidance movement has also been reported in plants under high air temperature in which the chloroplast moved from the cell surface to the inside walls of the cells (Masahiro *et al.,* 2002). This results to a decrease in the amount of light absorption by chloroplasts and avert the damage associated by high air temperature.

4.2.10. Effect of poly film on leaf area

Leaf area was significantly affected by cover and cultivar response (Appendix 15). The control poly film (UV-A clear) registered the lowest leaf area (1608.41 mm²) compared to IR 504 and UV-A 205/N which had a leaf area of 2022.95 mm² and 1914.80 mm² respectively. The leaf area was statistically similar for plants under IR 504 and UV-A 205/N poly films and the results were consistent for both flushes I and II. Significantly higher leaf area was recorded in flush II compare to flush I (Figure 14). Leaf area also varied among the rose cultivars, Red Calypso had significantly smaller leaves compared to Furiosa (Figure 15) in both flushes. Conventionally leaf area is affected by many factors ranging from environmental to inherent factors within the vegetative propagules.

The leaf area differed among the cultivars which could be attributed to the status of the propagule material in terms of accumulation and distribution of stored substances like starch and sugars at harvest. The rate of leaf formation is primarily influenced by effect of air temperature on leaf expansion especially the cell expansion region (Tamaki *et al*., 2002). Leaves growing in sunny environment are usually smaller compared to those under shade. Smaller leaf area was recorded under the UV-A clear poly film that exhibited high light intensity which could cause increased evaporation rate in plants and loss of water. The plants therefore, could have developed smaller leaves as a mitigation strategy to avert excessive water loss through transpiration.

Plants adjust to low light intensity by developing bigger leaves which can intercept light, this could explain why plants grown under the IR 504 and UV-A 205/N coloured poly films had

bigger leaf area compared to UV-A clear poly film. Leaf area increase as air temperature decrease and decrease as plants receive higher air temperature than the optimal (Shin *et al.,* 2001) Air temperature induce changes in plant anatomy and morphology hence differences in stomata size that occurred because of air temperature increase could be implicated in varying leaf area in this case. Air temperature also affect enzymatic activities, implying higher air temperature accelerates rate of leaf expansion and reduces duration to full leaf expansion leading to smaller leaf sizes. In addition, air temperatures beyond threshold is perceived as a stress factor in plants, physiologically its associated with production of reactive oxygen species (ROS) which causes reduction in growth.

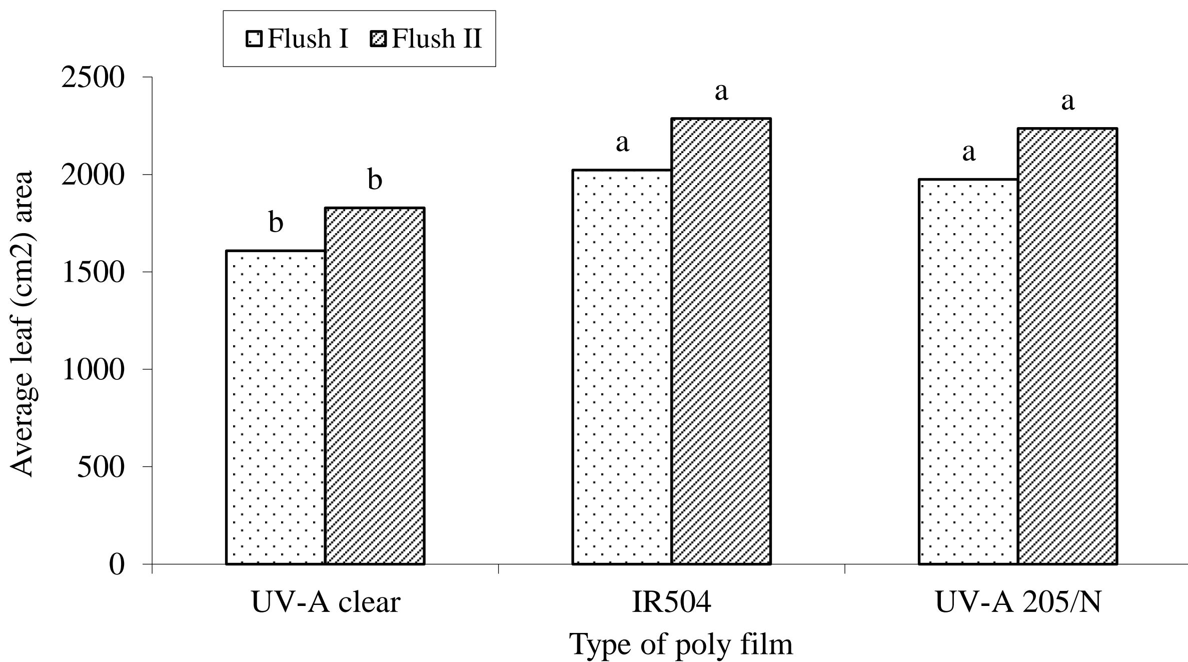


Figure 14: Effect of poly film cover on rose plant leaf area per flush for flushes I and II

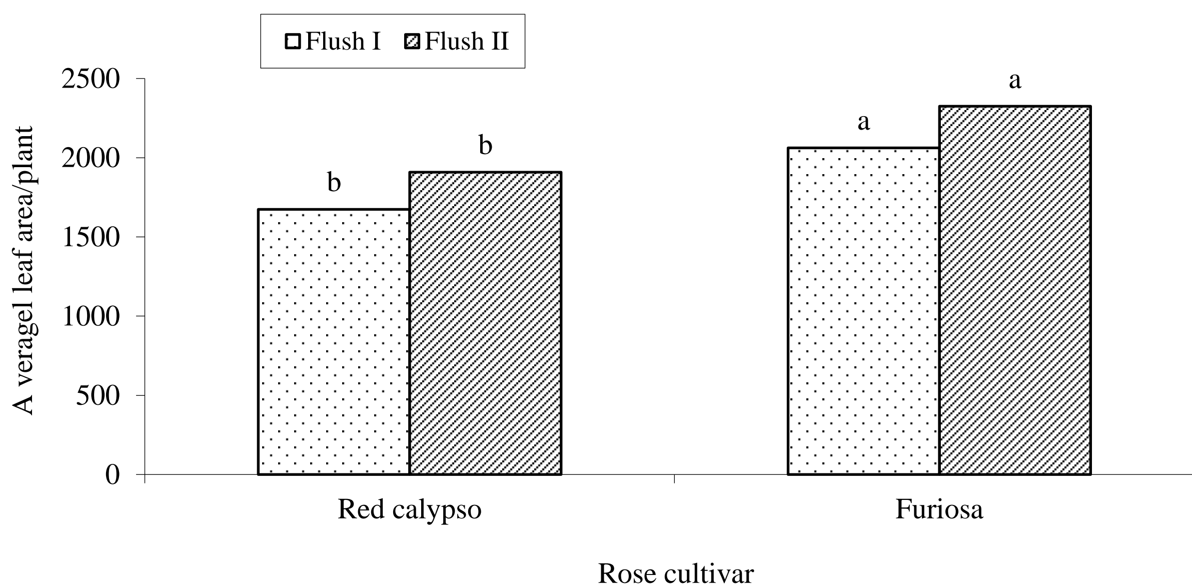


Figure 15: Effect of cultivar on rose plant leaf area per flush for flushes I and II, values are the means of the treatments (n = 3)

4.2.11 Effect of poly film covers on stomatal conductance

Stomatal conductance was significantly dependent on the poly film cover. Higher stomatal conductance of 253.02 mmol m^{-2} s^{-1} and 213.37 mmol m^{-2} s^{-1} was recorded under the UV-A clear and IR 504 poly films with leaf air temperature difference of 35.6 °C and 34.6 °C respectively. Stomata conductance decreased with increase in air temperature and was consistently high under the UV-A clear poly film during the 0800 hrs and 1600 hrs compared to the UV-A 205/N and IR 504 poly films. The highest stomatal conductance (253.02 mmol m^{-2} s^{-1}) was recorded under the UV-A clear poly film with air temperature of 35.6 °C. Although the UV-A 205/N poly film had almost similar air temperature of 34.6 °C stomata conductance under this poly film was slightly lower than the UV-A clear poly film. The average air temperature rose by day 42 and it was noted that IR 504 poly film had higher air temperature of 35.1 °C and high stomatal conductance of 213.01 mmol m^{-2} s^{-1} compared to UV-A clear. The lowest stomata conductance was recorded at 1200 hrs when light intensity and air temperature were equally high with low relative humidity (Figure 16).

Stomatal conductance varied with changes in the prevailing environmental conditions from time to time. The nature of plants is such that they adopt to the frequent changes in the

environment, as such the stomatal pores open and close depending on the environmental stimuli. The opening and closure of the stomata may increase or decrease the stomatal conductance that plays a pivotal role in the maintenance of the transpiration stream which is linked to the movement and absorption of the soil nutrients in plants. In addition the stomatal conductance also influences the carbon dioxide absorption that is key in photosynthesis. The greenhouse microclimate had significant effect on stomatal conductance as was evidenced from the results of the current study. Stomatal conductance was dependent on light intensity and air temperature. Poly film with high light intensity resulted in low stomatal conductance, under such conditions plants strive to maintain a balance between carbon dioxide intake and water loss through regulation of stomata aperture (Israelsson *et al.,* 2006).

Rose cultivars in the study differed significantly in their stomatal conductance potential. The rose cultivar Furiosa under the UV-A clear poly film recorded the highest mean stomata conductance (269.01 mmol m^{-2} s^{-1}) compared to Red Calypso under the same cover whose conductance was 237.03 mmol m^{-2} s^{-1}. Stomatal conductance for Red Calypso was statistically similar under all covers in the first flush. Stomatal conductance in rose cultivar Furiosa was significantly lower under the IR 504 poly film compared to the same cultivar under UV-A clear and UV-A 205/N. The lowest stomata conductance during the second flush was recorded under UV-A clear poly film (166.5 mmol m^{-2} s^{-1}) with Red Calypso compared to Furiosa under the same poly film which was significantly high (220.69 mmol m^{-2} s^{-1}). Stomata conductance for Furiosa in flush II was statistically similar under all poly film covers (Table 11).

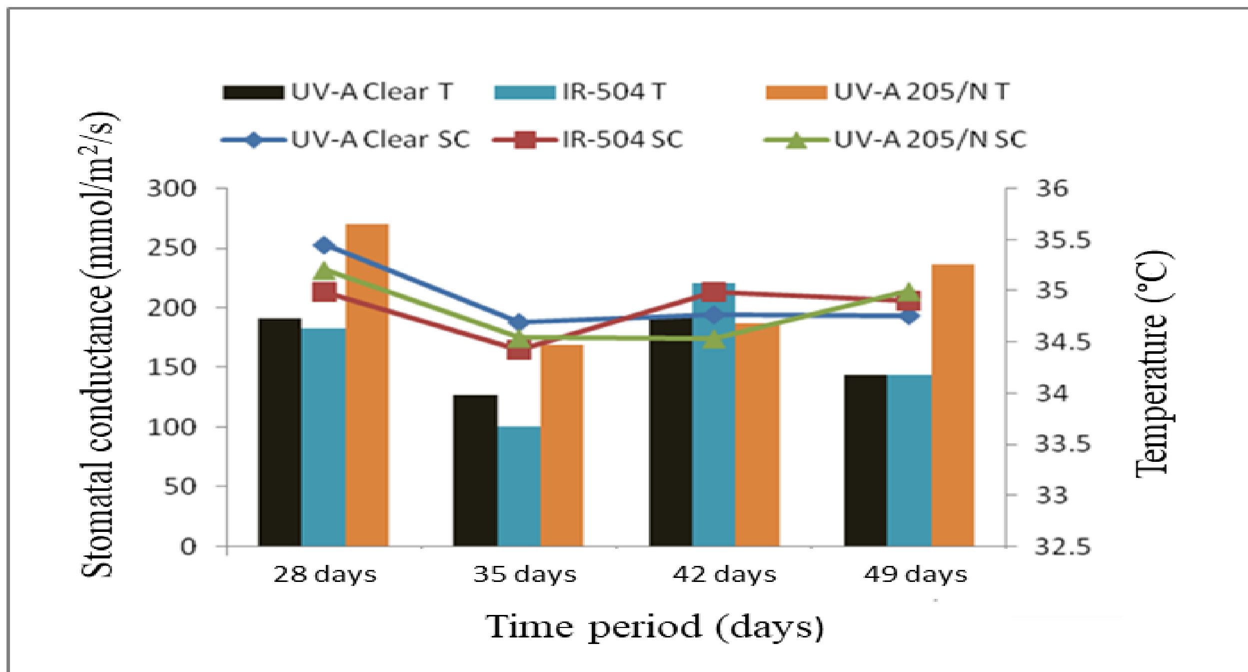


Figure 16: Change in stomatal conductance and air temperature under the poly film covers against number of days during plant growth.

Where T in the legend is air temperature and SC represents stomatal conductance

Stomatal conductance is influenced by temperature and light, from the results presented in figure 16 above stomatal conductance was noted to increase with increase in temperature till an optimal level. This could be explained by the results presented at day 42. The temperature was high and consequently the stomatal conductance did not increase proportionally with increase in temperature. Stomatal conductance also decreases with the decrease in temperature as observed from values presented by day 35. Stomatal conductance being a crucial determinant of the rate of photosynthesis it can therefore be linked to many physiological differences in plants that were noted in this study. High and low temperatures coincided with lower stomatal conductance that implies less photosynthesis. This could be the reason as to why plants under UV-A clear poly film exhibited shorter stems, low leaf chlorophyll content and dry matter content.

Table 11: Effect of poly film covers and cultivars on stomata conductance in flushes I and II

	Stomatal conductance (mmol m^{-2} s^{-1})	Flush I	Flush II
Cover / cultivar	UV-A clear	IR 504	UV - A 205 / N
Red Calypso	237.03 ab	223.56 ab	210.69 b
Furiosa	269.01 a	203.18 b	253.43 ab
		Flush II	
Red Calypso	166.52 b	221.63 a	203.71 ab
Furiosa	220.69 a	189.71 ab	224.7 a

[1]Means followed by different letter (s) are significantly different at 5 % level of significance according to Tukey's honestly significant difference test.

4.3. Poly film covers, cultivars and calcium on plant physiological disorders.

4.3.1. Effect of poly film covers on petal blackening

Petal blackening incidence was influenced by the poly film cover used (Appendix 16). Cultivars and calcium treatments had no significant effect on petal blackening under the conditions of the study. The incidence of petal blackening under UV-A clear and UV-A 205/N were statistically high compared to IR 504 during flush I. However, in flush II, the petal blackening incidence under UV-A 205/N and IR 504 were statistically similar though lower than UV-A clear. Although the occurrence of petal blackening was consistent under UV-A clear poly film in both flushes I and II, under UV-A 205/N and IR 504 poly films the incidence varied between flushes I and II, being more prevalent in flush I compared to flush II (Figure 17).

UV-A clear poly film could not retain high night air temperatures. Air temperature fluctuation was higher during the day and lower at night compared to the other poly films. Petal blackening was noted to be more prevalent during cold season especially when the night air temperatures were below 14 °C which was more in UV-A treated poly films. This could explain why petal blackening occurrence was high under UV-A clear and UV-A 205/N poly films. Conventionally, low air temperature reduces metabolism of various processes within the plant including reduced energy usage and increased sugar storage. It is possible that plant metabolic processes were slowed by the prevailing conditions in flush II causing increase in the occurrence of petal blackening under UV-A 205/N poly film compared to UV-A clear that remained stable.

The accumulation of the sugars in the flower petals could be the cause of discolouration seen as petal blackening under the UV-A 205/N cover. Cases of petal blackening occurrence were the highest and consistent under UV-A clear poly films in both flushes I and II since this poly film exhibited the highest temperature during the day and the lowest during the night. The drastic fluctuations in temperature could have enhance occurrence. The IR504 poly film recorded the lowest number of stems with petal blackening. This poly film maintained higher night temperature compared to UV-A treated poly films. Night temperature could also be a key factor to consider in minimising incidences of petal blackening as observed in this study.

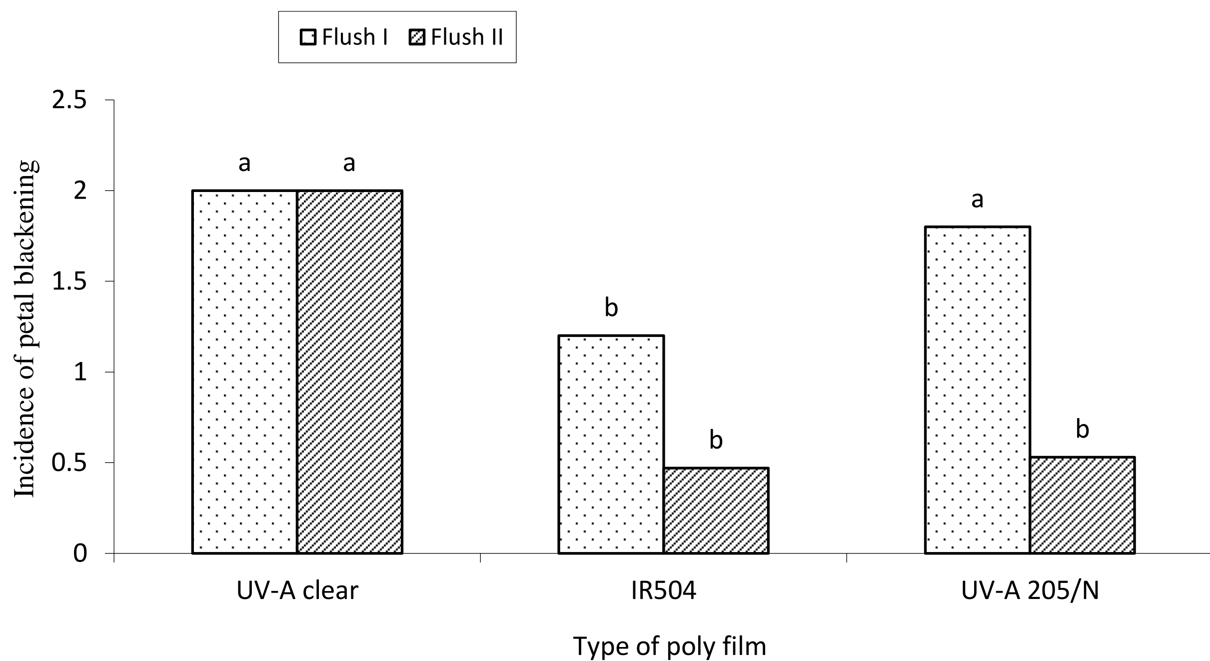


Figure 17: Effect of poly film covers on petal blackening occurrence

Zieslin and Mor (1990) investigated UV-induced blackening of rose petals during winter and established that blackening was prevented when rose plants were kept at air temperatures of 18 °C and higher his observation agrees with the findings of the current study. Besides air temperature, petal blackening could be induced by light quality and more so, the UV part of the spectra. It is expected therefore, that plants grown under IR 504 poly film would have recorded higher incidence of petal blackening since it was not treated against transmission of UV-radiation. However, the occurrence of petal blackening was lower under the IR 504 poly film which could be attributed to the positive characteristic of IR 504 poly film in maintaining higher night air

temperatures. The results of this study therefore confirm that blackening could be due to constant low air temperatures that were noted under the UV-A treated poly films.

4.3.2. Effect of cultivars and blind shoot occurrence

Poly film covers, and calcium foliar feed application had no significant effect on occurrence of blind shoots. Additionally, treatment interactions did not have any significant effect on incidences of blind shoot. The number of blind shoots was observed to differ among the cultivars. Red Calypso recorded the highest incidence of blind shoots averaging 3 stems / m^2 compared to Furiosa where on average 1 stem / m^2 was recorded. High incidence of blind shoot occurred in flush I compared to flush II. Generally, blind shoot occurrence was statistically similar across cultivars in flush I. Blind shoot seems to be more of varietal characteristics as noted in this experiment where one cultivar was more prone to the problem than the other (Figure 18).

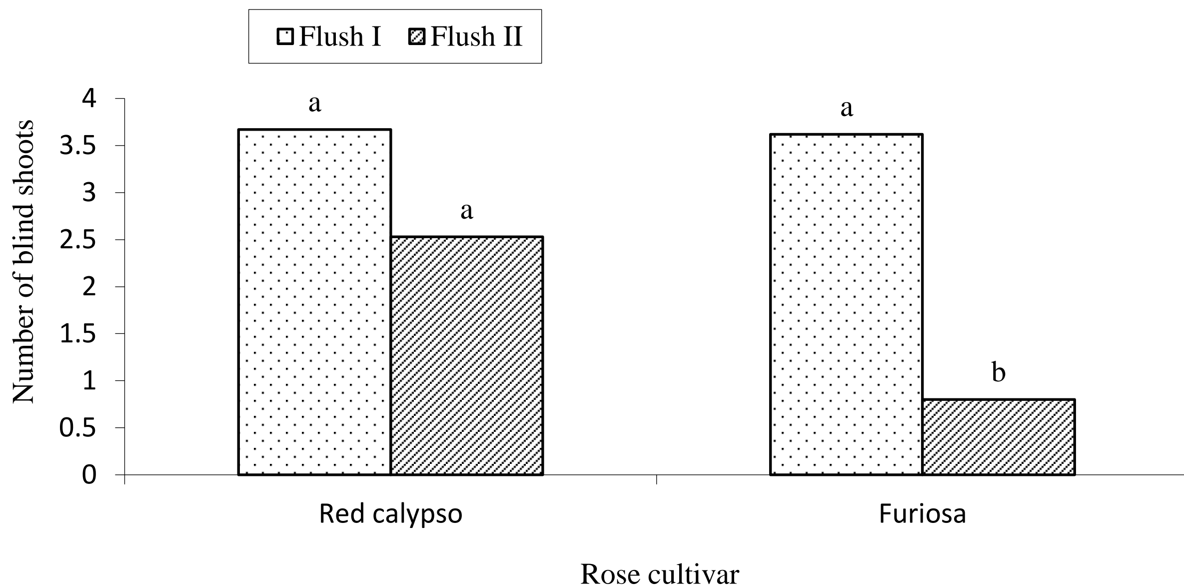


Figure 18: Effect of cultivar on rose plant blind shoot occurrence flushes I and II

The number of blind shoots were more in flush I which recorded high levels of solar radiation. Due to the high air temperatures plants may have accumulated less photosynthates to support growth since illumination hours and the plant nutrient affect plant growth and differentiation. Therefore, limited carbohydrate reserves within the plant tissues would be the most probable cause of higher number of blind shoots in flush I. High air temperature above 30 °C and greenhouse humidity less than 60 % resulted into poor quality stems with more blind shoots as observed in earlier research (Dayan *et al*., 2000). A decrease in illumination decreases

photosynthesis as it was observed in this study to reduce stomatal conductance. The effect of this could be reduced flower production and consequently it is expected that the number of blind shoots would have increased in flush II. On the contrary, the number of blind shoots decreased, an indication that plants were able to assimilate more photosynthates to support growth. This finding supports the work done by Atsushi *et al*. (2005) who reported that decreased illumination resulting in low air temperature decreased total shoot production and increased illumination consequently increased shoot production.

As noted earlier in this study, production of more shoots per square metre as observed under UV-A clear poly film which implies higher competition for the available carbohydrate reserves among the sprouted shoots. Due to high productivity under UV-A clear compared to IR 504 and UV-A 205/N there was a likelihood of competition for light, nutrients and other growth factors leading to poor quality stems leading to increased number of blind shoots. In addition, among the tested poly films UV-A clear exhibited the highest light transmission. Under such conditions of high air temperature and light transmission the rate of photosynthesis could be low as the leaf chlorophyll tends to be affected and the plants stomates close to reduce the rate of water loss hence accumulation of less photosynthates that could not support proper plant growth and development.

4.3.3. Effect of poly film covers on flower petals anthocyanin content

Poly films significantly affected the quantity and quality of anthocyanin accumulation in rose petals (Appendices 17, 18 19 and 20). Calcium foliar feed had no significant effect on rose petal anthocyanin content. Cyanidin 3 – 0 - glucoside was the most prevalent anthocyanin across all poly film covers and it was noted to be higher under the UV-A 205/N (110.95 ± 8.26) and IR 504 (109.69 ± 8.26) compared to UV-A clear (84.56 ± 8.26). The quantity of cyanidin 3-0-glactoside and cyanidin chloride were statistically similar under the IR 504 and UV-A 205/N poly films compared to UV-A clear. Interestingly the quantity of pelargonidin chloride was relatively stable across all the poly film treatments. Peonidin chloride was relatively low under the UV-A clear poly film compared to IR 504 and UV-A 205/N (Table 12 and 13).

Cyanidin 3 - 0 - glucoside and cyanidin 3 – 0 - galactoside were high in Red Calypso and lower in Furiosa implying that species of same plant accumulate different quantities of similar anthocyanins. Apparently, the quantities of delphinidin chloride and pelargonidin chloride were

not significantly different among the cultivars used in the study. The major anthocyanins were cyanidin 3 – 0 - glucoside and cyanidin 3 – 0 - galactoside while the minor anthocyanins were the chlorides of peonidin and pelargonidin. Previous works have established that the amount of coloured material in leaves or petals depend on cultivars, plant habitat and the climatic conditions encountered during the growing season (Kopsell *et al*., 2004).

Anthocyanin accumulation was substantially high in flush II compared to flush I which probably reflects influence of changes in the environmental conditions. Flush I was characterized by high irradiance consequently low anthocyanin content was observed in rose petals whereas low irradiance was recorded in flush II where petals were noted to accumulate more anthocyanins. Further differences were observed in specific anthocyanins, whereby peonidin, cyanidin and pelargonidin based anthocyanin increased with decrease in air temperature. 30.3 % more cyanidin – 3 – 0 - glucoside was recorded in flush II compared to flush I while cyanidin – 3 – 0 - galactoside increased by 33.3 %. This observation supports the finding of Laleh *et al*. (2006) who studied the effect of air temperature on anthocyanin among other factors and established a positive relationship between air temperature and anthocyanin. What is not clear from this study is the effect of other microclimatic factors like relative humidity on the anthocyanin since it was difficult to quantify their effect singly under field conditions.

A wide range of flavonoids and phenolic compounds are produced in plants in response to UV- radiation related stresses. Plants produce and accumulate these secondary products to protect themselves against UV light damage (Krizek, 2005). Other studies have shown that where both wave lengths UV-A and UV-B are excluded anthocyanin reduced drastically implying that both UV-A and UV-B light are essential in anthocyanin photo-induction. This observation could also explain the reason why there were less anthocyanins under the UV-A clear poly film cover that had high irradiance with substantial amount of UV-A and UV-B radiation transmitted.

Table 12: Effect of poly film covers on rose petal anthocyanin content in flush I

Flush 1	Anthocyanins [(mean ± SE) (µg – 5 g^{-1} DW)]				
Poly film	Cya 3 – 0 - glu	Cya - 3 – 0-gla	Cya - cl	Pela - cl	Peo - cl
UV-A clear	84.56 ± 8.26 b[1]	14.88 ± 1.23 b	2.99 ± 0.23 b	5.49 ± 0.5b	0.97 ± 0.17 b
IR 504	109.69 ± 8.26 a	20.58 ± 1.23 a	4.50 ± 0.23 a	7.89±0.51a	1.90 ± 0.17 a
UV-A205/N	110.95 ± 8.26 a	20.07 ± 1.23 ab	3.98 ± 0.23a	7.72±0.51a	2.09 ± 0.17 a

[1]Means followed by different letter (s) along the column for poly film covers are significantly different at 5 % level of significance according to Tukeys' honestly significant difference test. Where; Cya 3 – 0 - glu, Cya 3 – 0 - gla, Cya - cl, Pela - cl and Peo - cl are Cyanidin - 3 – 0 - glucoside, Cyanidin 3 – 0- galactoside, Cyanidin chloride, Pelargonadin chloride and Peonidin chloride respectively.

Table 13: Effect of cover on rose petal anthocyanin content flush II

Flush II	Anthocyanins	[(mean ± SE) (µg – 5 g^{-1} DW)]			
Poly film cover	Cya 3 – 0 - glu	Cya-3 -0 – gla	Cya - cl	Pela – cl	Peo - cl
UV-A clear	110.16 ± 13.02 b[1]	19.84 ± 1.77 b	4.32 ± 0.43 b	6.76 ± 1.4b	5.85 ± 0.72 a
IR 504	140.07 ± 13.02 a	28.44 ±1 .77 a	7.60 ± 0.43 a	9.82 ± 1.4 a	4.39 ± 0.72 b
UV-A205/N	139.54 ± 13.02 a	29.65 ± 1.77 a	7.58 ± 0.43 a	9.59 ± 1.4 a	4.84 ± 0.72 b

[1]Means followed by different letter (s) along the column for poly film covers are significantly different at 5 % level of significance according to Tukeys' honestly significant difference test. Where; Cya 3 – 0 - glu, Cya 3 – 0 - gla, Cya - cl, Pela - cl and Peo - cl are Cyanidin – 3 – 0 - glucoside, Cyanidin 3 – 0 - galactoside, Cyanidin chloride, Pelargonadin chloride and Peonidin chloride respectively.

Table 14: Effect of cultivars on rose petal anthocyanin content flush I and II

Flush I	**Anthocyanins**	**[(mean ± SE) (μg - 5 g^{-1} DW)]**			
Rose cultivars	Cya - 3 - 0 - glu	Cya - 3 -0 - gla	Cya - cl	Pela – cl	Peo - cl
Red-Calypso	142.93 ± 6.74 a	23.11 ± 1.0 a	4.40 ± 0.18 a	8.92 ± 0.41 a	2.18 ± 0.14 a
Furiosa	60.54 ± 6.74 b	13.91 ± 1.0 b	3.26 ± 0.18 b	5.14 ± 0.41 b	1.13 ± 0.14 b
Flush II	**Anthocyanins**	**[(mean ± SE) (μg - 5 g^{-1} DW)]**			
Red-Calypso	192.60 ± 10.63 a	29.23 ± 1.45 a	7.56 ± 0.35 a	9.01 ± 1.14 a	4.11 ± 0.59 a
Furiosa	67.25 ± 10.63 b	22.72 ± 1.45 b	5.45 ± 0.35 b	8.46 ± 1.14 a	4.61 ± 0.59 a

[1]Means followed by different letter (s) along the rows for cultivars are significantly different at 5 % level of significance according to Tukeys' honestly significant difference test. Where; Cya - 3 - 0 - glu, Cya - 3 – 0 - gla, Cya - cl, Pela - cl and Peo - cl are Cyanidin – 3 – 0 -glucoside, Cyanidin 3 – 0 - galactoside, Cyanidin chloride, Pelargonadin chloride and Peonidin chloride respectively.

Air temperature may impact negatively or positively on anthocyanin production. Dela *et al*. (2003), demonstrated that anthocyanins are induced by low air temperatures and reduced by high air temperatures. Anthocyanin biosynthesis has been extensively studied; several authors have observed and reported that the expression of genes for anthocyanin biosynthesis pathway increased at low air temperature (Steyn *et al*., 2005; Ubi *et al*., 2006; Hasdai *et al*., 2006 and Steyn *et al*., 2009). Further Ban *et al*. (2009) studied the effect of air temperature on apples and established that high air temperatures caused a decline in accumulation of cyanidin the major anthocyanin in Rosaceae family causing fluctuations in skin colour. When the same apples were subjected to cooler air temperatures there was renewed synthesis and improvement in colour (Steyn *et al*., 2005). In another study Yamane *et al*. (2006) conducted a study on grapes and established that mRNA levels of the main gene involved in anthocyanin biosynthesis as well as other related genes tested, were higher at a temperature of 20 °C than at 30 °C. Low air temperature cause high levels of ABA that act as a signal to influence the expression of the main genes involved in the anthocyanin biosynthesis pathway, resulting in an increase in anthocyanin levels (Yamane *et al*., 2006).

According to Lefebre *et al*. (2001) who studied the effect of calcium on mineral nutrient uptake and growth of tobacco they reported that; increase of calcium in the nutrient solution affected potassium and sodium. However, it caused decline in phosphorus and magnesium and positively correlated with micronutrients namely Iron, manganese, zinc, chloride and boron. The imbalance in macro and micronutrient trigger dynamic changes in pH that had impact in anthocyanin accumulation. Therefore, pre-harvest conditions obviously affect plant anthocyanin content.

Air temperature, light and water supply are primary factors that influence the synthesis of carbohydrate that favours red colour formation of leaves (Boo *et al*., 2002). Stability and quantity of anthocyanin pigments depends on its structure, concentration in the sample, pH, air temperature, light intensity and presence of other pigments. Anthocyanin destruction increases with increase in pH, and the flavylium salts are stable only in highly acidic conditions. Flavylium salts easily loose proton under high pH and transforms into unstable pigment that immediately bonds to water forming colourless compounds (Boo *et al*., 2002). The destruction is further accelerated under high air temperature through hydroxylation of 3 - glycoside structure which has a protective effect in unstable anthocyanin (Boo *et al*., 2002). This could explain why the quantity of anthocyanins was low under the UV-A clear poly film that was characterized by high light transmission and day air temperature.

The combination of light and air temperature accelerates the anthocyanin degradation process. The effect of high air temperature on anthocyanin has been attributed to the hydroxylation of 3- glycoside structure which confers protection to the unstable anthocyanin structure. low air temperatures on the other hand increase both anthocyanin content and the expression of genes of the anthocyanin biosynthetic pathway while high air temperature affect the activity of the phenylpropanoid pathway responsible for anthocyanin biosynthesis (Steyn *et al*., 2005, 2009; Ubi *et al*., 2006). In apple, accumulation of cyanidin and UDP-sugars Ban *et al*. (2009), has been reported to occur following a rapid reduction in anthocyanins after exposure to high air temperature causing fluctuations in skin colour (Steyn *et al*., 2005).

4.4. Effect of calcium foliar feed on growth and quality of rose flower

4.4.1. Effect of poly film covers, cultivar and calcium foliar feed on rose stem firmness

Cut flower stem firmness was affected by the treatments and their combined effects. Stem firmness was significantly high under the UV-A clear and UV-A 205/N poly films compared to IR 504 (Appendix 21). The combination of UV- A clear poly film, Red Calypso and 5.0 ml/L had the highest stem firmness (7.83 kg F). Red Calypso under the IR 504 poly film with no calcium treatment had stems with the least firmness (5.0 kg F). Furiosa under the UV-A clear poly film with no calcium treatment also recorded lowest stem firmness and it was statistically similar to 1.25 ml/L which was the lowest compared to 2.5 ml/L, 3.75 ml/L and 5.0 ml/L (Table 12).

Application of 5 ml/L of calcium foliar feed on Red Calypso and Furiosa gave the highest stem firmness under the UV-A clear and UV-A 205/N poly films respectively. Generally, low stem firmness was noted from all treatment combinations of poly films and cultivars with no calcium treatment. In flush II stem firmness was high under combined treatment IR 504 poly film with the cultivar Furiosa at calcium concentration 3.75 ml/L and 5.0 ml/L. Increase in stem firmness was noted in all treatment combinations in flush II. The treatment combination that recorded the highest stem firmness in flush I increased by 12 % in flush II, while Red Calypso under the poly film UV-A 205/N with distilled water recorded 11.4 % firmness in flush II compared to flush I (Table 15).

The UV-A clear poly film with the highest light transmission accompanied with high air temperature had firmer stems compared to IR 504. Plant development processes are hastened by high air temperature. It is most likely that the carbon from the photosynthesis process was assigned to the deposition of the cellulose microfibrils to strengthen the developing cell wall. Stem firmness increased proportionally with increase in the concentration of calcium foliar feed. Calcium foliar feed at an application rate of 5 ml/L had the highest stem strength compared to stems harvested from control plot where calcium was not applied. Stem firmness varied with environmental changes, such that it was higher in flush II compared to flush I. Stems harvested under the control treatment in flush II exhibited a similar trend to those harvested in flush I under the calcium concentration 2.5 ml/L and 3.75 ml/L (Table 16). Stem firmness also varied within the cultivar, Red Calypso recorded 30.0 %, 13.8 % and 19.4 % increase in firmness under UV-A clear, UV-A 205/N and IR 504 respectively in flush II compared to flush I.

Table 15: Effect of poly film covers, cultivars and calcium on stem firmness in flush1

		Calcium foliar feed concentrations				
Greenhouse cover	Cultivars	Distilled water	1.25 ml/L	2.5 ml/L	3.75 ml/L	5.0 ml/L
UV-A clear	Red Calypso	5.9 efg	6.8 abc	7.5 ab	7.3 abc	7.83 a
	Furiosa	5.2 ij	5.27 hij	5.8 efg	6.27 cde	5.9 efg
UV - A 205 / N	Red Calypso	5.0 j	5.57 fgh	5.37 hij	5.7 efg	6.2 cde
	Furiosa	5.33 hij	5.7 efg	6.07 def	5.43 ghi	6.6 bcd
IR 504	Red Calypso	5.77 efg	6.03 def	6.6 bcd	6.64 bcd	7.27 abc
	Furiosa	5.2 ij	5.57 fgh	6.4 bcd	6.66 abc	7.10 abc

[1]Means followed by different letter (s) along the column for calcium and along the row for poly films and cultivars are significantly different at 5 % level of significance according to Tukey's honestly significant difference test. Values are the means of the treatments (n = 3)

The efficiency of calcium in increasing stem firmness is affected by a range of factors. Plants response to calcium depends on species, fertilizer form applied, the quantity, frequency of application as well as stage of plant growth (Amir *et al*., 2009). Calcium absorption and utilization is influenced by the prevailing environmental conditions and it has been observed to increase with increase in air temperature to an optimal level and vice versa (Manganaris *et al*., 2007). Poly films altered the greenhouse microclimate resulting into varying growth conditions that would have led to differences in mineral nutrient absorption other than calcium. It is possible therefore that the increase in other nutrients absorbed through the root system created mineral nutrient imbalance that counteracted the effect of calcium treatment. The effect of poly films on stem firmness was strongly related to the microclimate differences under the covers. In this study where the air temperature was high stems were much firmer than under the poly film that exhibited lower air temperature. Lignification of the cell wall was therefore enhanced by an increase in air temperature which could be a mechanism to mitigate heat stress.

Table 16: Effect of poly film cover, cultivar and calcium on rose plant stem firmness in flush 1I

		calcium level				
Greenhouse cover	Cultivars	Distilled water	1.25 ml/L	2.5 ml/L	3.75 ml/L	5.0 ml/L
UV-A clear	Red Calypso	5.97 jkl	7.03 efg	7.63 bcd	8.37 abc	8.77 ab
	Furiosa	6.20 ijk	6.5 fgh	7.13efg	7.37 cde	8.5 abc
UV - A205 / N	Red Calypso	5.57 l	6.3 ghi	7.13efg	7.33 def	8.13 abc
	Furiosa	8.33 abc	5.5 l	6.4 ghi	6.6 fgh	7.4 cde
IR 504	Red Calypso	5.6 kl	6.23 hij	6.77 fgh	7.43 cde	8.4 abc
	Furiosa	5.77 kl	6.5 fghi	7.30 def	8.7 ab	8.9 a

[1]Means followed by different letter (s) along the column for calcium and along the row for poly films and cultivars are significantly different at 5 % level of significance according to Tukey's honestly significant difference test. Values are the means of the treatments (n = 3)

Calcium foliar feed strengthened the cut flower stems, although it varied with the treatment combinations. Pre- harvest application of calcium chloride fertilizer has been observed to increase stem firmness in addition to enhancing water uptake. Calcium is important in stabilizing the plant cell wall and protecting it from the activity of hydrolases cell wall degrading enzymes. This necessitated increase in stem firmness where calcium was applied compared to no calcium. Further in similar study calcium chloride application increased the uptake of nitrogen and enhanced growth (Swietlik, 2006). Many biochemical compounds present in the plant cells constitute nitrogen an essential element in formation of nucleic acids, proteins and amino acids. Processes that trigger uptake of nitrogen improves plant performance and in turn it is possible that these processes enhanced cell wall lignification resulting to increased firmness. Plants have their own mechanisms of sequestering active calcium in their tissues. The process is influenced by several factors including the fertilizer application methods making it difficult to quantitatively state the effect of calcium in plant response (Franceschi and Nakata, 2005).

Inconsistence was noted in stem firmness between flushes I and II results in response to poly film covers. It is possible that low air temperature might have had impact on nutrient absorption and utilization in flush II compared to flush I. Similar contradicting observation has been presented by Casero *et al*. (2009), who proposed that evaluation of the effectiveness of calcium should be considered a long side other nutrient. Under conventional production system it is difficult to separate the effect of calcium from other nutrients. In earlier studies it has been observed that the effect of calcium on firmness was expressed only when the potassium, nitrogen, phosphorus and magnesium to calcium ratios were high (Fallahi *et al*., 2006). There is also evidence that the cytosolic Ca^{2+} signature elicited by one environmental challenge can be modified by exposure to a contrasting one (Knight, 2000). Calcium transportation in plants is limited and relies on transpiration stream to move to young tissues. It is therefore expected that in flush I that exhibited higher light intensity plants absorbed more calcium than in flush II that was characterized by cold weather. However, the results show that plants were firmer in flush II which could probably be due to influence of air temperature on growth. Plants took an extra number of days ranging between 4 and 5 days to establish from visible bud to harvestable stem in flush II than flush I meaning they absorbed more calcium and had more time for cell wall lignification compared to flush I that took less time to maturity.

According to the study done by Li *et al*. (2012), they established that after subsequent foliar sprays the concentration of calcium was more in the upper part of the stem compared to the middle and the bottom section. Differences in the findings of this research may have occurred due to inconsistence in the section being sampled for analysis which could probably have received less calcium. While calcium was applied as foliar feed on leaves, sampling for stem firmness was done on the stems. Therefore, it is unlikely that increase in calcium levels resulted to increased stem firmness. Additionally, the prevailing air temperature in flush II were conducive for vigorous vegetative growth which might have triggered plant demand for calcium necessitating increased uptake for subsequent cell expansion resulting in increased stem firmness.

4.4.2. Effect of cover, cultivar and calcium on stem dry matter accumulation

Shoot dry matter was significantly affected by all treatments and their combined effects (Appendix 22). Dry matter accumulation was consistently high under UV-A 205/N poly film for in both flushes I and II. Furiosa had significantly higher stem dry matter accumulation compared

to Red Calypso in both flushes I and II. Dry matter recorded under Furiosa in flushes I and II was 7.9 % and 10.2 % higher than Red Calypso. Generally, dry matter accumulation was low in flush I compared to flush II. Dry matter increased by 1.5 % in flush II compared to flush I under Red Calypso while it increased by 3.63 % in Furiosa. Apparently, increase in dry matter could be related to increased sink strength. The decline in dry matter accumulation under the UV-A clear poly film partly could be because of the effect of air temperature on leaf chlorophyll content that is vital in photosynthesis. Stem dry matter accumulation was also significantly dependent on the cultivars (Appendix 22).

Reduction in dry matter accumulation under the UV-A clear poly film could be as a result of a decrease in leaf chlorophyll content leading to reduction in photosynthesis. These results concur with the finding of Inamoto *et al*. (2016) who studied the effect of day and night air temperature on dry matter accumulation of oriental hybrid lily by subjecting plants to different air temperatures at 28 °C, 24 °C and 20 °C and a constant relative humidity > 70 % and observed that plants grown under low day air temperature of 20 °C had higher dry matter compared to those under 24 °C and 28 °C. Dry matter accumulation therefore can be enhanced by promoting photosynthesis at low air temperature.

Negative correlation between PAR and dry weight has been reported in rose flower especially when optimum PAR levels were exceeded (Maas and Bakx, 1997). Lower stem dry matter accumulation was recorded under the UV-A clear poly film that transmitted high solar radiation and consequently higher PAR. They also found out that increasing PAR from 180 to 270 μmol m^{-2} s^{-1} did not affect partitioning of dry matter between leaf and stem tissues. This implies that beyond the PAR saturation point dry matter accumulation does not necessarily increase as was the case under UV-A clear cover compared to UV-A 205/N and IR 504. Additionally, plants under UV-A clear poly film received direct light without any kind of diffusion to reduce effect on the plant. The position of the harvested stem on the plant could also affect the dry matter accumulation yet this was not considered in the current study. Dry matter accumulation was high in the primary wood, followed by roots, lower shoot and least in the upper shoot (Maas and Bakx, 1997). They also observed that under conditions of high air temperature and low light starch content of the leaves increase and its evident from this study that plants under UV-A clear poly film where air temperature was high had the least dry matter accumulation.

Dry matter is not necessarily positively correlated with the calcium concentration in the nutrient solution but there is influence from other factors as well. The observation made in this study partly differ with previous reports according to Asfanani *et al*. (2008), they reported insignificant effect of calcium fertilizer on dry matter accumulation which agrees with findings recorded in flush 1 while it differs with the results of flush II. The results obtained for Furiosa showed that the dry matter accumulation was high under calcium treatment 3.75 ml/L compared to the control which had no calcium applied. This demonstrates that different cultivars vary in their potential accumulation of dry matter. This could also be attributed to the effect of calcium on availability of other nutrients. Lefebre *et al*. (2001), reported an antagonistic effect of calcium on Phosphorus in tobacco which is key in the absorption of other nutrients such as nitrogen (N) and carbon. The assimilation and portioning of carbon and nitrogen differ among the cultivars and depend on the organ analysed. Generally due to the hot conditions under which flush I was studied, the fertigation programme was quite frequent and consequently more nutrient were used which is likely to have masked the effect of calcium at different concentrations.

Table 17: Effect of cover, cultivar and calcium on stem dry matter accumulation flush I

Flush I		Calcium levels				
Greenhouse cover	Cultivar	D. water	1.25 ml/L	2.5 ml/L	3.75 ml/L	5.0 ml/L
UV-A clear	Red Calypso	12.39 def[1]	12.44def	13.51 def	10.77 f	11.74 ef
	Furiosa	14.01 bcd	13.1bcd	13.05 bcd	13.62 bcd	12.37 def
IR 504	Red Calypso	12.66 cde	13.2bcd	12.34 def	12.01 def	13.07 def
	Furiosa	12.09 def	13.66bcd	13.55 bcd	13.79 bcd	16.76 a
UV-A 205/N	Red Calypso	13.89 bcd	13.66 bcd	12.93 bcd	13.11 bcd	13.5 bcd
	Furiosa	13.82 bcd	13.92 bcd	14.64b	14.97 ab	13.13 de

[1]Means followed by different letter (s) along the column for calcium and along the row for poly films and cultivars are significantly different at 5 % level of significance according to Tukey's honestly significant difference test.

The observation made here emphasizes the effect of cultivar on dry matter accumulation. Apparently in most cases, calcium foliar feed was observed to have minimal influence on dry matter accumulation as opposed to cultivar and poly film cover. High levels of calcium foliar feed i.e. 3.75 ml/L and 5.0 ml/L had no significant influence on the dry matter accumulation under the UV-A clear poly film except for other covers IR 504 and UV- A 205/N (Table 18) in both flushes I and II. In the second flush it was observed that combination of UV-A clear poly film, Red Calypso and 5.0 ml/L had no significant difference in dry weight with the control experiment.

Table 18: Effect of poly film covers, cultivar and calcium on rose stem dry matter accumulation flush II

Flush II		Calcium level				
Greenhouse cover	Cultivar	Distilled water	1.25 ml/L	2.5 ml/L	3.75 ml/L	5.0 ml/L
UV-A clear	Red Calypso	12.09 fgh[1]	12.3 def	13.57 cde	10.59 h	11.31 gh
	Furiosa	14.32 bcd	13.4 cde	14.76 abc	14.36 bcd	12.65 cde
IR 504	Red Calypso	12.52 cde	13.59 cde	11.24 gh	12.3 def	14.62 abc
	Furiosa	12.14 efg	13.27 cde	13.79 cde	15.03 abc	16.61 ab
UV- A205/N	Red Calypso	13.33 cde	12.76 cde	15.04 abc	14.82 abc	14 bcd
	Furiosa	13.63 cde	13.72 cde	14.11 bcd	15.08 bc	16.99 a

Means followed by different letter(s) along the column for calcium and along the row for poly films and cultivars are significantly different at 5% level of significance according to Tukey's honestly significant difference test.

4.4.3. Effect of poly films, cultivars and calcium foliar feed levels on flower dry matter accumulation

Flower dry matter accumulation was significantly affected by the poly film covers, cultivars and calcium foliar feed treatments and their combined effect (Appendix 23). Under the UV-A clear poly film, calcium foliar feed had no significant effect on flower dry matter accumulation in Red Calypso, the results obtained were statistically similar across all the calcium treatments. Furiosa had high flower dry matter accumulation when grown under the IR 504 poly film with calcium foliar feed treatment at level 3.75 ml/L and 5.0 ml/L (Table 19).

Table 19: Effect of cover, cultivar and calcium on flower dry matter accumulation in flush I

		Calcium level				
Poly film	Cultivar	Distilled water	1.25 ml/L	2.5 ml/L	3.75 ml/L	5.0 ml/L
UV-A Clear	Red Calypso	6.07 def[1]	5.62 g	6.63 bcd	7.07 abc	6.13 def
	Furiosa	5.87 fg	7.49 abc	6.11 def	7.44 abc	7.45 abc
IR 504	Red Calypso	6.25 def	5.62 g	6.02 efg	6.13 def	6.77 bcd
	Furiosa	6.34 def	7.06 abc	8.24 ab	8.14 abc	8.43 a
UV-A 205/N	Red Calypso	7.55 abc	6.73 bcd	7.04 abc	6.69cde	7.25 abc
	Furiosa	6.31 def	6.75 bcd	7.68 abc	6.57 def	7.20 abc

[1]Means followed by different letter (s) across the row for poly films and cultivars and along the column for calcium are significantly different at 5 % level of significance according to Tukey's honestly significant difference test.

The lowest dry matter accumulation was recorded from Red Calypso under the IR 504 poly film with the lowest level of calcium treatment 1.25 ml/L. Calcium at level 3.75 ml/L resulted in high dry matter accumulation with Furiosa under the IR 504 poly film compared to UV-A clear and UV-A 205/N poly films. Poly film UV-A 205/N and UV-A clear with calcium level 2.5 ml/L

and 3.75 ml/L resulted in high flower dry matter accumulation with Furiosa compared to Red Calypso under similar treatments (Table 20). Flower head dry matter accumulation was high under the UV-A 205/N poly film compared to UV-A clear and IR 504 poly films in flush I. Apparently, it was noted that plants grown under the UV-A clear poly film had relatively smaller flower heads and were generally shorter in length contributing to low biomass. IR 504 and UV-A 205/N poly films had 4.4 % and 3.1 % higher dry matter content compared to the UV-A clear poly film. Flower head dry matter seems to be sensitive to environmental changes hence under UV-A clear it increased by 11.6 % between flushes and 1.4 % under IR 504 poly film. However, under UV-A 205/N poly film a decline of 3.1 % was recorded in dry matter accumulation between flushes I and II.

Flower head dry matter differed significantly among the rose cultivars (Appendix 18). Furiosa had significantly higher dry matter compared to Red Calypso in both flushes. The flower head dry matter was relatively constant for Red Calypso 6.49 g and 6.52 g for flushes I and II respectively compared to Furiosa where the dry weight was 7.09 g and 7.59 g for flushes I and II. An increase of 7.1 % in dry weight was recorded in flush II for the cultivar Furiosa.

Table 20: Effect of cover, variety and calcium interaction on flower dry matter accumulation flush II

Flush II		Calcium level				
Poly film covers	Cultivar	Distilled water	1.25 ml/L	2.5 ml/L	3.75 ml/L	5.0 ml/L
UV-A clear	Red Calypso	6.41 bcd[1]	7.25 abc	6.98 abc	6.67 bcd	7.39 abc
	Furiosa	6.65 bcd	7.73 abc	8.28 ab	8.16 abc	8.24 ab
IR 504	Red Calypso	5.38 e	6.37 bcd	6.18 bcd	6.98 abc	7.87 abc
	Furiosa	7.25 abc	6.26 bcd	7.15 abc	7.36 abc	9.20 a
UV-A 205/N	Red Calypso	5.7 de	5.68 de	6.17 bcde	5.3 cde	6.58 bcd
	Furiosa	5.76 cde	7.38 abc	7.40 abc	8.22 ab	8.38 ab

[1]Means followed by different letter (s) across the row for poly films and along the column for calcium foliar feed concentrations are significantly different at 5 % level of significance according to Tukey's honestly significant difference test.

The interaction of cultivar and calcium significantly affected dry matter accumulation in the flower. Dry matter was low under Furiosa and no calcium treatment but higher under 1.25 ml/L, 2.5 ml/L, 3.75 ml/L and 5.0 ml/L. An increase in flower dry weight was recorded in flush II compared to flush I. Furiosa accumulated high dry matter under the IR 504 poly film compared to Red Calypso under the same cover. The least dry matter was recorded from Red Calypso under the UV-A clear and IR 504 poly films. Furiosa had the highest dry matter (7.8 g) in flush II compared to flush I (7.64 g) in addition the quantities increased by 2.09 % compared to flush I (Table 20). Dry matter accumulation for the interaction between cultivar Red Calypso and no calcium reduced by 12.4 % in flush II compared to flush I. Dry matter consistently increased under the cultivar Furiosa although it was inconsistent among the cultivar Red Calypso

A decline of 12.4 % in dry matter accumulation was recorded under Red Calypso in flush I while a slight increase of 6.1 % was noted under the cultivar Furiosa. Dry matter decreased

by 18.1 % under the combined treatment of Red Calypso and UV - A 205 / N in flush II compared to flush I. Higher dry matter accumulation of 7.64 g and 7.8 g were recorded under Furiosa and IR 504 poly film in both flushes I and II respectively. Interaction of Furiosa and IR 504 poly film recorded the highest dry matter accumulation both flushes I and II (Table 17 and 18).

Differences in dry matter accumulation could have occurred due to differences in photosynthesis potential accruing from anatomic, morphological and genetic differences among the rose cultivars used in the experiment. The prevailing environmental conditions during flush I affected leaf chlorophyll content, which has direct impact on photosynthesis resulting to low dry matter during this flush. Plant leaf area and shoot length were significantly higher in flush II compared to flush I as earlier discussed. This contributed to higher dry matter accumulation in flush II since the assimilation area was big. During the second flush, also plants took almost 5 days from visible bud to harvestable stem consequently, the length of time taken would have played a substantial role in allowing more time for biomass accumulation. It followed that the longer the growth period the higher was the biomass accumulation.

A general tendency of higher amount of dry weight was recorded with increasing calcium concentration in the nutrient solution. The synergistic effect of higher calcium concentration could be attributed to uptake of other nutrients which resulted in better plant growth. This observation concurs with Hamdi *et al*. (2015) who studied the effect of levels of calcium nitrate addition on potato fertilizer and reported an increase in nitrogen, potassium and phosphorus levels with increasing calcium in the nutrient solution. Suggesting that application of calcium in nitrate form affected the uptake of other nutrients. Calcium has been implicated in numerous physiological processes such as stomatal regulation and closure under stress conditions. Specificity of the effect of calcium depends on the magnitude and duration of the stress factor, it's obvious that the magnitude of light intensity and air temperature varied under the different poly films in flushes I and II. This would have brought about difference in partitioning and quantity of the dry matter accumulation in various plant organs.

4.5. Effect of poly film covers, cultivars and calcium on post- harvest physiology and vase life of rose cut flowers

Evaluation of the pre- harvest factors such PAR and air temperature on post- harvest disorders showed both positive and negative relationships. Increase in PAR positively enhanced

the rate of post- harvest leaf wilting and the relationship was strong with $R^2 = 0.68$, however there was a negative relationship between increase in PAR and bent necks. Average increase in flower diameter was noted as PAR increased. Incidences of wilting were more as air temperatures increased ($R^2 = 0.60$) which implies that post- harvest leaf wilting is affected more by PAR than air temperature. Increase in PAR by I unit caused flower diameter to increase by 0.032 whereas leaf wilting increased by 0.0031. The occurrence of bent necks was negatively correlated with PAR and air temperature. As PAR and air temperature increased the number of bend necks reduced by –0.005 and -0.14 respectively (Figure 19). These results show that pre- harvest environmental conditions have a strong relationship with changes in the physiological characteristics of the plant and hence affect vase life.

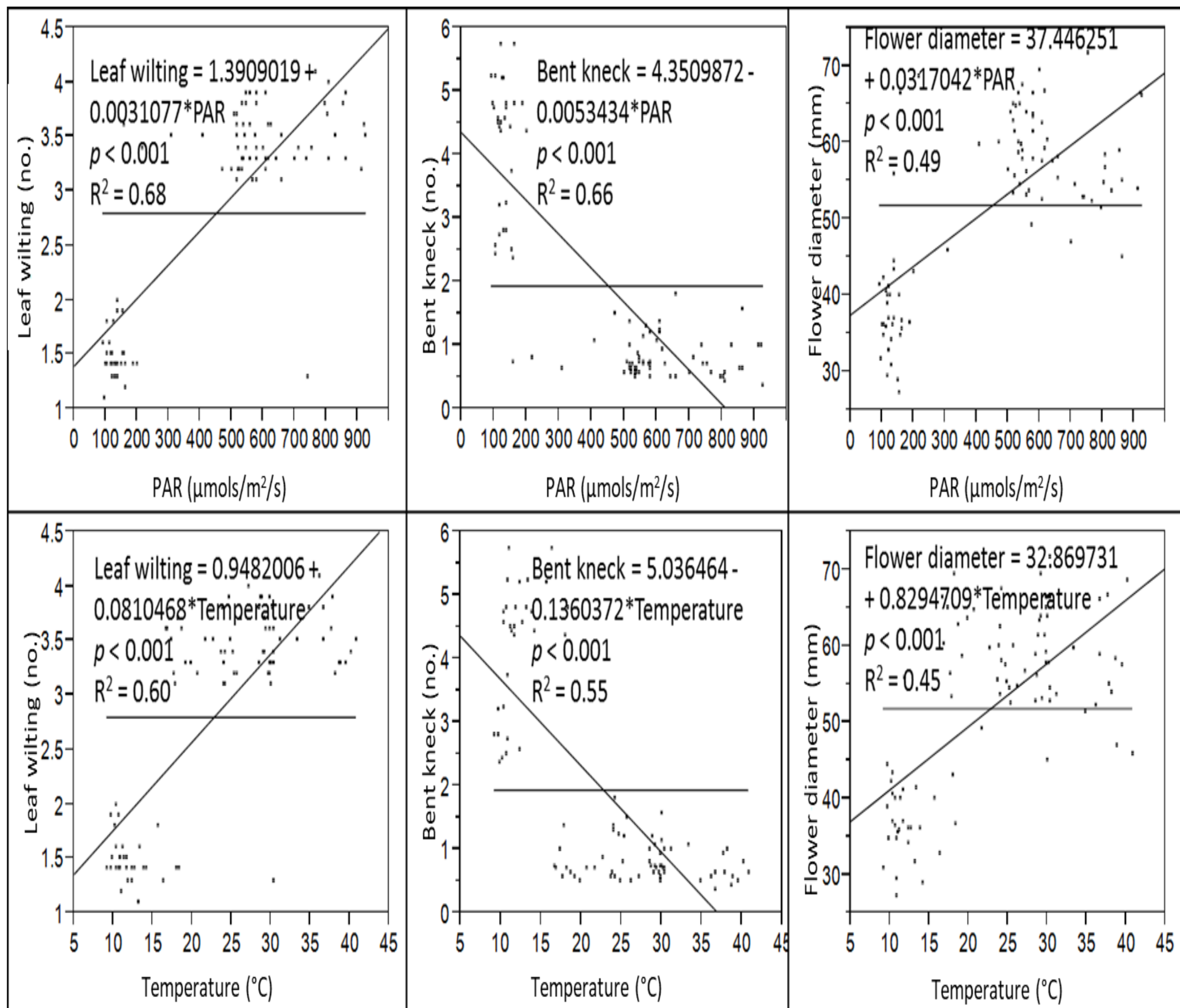


Figure 19: Relationships among leaf wilting, bent necks and flower diameter with PAR and air temperature

4.5.1. Effect of poly film covers on rose cut flower fresh weight

Cut flower fresh weight was affected by the type of poly film cover used during production (Appendices: 24, 25, 26 and 27). Increase in cut flower fresh weight was recorded during the first 3 days of the experiment across all treatments. A gradual decrease in fresh weight was noted as the cut flower continued being held in the vase. Flowers grown under the UV-A 205/N poly film had the highest fresh weight in both flushes I and II (13.79 g and 14.35 g respectively) compared to stems harvested under UV-A clear that had least weight of 12.7 g and 12.9 g for flushes I and II respectively. Generally, cut flowers obtained from the UV-A clear poly film had the least fresh weight (Table 21) in both flushes I and II. Cut flowers harvested from the UV-A 205/N poly film maintained the highest fresh weight by day 12 providing the best pre- harvest growth condition for vase life longevity.

Table 21: Effect of poly film covers on cut rose flower fresh weight (g day^{-1})

Treatments	Longevity (days)			
	3rd day	6th day	9th day	12th day
UV-A clear	8.83 c	8.47 b	7.67 b	6.44 c
IR 504	9.86 b	8.98 b	8.82 a	7.09 b
UV–A 205/N	10.03 a	9.73 a	8.61 a	7.67 a
Flush II				
UV-A clear	12.49 b	12.22 b	11.92 b	11.54 b
IR 504	12.96 b	12.81 b	12.53 a	11.98 b
UV–A 205/N	13.04 a	13.01 a	12.62 a	12.08 a

[1]Means followed by different letter (s) along the column for poly film covers by the number of days in the vase are significantly different at 5 % level of significance according to Tukeys' honestly significant difference test. Values are means n = 3

The decline in fresh weight could be associated with, among other factors the imbalance in water uptake and water loss through evapo-transpiration. Although cut flowers under the IR 504 poly film had low fresh weight compared to UV-A 205/N, its fresh weight was relatively higher compared to the control poly film (UV-A clear). Fresh weight for cut flowers harvested under UV-A clear poly film declined steadily, by the 6th day there was 0.47 % decline in cut flower fresh weight while for IR 504 and UV-A 205/N weight increased by 4.78 % and 13.14 %, respectively. Gradual decline of 21.1 % and 21.7 % was noted in fresh weight for plants harvested under IR 504 and UV-A 205/N compared to 24 % recorded under UV-A clear poly film. This observation emphasizes the effect of pre- harvest growth conditions on post- harvest quality of cut flowers. The growth conditions under the UV-A clear poly film were characterized by hot days and low relative humidity consequently the stems had the least dry matter accumulation (12.7 g). It is probable that the cut flower grown under the control poly film had low photo assimilates translating to low fresh weight.

Although in this experiment water uptake was not monitored, it is certain that the cut flowers imbibed water for a shorter time as evidenced by increase in fresh weight by day 3, followed by gradual water loss which is reflected by decrease in fresh weight at subsequent intervals of days 6, 9 and 12. Conventionally, water maintains the plant cell turgidity and its eventual loss predisposes the cut flowers to accelerated wilting, yellowing and subsequent ageing. The decrease in fresh weight could also be associated with breakdown of the accumulated sugar compounds in the cut flower stem tissues through the respiration process. Cut flowers were held in fresh distilled water with no carbohydrate source. It is probable that the cut flower must have broken down the starch content that had been accumulated for energy source.

Changes in fresh weight in this case could be because of physiological changes associated with senescence at cellular level. Huag and Chen (2002) conducted an experiment to measure the effect of pulse treatments of BA, sucrose and BA on vase life, they established that the number of days the flowers remained fresh in the vase was maintained especially where 4 % sugars were used in the vase solution. Sugar acts as a respiratory substrate and a good osmolite in maintenance of water balance in cut stem tissues. The presence of sugars in the holding solution has been reported to reduce stomata aperture in rose cut flower leaves thus reducing water loss. Although sugars were not included in the vase solution in the current study, it is assumed that plants accumulated different concentrations of sugars during growth which might have varied due to differences in

growth conditions. This could explain the reason as to why cut flowers harvested under the UV-A clear poly film had the least fresh weight and consequently short vase life than plants harvested under UV-A 205/N and IR 504 poly films that recorded higher dry matter content.

Plants under the UV-A clear poly film received higher day air temperature compared to those under UV-A 205/N and IR 504 poly film. Despite the high PAR recorded under the UV-A clear poly film, air temperature is likely to have triggered closure of the stomata aperture leading to reduced photosynthesis and low dry matter accumulation as earlier observed in this study resulting into low fresh weight. Although high light intensity and air temperature promoted faster growth of rose plants under the UV-A clear poly film causing faster maturity it negatively affected vase life a key postharvest quality attribute. Plants under the UV-A 205/N and IR 504 poly films stayed fresh by 3 days longer. During growth plants under UV-A 205/N and IR 504 poly film took 3 to 5 more day to flower maturity and it is possible they accumulated more starch. Plants grown under the poly film UV-A 205/N and IR 504 recorded higher dry matter. The accumulated carbohydrate content could have been broken down to supply sugar for cut flower nourishment hence extended vase life.

4.5.2. Effect of cultivar on cut flower fresh weight

Cut flower fresh weight differed significantly among the cultivars. Fresh weight was significantly higher under Furiosa compared to Red Calypso. Fresh weight increased by 4.9 % and 3.5 % between the first day (day 0) and 3rd day for Red Calypso and Furiosa respectively. In both cultivars, fresh weight declined between the third and sixth day although the decrease was noted to be high in Red Calypso by 1.3 % compared to 0.2 % in Furiosa. Fresh weight declined across all cultivars between sixth and ninth day and ninth and twelfth day (Table 22). 12.2 % and 22.6 % decline were noted in fresh weight for Red Calypso between sixth and ninth day and ninth and twelfth day respectively. The decline was gradual under Furiosa whereby between sixth and ninth and ninth and twelfth day percent fresh weight reduction was minimally at 8.4 % and 18.6 % respectively. Average fresh weight was high in flush II compared to flush I. Weight loss was relatively high in flush II whereby between the first and twelfth day 42.1 % loss in weight was recorded under Red Calypso while 31.3 % was recorded under Furiosa.

The number of days taken during production has appositive relationship with cut flower vase life. Rose cultivar Furiosa recorded the highest number of days from bud break to flower

development compared to Red Calypso in both flushes I and II, it is therefore likely that this cultivar accumulated more photosynthates. Consequently, Furiosa cut flowers stayed fresh for a longer time in the vase compared to Red Calypso. Despite the difference in growth period, some factors may be inherent. This observation supports Ferrante *et al*. (2007), who established that cut flower vase life of *Gebera jamesonii* varied between 5 to 12 days among cultivars of the same plant which illustrates the effect of genotypes grown under different environment on vase life.

Table 22: Weight loss among rose cultivars and its effect of vase life longevity

Cultivar	0	3rd day	6th day	9th day	12th day
Red Calypso	8.53 b	8.95 b	8.42 b	7.49 b	6.61 b
Furiosa	9.51 a	9.84 a	9.49 a	8.72 a	7.75 a
Flush II	**0**	**3rd day**	**6th day**	**9th day**	**12th day**
Red Calypso	11.57 b	11.24 b	10.03 b	8.28 b	6.71 b
Furiosa	12.63 a	12.22 a	11.17 a	9.58 a	7.92 a

[1]Means followed by different letter (s) along column for different cultivars by the number of days along the row are significantly different at 5 % level of significance according to Tukeys' honestly significant difference test. Values are means n = 3

4.5.3. Effect of calcium foliar feed on cut flower post- harvest fresh weight

Calcium foliar feed significantly affected cut flower fresh weight (Appendix 23). There was a noticeable increase in fresh weight across all calcium levels by day 3. This was followed by gradual decline in fresh weight across all the treatments by day 6. Fresh weight was significantly affected by the concentration of calcium in the foliar feed. Between the first and third day fresh weight increased by 2.8 %, 3.1 %, 4.9 %, 6.4 % and 4.2 % for control treatment and calcium treatments, 1.25 ml/L, 2.5 ml/L, 3.75 ml/L and 5.0 ml/L. respectively. Fresh weight between the third and sixth day increased with the following percentages; 4.8 %, 5.2 %, 4.9 %, 4.3 % and 4.2 % for calcium treatments 0 ml/L, 1.25 ml/L, 2.5 ml/L, 3.75 ml/L and 5.0 ml/L respectively. Cut flowers treated with higher concentration of calcium (5 ml/L) recorded 4.2 % increase in weight

between day 0 and day 3 while for control experiment weight increased by 2.8 % in flush I. Weight loss was high 22.4 % under the control experiment from the first and last day of the experiment while an average decline of 20.1 % was noted in most treatments 1.25 ml/L, 2.5 ml/L, 3.75 ml/L and 5.0 ml/L (Figure 20 and 21). Calcium levels 3.75 ml/L and 5.0 ml/L maintained steady fresh weight over a period of 12 days while drastic decline was observed by day 6 under the control treatment.

Water is very crucial in cut flower post-harvest and any slight imbalance may cause termination in vase life due to wilting. The amount of water retained in the stem segments and resistance to movement is influenced by the presence of calcium ions. Calcium is the main component of plant pectin which have high water absorption capacity. This could explain why the cut flowers treated with higher concentrations of calcium maintained higher fresh weight. Plants harvested under the control experiment retained the least fresh weight which could be because of high resistance to water movement across the xylem vessels. Besides the effect of calcium levels within the plant, water uptake by cut flowers may be limited by the blockage of the vessels by microorganisms or air embolism.

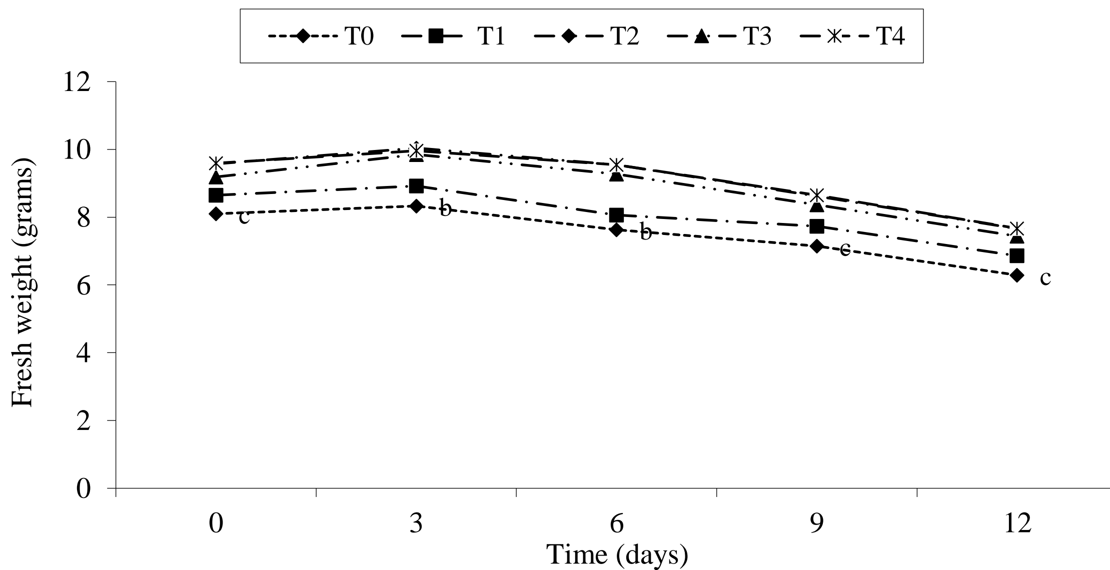


Figure 20: Effect of calcium pre-treatment on cut flower fresh weight in flush 1

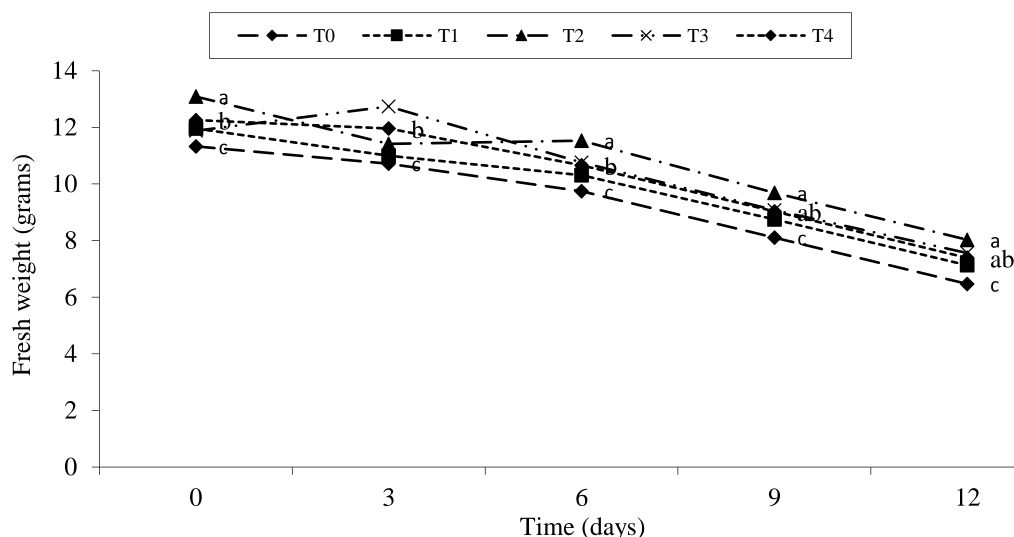


Figure 21: Effect of calcium pre - treatment on cut flower fresh weight in flush II.

Cut flower vase life may be enhanced by reducing the loss in fresh weight. When the rate of water uptake is lower than transpiration, cut flower stems loss turgidity and cells collapse. Pre- and post- harvest calcium treatments have been reported to enhance water uptake at post - harvest (Capdeville *et al*., 2005). The use of calcium sulphate at post- harvest has been reported to increase the longevity by 37 % in Rose cultivar Kiss compared to the control where there was no calcium. According to the findings of another study carried out by Bagheri *et al*. (2015) in apple fruits, they established that fruits treated with 2 % calcium chloride had decreased weight loss during the evaluation period compared to the control. This implies that, calcium treatment enables the plants to uptake more water. Similar observation was made by Shirzadeh *et al*. (2011) who observed that apple fruits treated with calcium chloride had impressively slow decrease in weight compared to the non-treated.

Calcium enhances water uptake by reducing the flow resistance, Ieperen *et al*. (2006) compared water flow in chrysanthemum and Prunus treated with calcium chloride (1 mM) and observed decreased flow resistance by up to 87 % in Chrysanthemum stems and by 95 % in *Prunus* L., compared to distilled water. Calcium therefore, increased fresh weight and delayed water loss. Calcium concentration in the leaf tissues increases with increase in the concentration. However, comparison of the two flushes showed that fresh weight varied with changes in air temperature, irradiance and relative humidity besides calcium. The rate of water loss under calcium treatment

2.5 ml/L and 3.75 ml/L between day 6 and 12 was 19.8 % which was statistically similar to calcium treatment 5.0 ml/L that had lost 19.7 %.

Little information is available to explain this phenomenon however it has been speculated that increasing the level of calcium in the plant nutrition can pose chances of photo-toxicity which may inhibit plant water uptake. This could explain the reason as to why the highest level of calcium concentration 5.0 ml/L did not result to steady increase in fresh weight. The ability of cut flower stems to maintain fresh weight may also be compounded by other factors that may not easily be measurable such as the initial status of the plant. Cut flowers should be harvested at full maturity stage when they have accumulated adequate quantity of carbohydrate in the leaves, stem and petals to support vase life. The complexity of water uptake at post- harvest level therefore may not be limited to plant nutrition status and hydrating solutions.

4.5.4. Effect of calcium foliar feed and cultivar on cut flower fresh weight

Calcium and cultivar interaction significantly affected the post- harvest fresh weight. By day 3 the combined treatment 1.25 ml/L and Furiosa, 5.0 ml/L and both cultivars Red Calypso and Furiosa recorded the highest fresh weight compared to Red Calypso and Calcium level 1.25 ml/L. The cultivar Furiosa positively responded to the lowest calcium treatment 1.25 ml/L and recorded the highest percent fresh weight. By day 12 this treatment recorded more days in the vase than the highest concentration of calcium recorded (Table 23).

The results of flush II were relatively inconsistent, fresh weight was not significantly different under all calcium treatment levels with cultivar Furiosa. Combined treatment between calcium 3.75 ml/L and Furiosa also had high fresh weight from day 3 and other successive days compared to 3.75 ml/L and Red Calypso. All treatment combinations of cultivars with 1.25 ml/L and 2.5 ml/L were statistically similar. The highest concentration of calcium had the least fresh weight under Red Calypso compared to Furiosa in flush II (Table 24).

Table 23: Effect of cultivar and calcium foliar feed interaction on fresh weight flush I

Flush 1		Vase life longevity (days)				
Calcium	Cultivars	0	3	6	9	12
Distilled water	Red Calypso	9.05 abcd[1]	9.48 ab	8.94 abc	7.74 bcd	6.82 ab
	Furiosa	10.27 a	10.41 a	10.03 a	9.26 a	8.22 ab
1.25 ml/L	Red Calypso	8.77 bcd	9.13 ab	8.52 abc	7.81 abcd	6.95 ab
	Furiosa	9.63 abc	9.91 a	9.60 ab	8.76 abc	9.59 a
2.5 ml/L	Red Calypso	8.60 c	9.13 ab	8.52 abc	7.84 abcd	6.91 ab
	Furiosa	8.77 bcd	9.10 ab	8.78 abc	8.01 abcd	7.07 ab
3.75 ml/L	Red Calypso	8.33 cd	8.87 ab	8.39 bc	7.43 cd	6.54 b
	Furiosa	9.11 abcd	9.53 ab	9.17 ab	8.55 abc	7.68 ab
5.0 ml/L	Red Calypso	7.83 d	8.14 b	7.56 c	6.62 d	5.85 b
	Furiosa	9.78 ab	10.28 a	9.86 ab	9.01 ab	7.98 ab

[1]Means followed by different letter (s) along the column for different rates of calcium foliar feed by the number of days in the vase and along the row for cultivar are significantly different at 5 % level of significance according to Tukeys' honestly significant difference test. Values are means n = 3

Table 24: Effect of cultivar and calcium foliar feed interaction on fresh weight (Flush II)

		Vase life longevity (days)				
Calcium	Cultivar	0	3	6	9	12
Distilled water	Red Calypso	12.2 abc[1]	11.81 ab	10.58 abc	8.57 bcd	6.94 abc
	Furiosa	13.43 a	12.74 a	11.18 a	10.09 a	8.33 a
1.25 ml/L	Red Calypso	11.92 bc	11.46 ab	10.17 abc	8.64 abcd	7.07 abc
	Furiosa	12.43 abc	12.23 a	11.25 ab	9.59 abc	8.05 ab
2.5 ml/L	Red Calypso	11.84 bc	11.46 ab	10.31 abc	8.67 abcd	7.03 abc
	Furiosa	11.92 bc	11.43 ab	10.43 abc	8.83 abcd	7.19 ab
3.75 ml/L	Red Calypso	11.13 c	11.20 ab	10.04 bc	8.25 cd	6.66 bc
	Furiosa	12.26 abc	11.86 ab	10.82 ab	9.38 abc	7.80 ab
5.0 ml/L	Red Calypso	10.97 c	10.47 b	9.21 c	7.45 d	5.97 c
	Furiosa	12.93 ab	12.61 a	11.51 ab	9.83 ab	8.10 a

[1]Means followed by different letter (s) along the column for different rates of calcium foliar feed by the number of days in the vase and along the row for cultivar are significantly different at 5 % level of significance according to Tukeys' honestly significant difference test. Values are means n = 3

Calcium plays a critical role in maintaining cellular turgidity. This aids in keeping the flowers fresh for longer time. Calcium as a post- harvest treatment did not contain a carbon source since sugars were not included in the post- harvest solution thus chances of bacterial growth were minimal. The results of this study further confirm that pre - harvest calcium application affect cut flower post- harvest. Red calypso was noted to have less dry matter under the UV-A clear poly

film. The least fresh weight recorded at post- harvest could be because of rapid carbohydrate depletion since the cultivar had the least reserves of photo assimilates as observed by the lowest dry matter content recorded from this treatment.

Pre- harvest factors may directly affect vase life, for example the cultivar used could influence post- harvest with regard to ethylene sensitivity. Rose cultivars have different levels of resistance to bacteria infestation one of the most devastating factors of cut flower post- harvest. If the cultivar has better resistance the flowers will stay longer in the vase compared to a cultivar that has low resistance. The potential accumulation of carbohydrate was also identified to differ among the cultivars used in the study. Furiosa had consistently higher dry matter accumulation compared to Red Calypso and this was exhibited at post-harvest whereby the lowest fresh weight was recorded under the cultivar Red Calypso.

4.5.5. Effect of flower bud opening under different poly films on rose cut flower vase life

The increase in flower diameter was significantly affected by the poly film type under which the rose cultivars were grown (Figure 22). UV-A clear recorded the lowest percent increase in flower size between the first and third day of 26.95 %, while for UV-A 205/N and IR 504 bloom size increased by 31.9 % and 27 % respectively. Bloom size increased by 13.07 % between the day 3 and day 6 under the UV-A clear poly film compared to 16.3 % under the UV-A 205/N. Flower opening decreased progressively, by day 12 flowers under UV-A clear recorded the lowest increase in size by 3.9 % compared to IR 504 and UV-A 205/N which increased by 5.0 % and 7.88 %. The rate of opening for cut flowers harvested under the UV- A clear and UV-A 205/N was statistically similar between day 3 and day 6. However, bloom size drastically declined under IR 504 between day 6 and 9 compared to UV-A 205/N. Generally, increase in bloom size was significantly high between day 3 and 6 and gradually reduced for subsequent days (Figure 23).

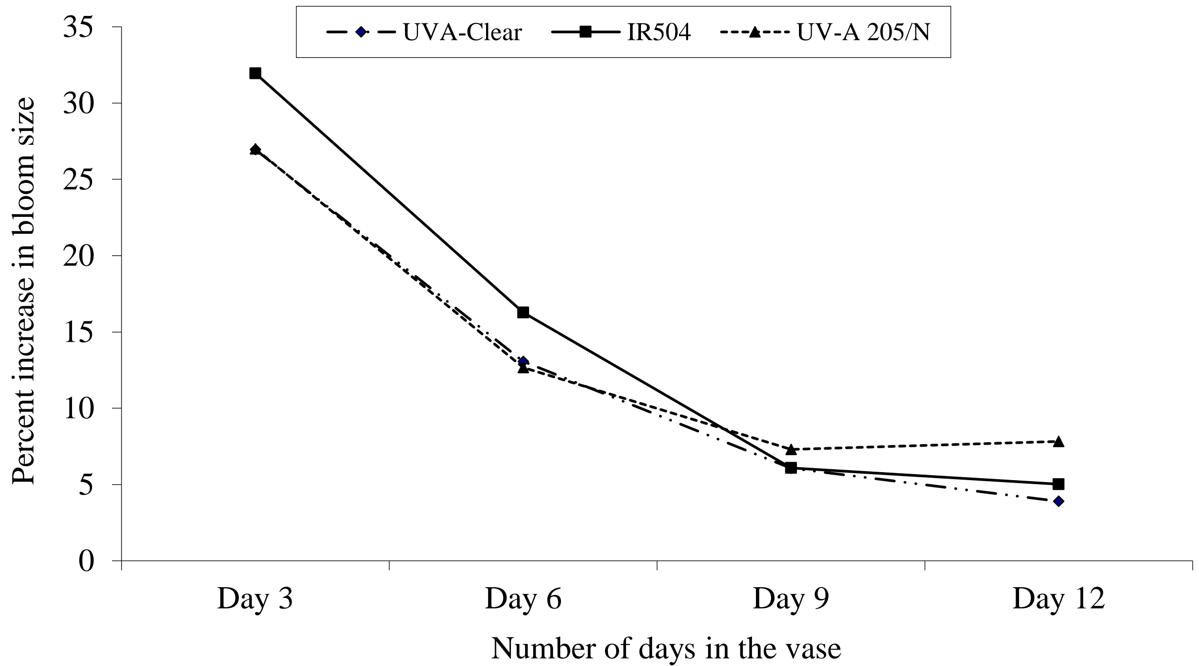


Figure 22: Effect of poly film covers on number of days to flower bud opening in flush I

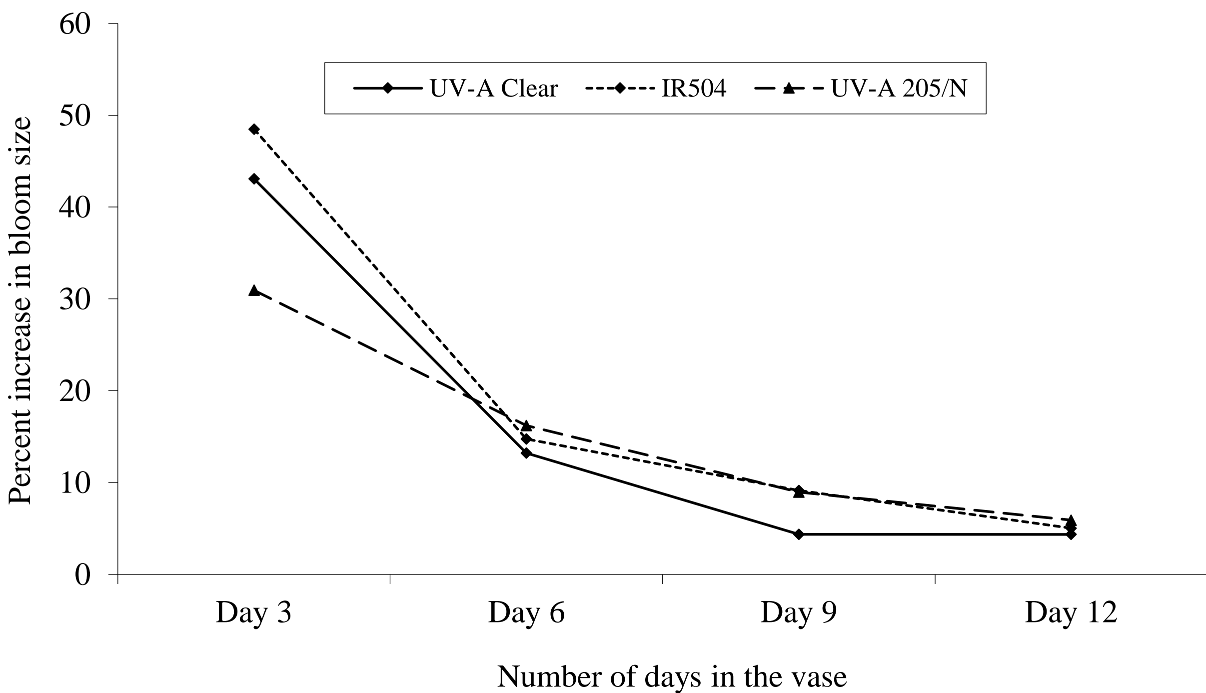


Figure 23: Effect of greenhouse cover on number of days to flower bud opening in flush II

Cut flowers grown under the UV-A 205/N displayed better bloom sizes by the 12th day. Flowers harvested under UV-A clear poly film drastically reduced in size by day 9 and recorded least increase between day 9 and 12. Flower petals contain a considerable amount of starch, during development, flower head expansion is accompanied by rapid break down of starch into glucose and fructose to support growth. The difference in flower head expansion reflect the different quantities of starch that could have been accumulated in the flowers. Starch accumulation however, is affected by the position of the shoot on the plant which could cause differences at post- harvest. The base of the shoot has more starch than the tip which is still young and succulent.

UV-A clear poly film recorded low dry matter of 6.61 g compared to UV-A 205/N that had 6.91 g in flush I. During the second flush dry matter increased under UV-A clear and IR 504 while there was a decline under UV-A 205/N. Considering the quantities of dry matter reported earlier, it is possible that in flush I plants grown under UV-A clear poly film had less starch since they were subjected to higher air temperature and in flush II there was better light transmission hence more assimilation of photosynthates compared to the UV-A 205/N coloured poly film. Air temperature influences carbohydrate synthesis and metabolism which is closely related to post-harvest.

Although vase life was carried out at room conditions, it is possible that the prevailing environmental conditions affected the rate of water loss from the cut flowers. Water relations affect vase life and it is directly linked to the prevailing air temperature. The higher the surrounding air temperature, the higher the transpiration rate. Fresh weight steadily declined under the UV-A clear poly film which was characterized by high day air temperature and low relative humidity. These conditions induced the closure of the stomata resulting in reduced photosynthesis. It further implies that the movement of calcium in the plant tissues was also limited under UV-A clear as opposed to IR 504 and UV-A 205/N poly films.

The results of the current study demonstrate that air temperature has direct and indirect effect on cut flower post- harvest physiology. Higher air temperature impact on starch accumulation which affects vase life. Increase in air temperature, increases respiration, moisture loss and flowers eventually become susceptible to ethylene damage and wilting. Celikel and Reid (2005) observed that respiration increased exponentially with increasing air temperature. Cut flowers respired three times faster at air temperature of 10 °C compared to 0 °C. Respiration is one

of the key factors that reduce vase life for example within 5 days interval vase life reduced by 40 % for roses and 38 % for gerbera due to high respiration (Celikel and Reid, 2005).

4.5.6. Effect of cultivars on change in bloom size

Change in bloom size was significantly dependent on cultivar. Initial flower diameter increased significantly by day 3 by 34.35 % under Red Calypso compared to 23.46 % under Furiosa. Further on between day 3 and 6 increase in bloom size reduced to 15.2 % and 9.47 % under Red Calypso and Furiosa respectively. By the 12th day increase in bloom size was 4.48 % under Red Calypso being significantly lower than Furiosa that increased by 6.68 %. Bloom size increased faster under Red Calypso and expansion started decreasing by the 9th day. However, under Furiosa expansion was gradual and steady over time hence flowers appeared fresh in the vase for longer duration. Furiosa displayed better blooms by day 12 compared to Red calypso that maintained quality for 9 days thus Furiosa had better vase life longevity (Figure 24 and 25).

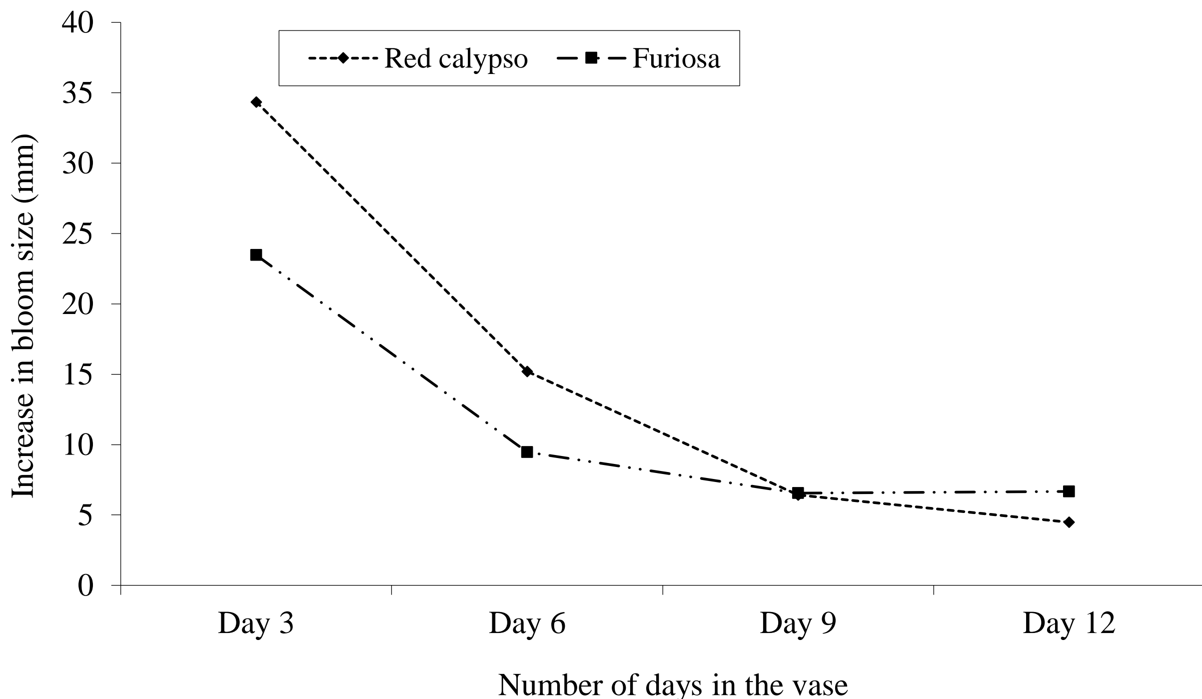


Figure 24: Effect of cultivar on vase life flower bloom diameter flush I

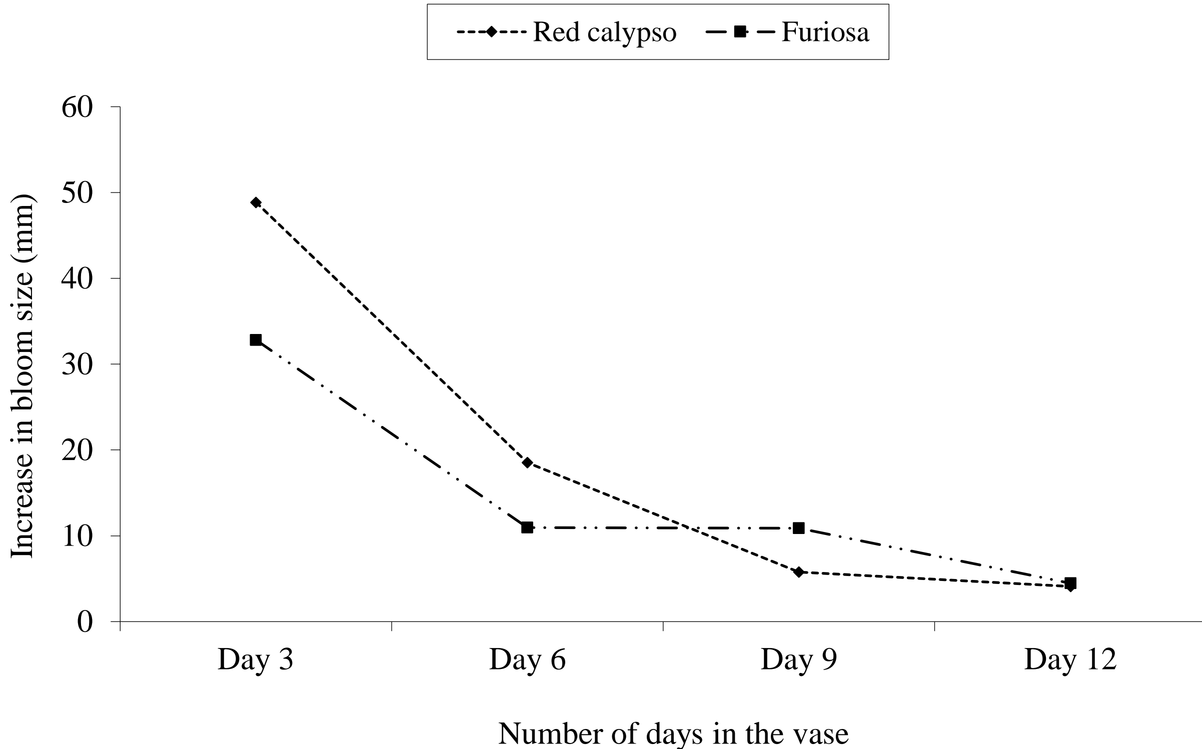


Figure 25: Effect of cultivar on vase life flower bloom diameter flush II

The results of this study illustrate that different cultivars tolerate fluctuating light intensity and air temperatures differently. Furiosa was more tolerant to changes in air temperature and could not substantially affect vase life compared to Red Calypso in flushes I and II. Under conditions of this study where by flush I was carried out at average room air temperature of 15 °C ± 2 °C and 63 % relative humidity while flush II was carried out at 10 °C ± 4 °C at 75 % reative humidity increase in bloom size showed higher variation in flush I compared to flush II. Flower opening is directly affected by the amount of carbohydrates in the flower, Red Calypso exhibited the least increase in bloom size possibly due to shortage of soluble carbohydrates.

Cultivars respond differently to changes in the environment. Their response determines how plant growth and development vary with varying environmental conditions. Changes in air temperature causes changes in carbohydrate content in the plant tissues that is key for vase life enhancement. It is possible therefore, that under the conditions of the study, Furiosa accumulated more carbohydrates to support vase life compared to Red Calypso. Additionally, pre- harvest evaluation of the two cultivars under different poly films showed that Red Calypso had smaller leaves. Larger leaf area is suitable for light interception leading to a higher rate of photosynthesis

which implies more biomass accumulation that could support vase life. This could explain further why flower opening was low for the cultivar Red Calypso compared to Furiosa.

4.5.7. Effect of cover and calcium on post- harvest bent neck occurrence

Cover and calcium significantly affected post- harvest bent neck occurrence. Combined treatment of UV-A clear and no calcium registered 39.5 % bent necks by day 6 while UV-A 205/N and no calcium recorded 33 % (Table 25 and 26). Combined treatment of UV-A clear and higher calcium concentrations 3.75 ml/L and 5.0 ml/L delayed bent neck occurrence till day 9. Bent necks occurred by day 6 under low concentrations of calcium at 1.25 ml/L, 2.5 ml/L and no calcium under the same poly film cover. Poly film cover IR 504 and no calcium treatment recorded 17 % bent necks by day 6 while there were no cases of bent necks under UV-A 205/N. Cut flower quality was good under IR 504 and 5.0 ml/L combined treatment and there were no incidences of bent necking recorded within the first 12 days in the vase under this treatment (Table 25 and 26). Difference in the occurrence of bent necks were apparent on the cut flowers after 6 days in the vase in flush I, however it was insignificant in flush II at a similar time (Table 25).

The number of bent necks appeared earlier (day 6) in the vase during flush I while in flush II bent neck occurrence delayed by 3 days (Appendix 28). By the 15th day the number of bent necks was relatively high under the UV-A clear and IR 504 poly films averaging 2 out of 3 cut flowers in the vase. UV-A 205/N poly film consistently recorded the lowest number of bent necks in both flushes I and II. Bent neck occurrence was significantly dependent on the level of calcium foliar feed applied. By end of the third day the number of bent necks were significantly high under the control compared to cut flowers treated with different concentrations of calcium foliar feed. In flush I, bent necking increased under calcium treatments 1.25 ml/L, 2.5 ml/L and no calcium with 67 %, 40.5 % and 47.6 %, respectively. Occurrence of bent necks was delayed by 9 and 12 days respectively under calcium concentration 3.75 ml/L and 5.0 ml/L.

Cut flowers treated with calcium levels 1.25 ml/L and 2.5 ml/L with no calcium developed bent necks by day 3 of the experiment. Higher concentration of calcium foliar feed-maintained flower quality by day 9 there were no incidences of bent necking recorded under calcium treatment with concentration of 5.0 ml/L while 73 % of the cut flower under the control experiment had bent necks by day 9.

Table 25: Effect of cover and calcium foliar feed on post- harvest bent necks occurrence (Flush I)

Flush 1		Calcium concentration ml/L				
Time (days)	Covers	Distilled water	1.25	2.5	3.75	5.0
3	UV-A clear	1.67 a	1.0 b	1.0 b	1.0 b	1.0 b
	IR 504	1.17 b	1.33 a	1.0 b	1.0 b	1.0 b
	UV - A205 / N	1.0 a	1.0 a	1.0 a	1.0 a	1.0 a
6	UV-A clear	2.33 a	1.5 abc	1.17bc	1.0 c	1.0 c
	IR 504	2.0 ab	2.0 ab	1.17 bc	1.0 c	1.0 c
	UV - A205 / N	1.33 bc	1.17 bc	1.0 c	1.0 c	1.0 c
9	UV-A clear	2.5ab	2.83 a	1.67 cd	1.0 d	1.0 d
	IR 504	1.5 bcd	1.67 cd	1.33 cd	1.0 d	1.0 d
	UV - A205 / N	3.17 a	2.33 abcd	1.5 de	1.17 e	1.17 e
12	IR 504	3.17 a	2.83ab	1.5 de	1.5 de	1.0 e
	UV - A205 / N	1.67 cde	2.67 ab	1.83 bcde	1.17 e	1.17 e
	UV-A clear	3.67 a	2.83 abc	2.83 abc	1.83c	1.83 c
15	IR 504	3.5 a	3.17 ab	2.17 bc	2.17 bc	1.83 c
	UV - A205 / N	2.83 abc	2.17 bc	3.82 abc	2.17 bc	2.17 bc

[1]Means followed by different letter (s) for poly films along the column and calcium foliar feed across the row are significantly different at 5 % level of significance according to Tukeys' honestly significant difference test.

Table 26: Effect of cover and calcium foliar feed on post- harvest bent neck occurrence (Flush II)

Flush I		Calcium concentration ml/L				
Time (days)	Covers	Distilled water	1.25	2.5	3.75	5.0
6	UV-A clear	2.17 a	1.83 abc	1.17 bc	1.0 c	1.0 c
	IR 504	1.83 ab	1.83 ab	1.17 bc	1.0 c	1.0 c
	UV – A 205 / N	1.5 bc	1.17 bc	1.17 bc	1.0 c	1.0 c
9	UV-A clear	3.17 a	2.5 ab	1.33 bc	1.33 bc	1.67 bc
	IR 504	2.0 abc	2.0 abc	1.33 bc	0.83 c	0.83 c
	UV – A 205 / N	1.33 bc	1.16 c	1.33 bc	1.0 c	1.33 bc
12	UV-A clear	3.33 a	2.17 bc	1.5 cd	1.17 cd	1.0 d
	IR 504	2.67 ab	2.67 ab	1.5 cd	1.17 cd	1.0 d
	UV – A 205 / N	1.83 bcd	1.17 cd	1.83 bcd	1.0 d	1.17 cd
15	UV-A clear	3.5a	2.33 abcd	1.5 cde	1.33 de	1.0e
	IR 504	2.83 ab	2.67 abc	1.5 cde	1.33 de	1.0 e
	UV - A205 / N	1.67 bcde	1.17 de	1.83 bcde	1.0 e	1.17 de

[1]Means followed by different letter (s) for poly films along the column and calcium foliar feed across the row are significantly different at 5 % level of significance according to Tukeys' honestly significant difference test.

Bent neck is a phenomenon that occurs from bending of the floral axis just below the flower head. It occurs mostly due to wilting of the flower and lack of cell turgidity to support flower stems in an upright position. Bent neck occurrence is perceived as senescence disorder that accompanies aging of cut flowers in the vase. Bent neck occurrence was probably lower under high calcium treatment since calcium enhances fresh weight through accelerated water uptake minimising subsequent loss and wilting. It can also be implicated to decline in carbohydrate reserves in the cut flower that is converted to glucose and sucrose to support growth. However, it

is not easy to ascertain which environmental factor greatly contributed to occurrence of post-harvest bent necks. In the current study, it was observed that dry matter accumulation was higher in flush II compared to flush I and consequently the number of bent necks by day 12 were relatively lower in flush II compared to flush I. It is therefore evident that if the preharvest conditions affect starch accumulation then the vase life will also be affected.

Conventionally, carbohydrates are broken down to support growth and for the metabolic processes to be complete there is need of adequate water within the cut stems. Interruption with moisture uptake interferes with cell turgidity leading to bent necking. It is therefore possible that where there was less dry matter correlating to less starch content there was less water uptake and consequently faster occurrence of bent necks. It is also interesting to note that plants under the UV-A 205/N registered the least amount of dry matter in flush II but lowest number of bent necks contradicting the assumption that high starch content minimizes post-harvest bent necking. Resistance of the peduncle to bending symptoms depends on lignification of the plant cell wall and addition of external sugar source that act as an enery source. Exogenous sucrose has been used as a substrate for respiration which helps to maintain osmotic potential of the flower petals and cell turgidity resulting to vase life extension (Sujata *et al.,* 2003).

Bent necking is a common symptom were water absorption is limiting during post- harvest handling. Cut flowers may fail to absorb water due to bacterial growth within the cut stem vascular vessels. Cut flowers in this study were held in plain water with no germicide and chances of bacterial growth were relatively high. It is possible that bent necks occurred due to limited water uptake has a result of bacterial blockages and other inherent factors that occurred during production.

Insufficient light intensity and high relative humidity during growth result to reduced vase life in cut flowers. Ottosen *et al*. (2002) experimented on the effect of relative humidity on post-harvest of two rose cultivars Mercedes and Baroness and established that at 90% RH the rose cultivar had shorter vase life. Relative humidity affects calcium absorption a major nutrient in vase life extension. Calcium is absorbed when there is low relative humidity that triggers water movement along the transpiration stream. Calcium reduces ethylene production at post-harvest stage and enhances vase life. This could be the reason why cut flowers in flush II could not delay bent necks for longer time as was the case in flush I since flush II was carried out at a time when relative humidity was high implying less calcium was absorbed. Calcium is also reported to

improve flower longevity by delaying occurrence of physiological activities related to senescence. Nabigol (2012) observed a decreased rate of electrolyte leakage from rose flower petals following a pre- harvest application of calcium sulphate. He also observed that rose petals treated with calcium produced less ethylene than their untreated counterparts. Calcium enhance water uptake and maintain the integrity of the cut flower cell wall limiting chances of bent necking.

4.5.8. Effect of calcium foliar feed on post-harvest leaf wilting

The poly film covers, and cultivars had no effect on leaf wilting at post- harvest. Wilting was influenced by different concentrations of calcium foliar feed. There were no cases of leaf wilting recorded for the first 6 days across all calcium treatments. Leaf wilting started by day 6 onwards and was significantly high under the treatment with no calcium and the lowest calcium concentration 1.25 ml/L compared to 5.0 ml/L. Cut flower quality declined progressively with time a cross all the treatments, by the 9th day wilting incidence was statistically higher under the control treatment and the lowest calcium concentration 1.25 ml/L and 2.5 ml/L compared to concentration 3.75 ml/L and 5.0 ml/L. Severe wilting occurred under treatment with no calcium (3.61 a) compared to 5.0 ml/L (2.22 c) by the 12th day. Wilting was more rapid under the control treatment where it increased by 24.9 % between the 9th to 12th day as opposed to 5.0 ml/L where by 11 % rise was recorded. Calcium concentration 5.0 ml/L was the best treatment in extending cut flower longevity in relation to leaf wilting (Table 27).

Basically, post- harvest leaf wilting is accelerated by water stress and blockage of the xylem vessels. Application of calcium increased cut flower stem firmness as earlier observed in this study. Plants treated with calcium concentration 3.75 ml/L and 5 ml/L presented stems with higher strength compared to the control treatment. This limited the chances of micro-organism attack that is the probable cause of vessel blockage resulting to leaf wilting compared to the control treatment with no calcium. Furthermore, calcium plays a significant role in post- harvest by controlling ethylene production and strengthening of the cell wall. This explains the reason why high concentration of calcium delayed occurrence of bent neck symptoms.

Generally, the wilting incidence was high in flush I where the experiment was carried out at high air temperature and low relative humidity. The roses grown at high relative humidity wilted faster after harvest, probably because of less sugar reserves and water stress caused by reduced stomata function (Torre and Fjeld, 2001). Post - harvest wilting may also occur due to blockage

which may arise from the exudation of latex and mucilage from the cut surface. During harvesting, cases of air embolisms have been reported to occur causing wilting.

Table 27: Effect of calcium foliar feed on post- harvest leaf wilting occurrence

	Flush I					Flush II				
Days	Calcium concentration ml/L									
	Distilled water	1.25	2.5	3.75	5.0	Distilled water	1.25	2.5	3.75	5.0
6	2.28 a	2.0 a	1.72 ab	1.72 ab	1.33 b	1.61 a	1.06 a	1.50 a	1.39 ab	1.0 b
9	2.89 a	2.67 ab	2.39 ab	2.17 b	2.00 b	2.39 a	2.06 ab	1.94 ab	1.78 b	1.61 b
12	3.61 a	3.11 ab	2.83 bc	2.67 bc	2.22 c	2.94 a	2.72 ab	2.33 abc	2.28 bc	1.94 c

[1]Means followed by different letter (s) along the row for calcium foliar feed are significantly different at 5 % level of significance according to Tukeys' honestly significant difference Test.

4.5.9. Effect of cultivar on post-harvest leaf defoliation

The incidences of defoliation were recorded after the first 6 days of holding flowers in the vase. Leaf defoliation was significant among the cultivars in flush I it was evident from day 9 although this observation was not consistent in flush II. The effect of poly film cover and calcium foliar feed were not significant in flush I. Leaf defoliation was cultivar dependent. Red Calypso had higher incidence of defoliation compared to Furiosa. Between day 9 and day 12 leaf defoliation increased by 24.8 % and 38.9 % under Furiosa and Red Calypso respectively (figure 26).

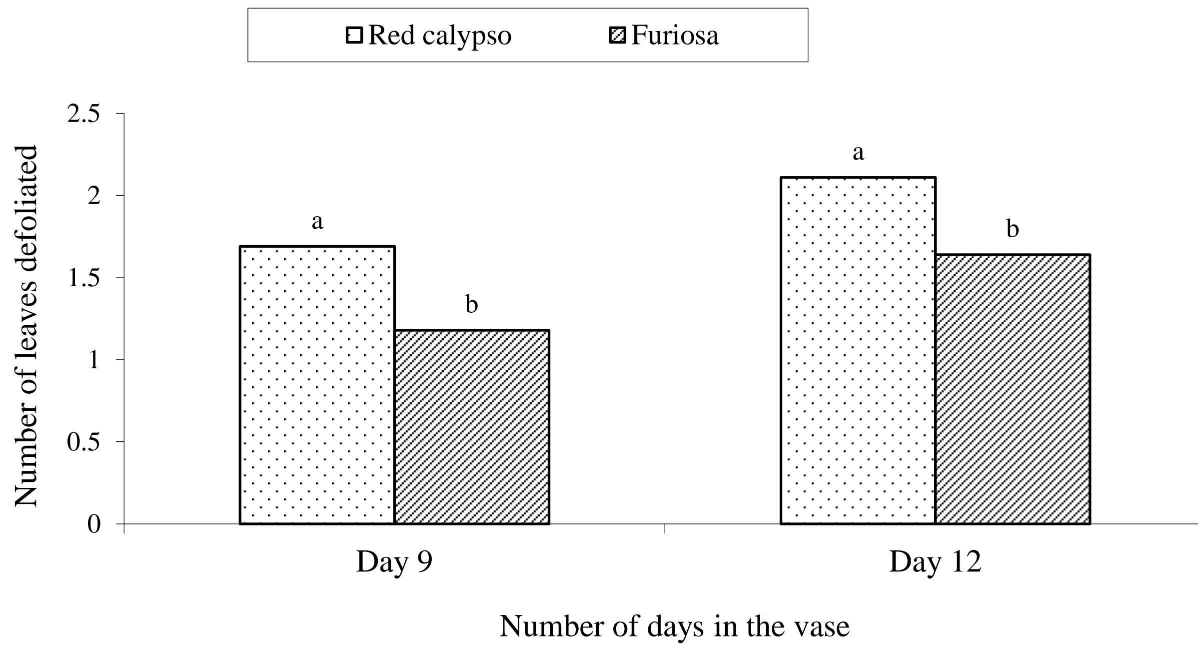


Figure 26: Effect of the pre- harvest treatments on leaf defoliation during vase life

CHAPTER FIVE
CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

1. The transmission of photosynthetic active radiation was high under UV-A clear poly film, followed by UV-A 205/N and IR504, the quantity of light transmitted was 1492 µmol m^{-2} s^{-1}, 1064 µmol m^{-2} s^{-1} and 984 µmol m^{-2} s^{-1} for the respective covers. The average relative humidity of 69.2 %, 76.1 % and 71 % with air temperatures of 31.2 °C, 31.1 °C and 30.9 °C were recorded during the day under UV-A clear, UV-A 205/N and IR 504 poly films respectively. The quantities of Cyanidin-3-0- glucoside the main anthocyanin in rose petals was higher under UV-A 205/N (110.95 ± 8.26), IR 504 (109.69 ± 8.26) and UV-A clear (84.56 ± 8.26).
2. Leaf area was affected by the poly film covers, it averaged at 2022.95 mm^2, 1914.8 mm^2 and 1608.41 mm^2 under UV-A clear, IR 504 and UV-A 205/N. The combined treatment of UV-A clear and cultivar Red Calypso recorded the highest number of bud breaks (3.47) and stem productivity per square metre. The least number of stem production was recorded under UV-A 205/N cover and cultivar Furiosa. UV-A clear poly film recorded faster growth rate followed by IR 504 and UV-A 205/N. UV-A clear recorded the highest number of short stems in category < 40 cm at 12.1%, 6.2% under UV-A 205/N and 5.0 under IR504. The time taken to maturity by the cultivar Red Calypso was 46, 49 and 47 days while Furiosa to 52, 54 and 56 days respectively under poly films UV-A clear, IR 504 and UV-A 205/N.
3. Leaf chlorophyll content (59.68) was highest under the IR 504 with calcium treatment 3.75 ml/L. The least values of leaf chlorophyll content (41.68) were recorded under UV-A clear with no calcium treatment. The combined effect of UV-A 205/N and IR 504 poly films with calcium concentrations 3.75 ml/L and 5.0 ml/L increased dry matter accumulation. Application of 5 ml/L of calcium foliar feed on Red Calypso and Furiosa gave the highest stem firmness under the UV-A clear and UV-A 205/N poly films respectively.
4. Petal blackening incidences were high under UV-A clear and UV- A 205/N and low under poly film IR 504. Red Calypso recorded the highest number of blind shoots (3.67 stems/m^2). The prevalence of petal blackening was high in flush I (3.64 stems/m^2) and low in flush II (0.8 stems/m^2) for the cultivar Furiosa.

5. Calcium foliar feed at concentration 2.5 ml/L was as effective as concentrations 3.75 ml/L and 5.0 ml/L in enhancing vase life by delaying bent neck occurrence, leaf wilting and leaf defoliation. Average vase life of 12 days was achieved under UV-A 205/N and IR 504 poly films, with the cultivar Furiosa and calcium concentrations of 3.75 ml/L and 5.0 ml/L in isolation.

5.2. Recommendations

1. Poly film transmission properties greatly influence the greenhouse microclimate. The results of this study could be used by rose cut flower growers to choose most appropriate poly film covers for their growing conditions. Coloured poly film covers can be used under conditions of high air temperature since they reduce irradiance and air temperature within the structure. Clear poly films on the other hand would be suitable for areas prone to overcast conditions since they are the potential to transmit high light intensity.
2. Rose cultivars exhibit differences in growth and quality even under similar growth conditions. Growers should continuous carry out on farm trials to establish those cultivars that are suitable for their areas and screen for quality to satisfy customer requirements. It is also essential for purposes of timing peak market period that growers screen several cultivars to know the duration taken to maturity under their production conditions. This information is handy in synchronizing farm activities during planning with peak market seasons for profitability.
3. The effect of calcium foliar feed on growth and quality of rose flower depends on several factors among them being the prevailing weather conditions. The current study was carried out under natural conditions and it was not easy to separate the effect of various factors singly. Tests should be done in combination with other factors like different concentrations of nutrients in the fertigation solution and different stages of growth to establish the right concentration of calcium that will have effect on post- harvest vase life with minimal or no wastage.
4. Results of different interactions under natural conditions were inconsistent, further research should be carried out under controlled conditions in growth chambers to establish the optimal levels of light, air temperature and calcium that would positively enhance vase life.
5. The eco-physiological studies that offer an understanding of how plants and the environment interact should be done to determine the pattern of plant growth and development. The effects of high light intensity could not be quantified due to the limitations of this field data.

6. Photodegradation of poly films was evident after 12 months of usage. Currently, with challenges in climate change there is need for an intervention to enhance poly film durability against UV-transmission to lengthen their life span and minimize plastic pollution generated from protected agriculture.

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APPENDICES

Appendix 1: Quantity of nutrients in the foliar feed per single application

Fertilizer	mMol/L	Ca^{2+} 1-5	Mg^{2+} 0.83-4.17	Mn^{2+} 0.004-0.05	Fe^{2+} 0.005-0.05	B 0.009-0.07	Cu^{2+} 0.0002-0.008	Zn^{2+} 0.0005-0.05	Mo 0.0001-0.001	No_3^- 2.5-10.7
T1 0.333		1.25	0.28	0.006	0.003	0.015	0.002	0.001	0.00003	2.38
Urea	7.50									7.50
										9.88
T2 0.333		1.25	0.28	0.006	0.003	0.015	0.002	0.001	0.00003	2.38
$Ca(NO_4)_2.4H_2O$		1.25								2.50
Urea	5.0									5.00
										9.88
T3 0.333		1.25	0.28	0.006	0.003	0.015	0.002	0.001	0.00003	2.38
$Ca(NO_4)_2.4H_2O$		2.50								5.00
Urea	2.5									2.50
										9.88
T4 0.333		1.25	0.28	0.006	0.003	0.015	0.002	0.001	0.00003	2.38
$Ca(NO_4)_2.4H_2O$		3.75								7.50
										9.88

NB: Urea was used to balance N and ensure its uniformity in all applications. The concentration 0.333ml/L of Calmag is the lowest possible rate of the foliar fertilizer that can be used while at the same time allowing for modification of calcium levels at various concentrations.

Hence the Quantities of $Ca(NO_4)_2.4H_2O$ and Urea to be used are as follows:

T2 $Ca(NO_4)_2.4H_2O$ = 1.25x 236(RMM of $Ca(NO_4)_2.4\ H_2O$) = 295 mg / L

T3 $Ca(NO_4)_2.4H_2O$ = 2.5x 236(RMM of $Ca(NO_4)_2.4\ H_2O$) = 590 mg / L

T4 $Ca(NO_4)_2.4H_2O$ = 1.25x 236(RMM of $Ca(NO_4)_2.4\ H_2O$) = 885 mg / L

Urea to be used for balancing T1, T2 and T3 to be at per with T4 will

T1 7.5 x 60=450 mg / L

T2 5.0 x 60= 300 mg / L

T3 2.5 x 60= 150 mg /

Appendix 2: ANOVA table for number of bud breaks in flushes I and II

		Flush I			Flush II		
Source of variation	DF	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F
Cover	2	7.4666667	22.4000	<.0001*	8.066667	9.3077	0.0003*
Cultivar	1	4.9000000	29.4000	<.0001*	0.544444	1.2564	0.2668
Calcium	4	0.6222222	0.9333	0.4508	1.622222	0.9359	0.4494
Calcium*Cover	8	0.9777778	0.7333	0.6617	1.377778	0.3974	0.9177
Cultivar *Calcium	2	5.6000000	16.8000	<.0001*	16.155556	18.6410	<.0001*
Cover* Cultivar	4	1.3777778	2.0667	0.0964	0.955556	0.5513	0.6988
Cover*Cultivar*Calcium	8	3.9555556	2.9667	0.0073*	6.177778	1.7821	0.0985
Rsq 0.71					Rsq 0.57		
Root MSE 0.4082					Root MSE 0.6583		

Appendix 3: ANOVA table for rose cut flower stem production per square metre

		Flush I				Flush II			
Source of variation	DF	Type III SS	Mean Square	F Value	Pr > F	Type I SS	Mean Square	F Value	Pr > F
Rep	2	45.2666667	22.6333333	3.06	0.0563	9.688889	4.844444	0.92	0.4045
Cover	2	241.8000000	120.9000000	16.33	<.0001	1150.422222	575.211111	109.51	<.0001
Rep*Cover	4	37.5333333	9.3833333	1.27	0.2958	49.977778	12.494444	2.38	0.0648
Cultivar	1	739.6000000	739.6000000	99.91	<.0001	2016.400000	2016.400000	383.87	<.0001
Cover* Cultivar	2	48.2000000	24.1000000	3.26	0.0472	345.800000	172.900000	32.92	<.0001
Rep*Cover* Cultivar	6	35.2000000	5.8666667	0.79	0.5803	48.200000	8.033333	1.53	0.1889
Calcium	4	15.2888889	3.8222222	0.52	0.7241	26.933333	6.733333	1.28	0.2903
Cover*Calcium	8	68.6444444	8.5805556	1.16	0.3430	167.800000	20.975000	3.99	0.0011
Cultivar *Calcium	4	160.1777778	40.0444444	5.41	0.0011	22.711111	5.677778	1.08	0.3765
Cover* Cultivar *Ca	8	35.3555556	4.4194444	0.60	0.7755	110.422222	13.802778	2.63	0.0179

Flush	R-Square	Coeff Var	Root MSE
Flush I	0.939975	6.454019	2.291894
Flush II	0.800643	14.68060	2.720805

Appendix 4: ANOVA table rose cut flower plant height on 10th day for flushes I and II

		Flush I				Flush II			
Source of variation	DF	Type III SS	Mean Square	F Value	Pr > F	Type I SS	Mean Square	F Value	Pr > F
Rep	2	1.30755556	0.65377778	7.06	0.0020	115.185722	57.592861	1.92	0.1576
Cover	2	0.80288889	0.40144444	4.34	0.0186	1151.566722	575.783361	19.20	<.0001
Rep*Cover	4	0.02444444	0.00611111	0.07	0.9917	249.498444	62.374611	2.08	0.0980
Cultivar	1	0.69344444	0.69344444	7.49	0.0087	2720.850250	2720.850250	90.74	<.0001
Cover* Cultivar	2	0.18288889	0.09144444	0.99	0.3798	35.704500	17.852250	0.60	0.5554
Rep*Cover* Cultivar	6	0.21866667	0.03644444	0.39	0.8794	290.083500	48.347250	1.61	0.1642
Calcium	4	0.29733333	0.07433333	0.80	0.5293	130.996556	32.749139	1.09	0.3711
Cover*Calcium	8	1.30600000	0.16325000	1.76	0.1080	145.770778	18.221347	0.61	0.7668
Cultivar *Calcium	4	0.90933333	0.22733333	2.46	0.0582	166.561000	41.640250	1.39	0.2520
Cover* Cultivar*Calcium	8	1.35266667	0.16908333	1.83	0.0950	111.313000	13.914125	0.46	0.8752

R-Square	CV	Root MSE	R-Square	Coeff Var	Root MSE
0.614950	23.06710	0.304229	0.780499	11.22164	5.475724

Appendix 5: ANOVA table rose cut flower plant height on 17th day for flushes I and II

	Flush I					Flush II			
Source of variation	DF	Type III SS	Mean Square	F Value	Pr > F	Type I SS	Mean Square	F Value	Pr > F
Rep	2	10.46422222	5.23211111	2.86	0.0668	7.256000	3.6280000	2.65	0.0810
Cover	2	2.96288889	1.48144444	0.81	0.4504	17.114000	8.55700000	6.25	0.0039
Rep*Cover	4	5.18177778	1.29544444	0.71	0.5896	3.74000000	0.93500000	0.68	0.6073
Cultivar	1	72.36100000	72.36100000	39.62	<.0001	1.70844444	1.70844444	1.25	0.2695
Cover* Cultivar	2	1.98866667	0.99433333	0.54	0.5837	2.04688889	1.02344444	0.75	0.4790
Rep*Cover* Cultivar	6	11.03533333	1.83922222	1.01	0.4319	12.69066667	2.11511111	1.54	0.1841
Calcium	4	2.64066667	0.66016667	0.36	0.8348	7.91222222	197805556	1.44	0.2338
Cover*Calcium	8	7.25933333	0.90741667	0.50	0.8524	14.12711111	1.76588889	1.29	0.2713
Cultivar *Calcium	4	3.49177778	0.87294444	0.48	0.7517	27.61266667	6.90316667	5.04	0.0018
Cover* Cultivar *Ca	8	13.24022222	1.65502778	0.91	0.5193	20.49200000	2.56150000	1.87	0.0869
R-square	CV	RMSE				R-square	CV	RMSE	
0.598	12.28	1.35				0.636	23.72	1.17	

Appendix 6: ANOVA table rose cut flower plant height on 24th day for flushes I and II

		Flush I				Flush II			
Source of variation	DF	Type III SS	Mean Square	F Value	Pr > F	Type I SS	Mean Square	F Value	Pr > F
Rep	2	67.4675556	33.7337778	5.54	0.0068	99.574888	49.7874444	3.17	0.0511
Cover	2	3.3182222	1.6591111	0.27	0.7625	107.0513889	53.5256944	3.40	0.0414
Rep*Cover	4	18.4357778	4.6089444	0.76	0.5580	219.8311111	54.9577778	3.50	0.0139
Cultivar	1	233.6111111	233.6111111	38.40	<.0001	45.2271111	45.2271111	2.88	0.0964
Cover* Cultivar	2	8.0035556	4.0017778	0.66	0.5226	67.3027222	33.6513611	2.14	0.1287
Rep*Cover* Cultivar	6	41.1713333	6.8618889	1.13	0.3606	37.0146667	6.1691111	0.39	0.8803
Calcium	4	6.0973333	1.5243333	0.25	0.9079	58.857388	14.7143472	0.94	0.4513
Cover*Calcium	8	25.4006667	3.1750833	0.52	0.8341	106.458611	13.3073264	0.85	0.5673
Cultivar *Calcium	4	12.6977778	3.1744444	0.52	0.7201	160.5009444	40.1252361	2.55	0.0509
Cover* Cultivar *Ca	8	37.4375556	4.6796944	0.77	0.6313	111.8783889	13.9847986	0.89	0.5325
R-square	CV	RMSE				R-square	CV	RMSE	
0.608	13.11	2.47				0.573	26.88	3.96	

Appendix 7: ANOVA table rose cut flower plant height on 31st day for flushes I and II

		Flush I				Flush II			
Source of variation	DF	Type III SS	Mean Square	F Value	Pr > F	Type I SS	Mean Square	F Value	Pr > F
Rep	2	276.0482222	138.0241111	5.60	0.0065	248.82288	124.41144	4.87	0.0118
Cover	2	82.3415556	41.1707778	1.67	0.1992	98.90688	49.45344	1.94	0.1551
Rep*Cover	4	84.9077778	21.2269444	0.86	0.4944	85.3791111	21.3447778	0.84	0.5089
Cultivar	1	726.4721111	726.4721111	29.45	<.0001	772.055111	772.0551111	30.25	<.0001
Cover* Cultivar	2	100.0442222	50.0221111	2.03	0.1427	95.320222	47.6601111	1.87	0.1656
Rep*Cover* Cultivar	6	238.3146667	39.7191111	1.61	0.1649	240.3566667	40.0594444	1.57	0.1765
Calcium	4	8.2688889	2.0672222	0.08	0.9870	5.5706667	1.3926667	0.05	0.9943
Cover*Calcium	8	99.9284444	12.4910556	0.51	0.8455	105.13866	13.1423333	0.51	0.8392
Cultivar *Calcium	4	53.6017778	13.4004444	0.54	0.7047	59.2026667	14.8006667	0.58	0.6786
Cover* Cultivar *Ca	8	29.0302222	3.6287778	0.15	0.9963	32.2120000	4.0265000	0.16	0.9953
R-square	CV	RMSE				R-square	CV	RMSE	
0.589	14.16	4.97				0.587	13.88	5.05	

Appendix 8: ANOVA table rose cut flower plant height on 38th day for flushes I and II

		Flush I				Flush II			
Source of variation	DF	Type III SS	Mean Square	F Value	Pr > F	Type I SS	Mean Square	F Value	Pr > F
Rep	2	831.801556	415.900778	12.04	<.0001	115.185722	57.592861	1.92	0.1576
Cover	2	555.708222	277.854111	8.04	0.0010	1151.56672	575.78336	19.20	<.0001
Rep*Cover	4	377.463111	94.365778	2.73	0.0397	249.498444	62.374611	2.08	0.0980
Cultivar	1	1629.026778	1629.026778	47.16	<.0001	720.850250	2720.850250	90.74	<.0001
Cover* Cultivar	2	130.526889	65.263444	1.89	0.1623	35.704500	17.852250	0.60	0.5554
Re*Cover* cultivar	6	380.731333	63.455222	1.84	0.1117	290.083500	48.347250	1.61	0.1642
Calcium	4	85.183778	21.295944	0.62	0.6529	130.996556	32.749139	1.09	0.3711
Cover*Calcium	8	437.172889	54.646611	1.58	0.1553	145.770778	18.221347	0.61	0.7668
Cultivar *Calcium	4	40.037111	10.009278	0.29	0.8832	166.561000	41.640250	1.39	0.2520
Cover*Cutivar *Ca	8	250.420889	31.302611	0.91	0.5193	111.31300	13.914125	0.46	0.8752
R-squared	CV	RMSE				R-squared	CV	RMSE	
0.740	10.37	5.88				0.780	11.22	5.47	

Appendix 9: ANOVA table for different rose cut flower stem grades flushes I and II

		Flush I				Flush II			
Source of variation	DF	Type III SS	Mean Square	F Value	Pr > F	Type III SS	Mean Square	F Value	Pr > F
Rep	2	27.2888889	133.0222222	2.07	0.1370	1.15555556	0.57777778	0.34	0.7100
Cover	2	32.2888889	16.1444444	2.45	0.0968	67.35555556	33.67777778	20.11	<.0001
Rep*Cover	4	22.7777778	5.6944444	0.86	0.4918	3.77777778	0.94444444	0.56	0.6900
Cultivar	1	19.6000000	19.6000000	2.98	0.0909	24.54444444	24.54444444	14.65	0.0004
Cover* Cultivar	2	34.2000000	17.1000000	2.60	0.0849	40.15555556	20.07777778	11.99	<.0001
Rep*Cover* Cultivar	6	18.6000000	3.1000000	0.47	0.9265	8.00000000	1.33333333	0.80	0.5777
Calcium	4	81.1777778	20.2944444	3.08	0.0244	43.08888889	5.38611111	3.22	0.0053
Cover*Calcium	8	80.4888889	10.0611111	1.53	0.1725	43.08888889	5.38611111	3.22	0.0053
Cultivar *Calcium	4	8.5111111	2.1277778	0.32	0.8610	17.17777778	4.29444444	2.56	0.0501
Cover*Cultivar *Calcium	8	133.0222222	16.6277778	2.53	0.0222	34.28888889	4.28611111	2.56	0.0207

R-Square	Coeff Var	Root MSE	Mean	R-Square	Coeff Var	Root MSE	Mean
0.752911	20.25732	1.294218	6.388889	0.591708	22.46324	2.565801	11.42222

Appendix 10: ANOVA table for Number of days taken to flowering flushes I and II

		Flush I				Flush II			
Source of variation	DF	Type III SS	Mean Square	F Value	Pr > F	Type I SS	Mean Square	F Value	Pr > F
Rep	2	2.0666667	1.0333333	1.68	0.1979	5.0888889	2.5444444	5.59	0.0066
Cover	2	112.4666667	56.2333333	91.19	<.0001	83.3555556	41.6777778	91.49	<.0001
Rep*Cover	4	2.6666667	0.6666667	1.08	0.3764	0.7777778	0.1944444	0.43	0.7885
Cultivar	1	694.4444444	694.4444444	1126.13	<.0001	960.4000000	960.4000000	2108.20	<.0001
Cover* Cultivar	2	12.2888889	6.1444444	9.96	0.0002	4.8666667	2.4333333	5.34	0.0080
Rep*Cover* Cultivar	6	5.6666667	0.9444444	1.53	0.1882	6.9333333	1.1555556	2.54	0.0325
Calcium	4	10.1555556	2.5388889	4.12	0.0060	8.1555556	2.0388889	4.48	0.0037
Cover*Calcium	8	10.9777778	1.3722222	2.23	0.0417	2.3111111	0.2888889	0.63	0.7451
Cultivar *Calcium	4	10.1111111	2.5277778	4.10	0.0062	7.4888889	1.8722222	4.11	0.0061
Cover*Cultivar *Calcium	8	7.1555556	0.8944444	1.45	0.2006	6.5777778	0.8222222	1.80	0.0994

Flush	R-Square	Coeff Var	Root MSE
Flush I	0.980262	1.348698	0.674949
Flush II	0.967023	1.633734	0.785281

Appendix 11: ANOVA table for cut flower stem diameter flushes I and II

		Flush I			Flush II		
Source of variation	DF	Type III SS	F Value	Pr > F	Type III SS	F Value	Pr > F
Cover	2	1.7077400	2.2316	0.1162	0.4943089	0.8075	0.4508
Cultivar	1	4.8348844	12.6362	0.0007*	6.2779211	20.5113	<.0001*
Calcium	4	3.0836067	2.0148	0.1038	0.3737044	0.3052	0.8734
Cover* Cultivar	2	0.1355756	0.1772	0.8381	0.6524822	1.0659	0.3508
Cover*Calcium	8	3.6053933	1.1779	0.3274	2.8251356	1.1538	0.3419
Cultivar *Calcium	4	2.3092378	1.5088	0.2110	1.9372733	1.5824	0.1906
Cover* Cultivar *Calcium	8	2.3539356	0.7690	0.6312	2.3524067	0.9607	0.4750
R-Square	RMSE				R-Square	RMSE	
0.45	0.619				0.45	0.553	

Appendix 12: ANOVA table for cut flower bloom diameter flushes I and II

	Flush I				Flush II		
Source of variation	DF	Type I SS	F Value	Pr > F	Type I SS	F Value	Pr > F
Cover	2	78.82510	4.2703	0.0185*	9.31097	0.1868	0.8301
Cultivar	1	2.45355	0.2658	0.6080	8.19628	0.3289	0.5684
Calcium	4	42.12798	1.1411	0.3459	20.10448	0.2017	0.9365
Cover* Cultivar	2	15.01772	0.8136	0.4481	13.75654	0.2760	0.7597
Calcium* Cover	8	102.12274	1.3831	0.2224	21.14594	0.1061	0.9989
Cultivar * Calcium	4	55.25039	1.4966	0.2146	88.21684	0.8851	0.4784
Cultivar * Calcium* Cover	8	51.32837	0.6952	0.6943	205.61437	1.0315	0.4229
R-Squared	RMSE				R-Squared	RMSE	
0.39	3.038				0.20	4.99	

Appendix 13: ANOVA table for Petal blackening in rose plants flushes I and II

		Flush I			Flush II		
Source of variation	DF	Type III SS	F Value	Pr > F	Type III SS	F Value	Pr > F
Cover	2	11.666667	17.5000	<.0001*	45.066667	53.3684	<.0001*
Cultivar	1	0.711111	2.1333	0.1493	0.711111	1.6842	0.1993
Calcium	4	0.777778	0.5833	0.6759	1.333333	0.7895	0.5366
Cover* Cultivar	2	2.222222	0.8333	0.5769	1.600000	0.4737	0.8700
Cover*Calcium	8	1.177778	0.8833	0.4794	1.288889	0.7632	0.5534
Cultivar *Calcium	4	0.822222	1.2333	0.2986	5.955556	7.0526	0.0018*
Cover* Cultivar *Calcium	8	2.622222	0.9833	0.4580	0.711111	0.2105	0.9879

R-Squared	RMSE	P	R-Squared	RMSE	P
0.55	0.577	.0089	0.69	0.649	< .0001

Appendix 13: ANOVA table for effect of poly film covers, cultivars and calcium foliar feed on leaf chlorophyll flushes I and II

		Flush I				Flush II			
Source of variation	DF	Type III SS	Mean Square	F Value	Pr > F	Type I SS	Mean Square	F Value	Pr > F
Rep	2	102.9202222	51.4601111	29.73	<.0001	119.910222	59.955111	25.15	<.0001
Cover	2	389.8835556	194.9417778	112.63	<.0001	324.989556	162.494778	68.16	<.0001
Rep*Cover	4	50.8297778	12.7074444	7.34	0.0001	25.377111	6.344278	2.66	0.0438
Cultivar	1	955.8321111	955.8321111	552.25	<.0001	435.600000	435.600000	182.72	<.0001
Cover* Cultivar	2	1.1902222	0.5951111	0.34	0.7108	7.572667	3.786333	1.59	0.2148
Rep*Cover* Cultivar	6	66.8246667	11.1374444	6.43	<.0001	60.007333	10.001222	4.20	0.0018
Calcium	4	810.7955556	202.6988889	117.11	<.0001	1061.894000	265.473500	111.36	<.0001
Cover*Calcium	8	53.9797778	6.7474722	3.9	0.0013	78.349333	9.793667	4.11	0.00009
Cultivar *Calcium	4	104.3906667	26.0976667	15.08	<.0001	80.830000	20.207500	8.48	<.0001
Cover* Cultivar *Ca	8	18.0753333	2.2594167	1.31	0.2636	11.110667	1.388833	0.58	0.7871

R-Square	Coeff Var	Root MSE	CH1 Mean	R-Square	Coeff Var	Root MSE	CH2 Mean
0.950677	2.839898	1.544021	54.36889	0.968505	2.611759	1.315601	50.37222

Appendix 14: ANOVA table for effect of poly film covers, cultivars and calcium foliar feed on leaf area flushes I and II

		Flush I			Flush II		
Source of variation	DF	Type III SS	F Value	Pr > F	Type I SS	F Value	Pr > F
Cover	2	3793736.9	9.9258	0.0002*	3084031.6	9.5539	0.0003*
Cultivar	1	3903675.4	20.4269	<.0001*	3391241.5	21.0112	<.0001*
Calcium	4	1012895.4	1.3251	0.2710	835085.3	1.2935	0.2828
Cover* Cultivar	2	102058.0	0.2670	0.7666	92736.4	0.2873	0.7513
Cultivar * Calcium	4	307156.3	0.4018	0.8066	252875.6	0.3917	0.8138
Calcium* Cover	8	1776863.3	1.1622	0.3368	1480890.1	1.1469	0.3462
Calcium* Cover* Cultivar	8	180204.4	0.1179	0.9984	152722.4	0.1183	0.9983

R-Square	RMSE	P	R-Square	RMSE	P
0.49	437.1	0.012	0.49	0.49	0.0127

Appendix 15: ANOVA table for effect of poly film covers, cultivars and calcium foliar feed on stem firmness flushes I and II

		Flush I			Flush II		
Source of variation	DF	Type III SS	F Value	Pr > F	Type I SS	F Value	Pr > F
Cover	2	5.962667	21.9395	<.0001*	3.512889	12.6363	<.0001*
Cultivar	1	5.625000	41.3941	<.0001*	0.000111	0.0008	0.9775
Calcium	4	21.887778	40.2678	<.0001*	57.183333	102.8477	<.0001*
Cover* Cultivar	2	15.200000	55.9280	<.0001*	2.752889	9.9025	0.0002*
Calcium*cover	8	2.296222	2.1122	0.0484*	12.252667	11.0186	<.0001*
Calcium* Cultivar	4	0.250000	0.4599	0.7648	6.741556	12.1251	<.0001*
Calcium* Cultivar*cover	8	1.450000	1.3338	0.2447	11.317111	10.1773	<.0001*
R-Squared	RMSE				R-Squared	RMSE	
0.87	0.37				0.92	0.37	

Appendix 16: ANOVA table for effect of poly film covers, cultivars and calcium foliar feed on shoot dry matter accumulation flushes I and II

		Flush I				Flush II			
Source of variation	DF	Type III SS	Mean Square	F Value	Pr > F	Type I SS	Mean Square	F Value	Pr > F
Rep	2	2.57352667	1.28676333	3.44	0.0403	3.42628222	1.71314111	2.92	0.0638
Cover	2	16.81372667	8.40686333	22.45	<.0001	30.29613556	15.14806778	25.79	<.0001
Rep*Cover	4	2.07620667	0.51905167	1.39	0.2528	5.93631111	1.48407778	2.53	0.0527
Cultivar	1	23.22576000	23.22576000	62.03	<.0001	39.11165444	39.11165444	66.59	<.0001
Cover* Cultivar	2	1.55660667	0.77830333	2.08	0.1362	5.50277556	2.75138778	4.68	0.0139
Rep*Cover* Cultivar	6	3.22813333	0.53802222	1.44	0.2203	2.25590000	0.37598333	0.64	0.6975
Calcium	4	1.76845556	0.44211389	1.18	0.3312	20.70514889	5.17628722	8.81	<.0001
Cover*Calcium	8	30.09645111	3.76205639	10.05	<.0001	58.85723111	7.35715389	12.53	<.0001
Cultivar *Calcium	4	10.06954000	2.51738500	6.72	0.0002	11.47778444	2.86944611	4.89	0.0022
Cover* Cultivar *Ca	8	20.88899333	2.61112417	6.97	<.0001	22.88456889	2.86057111	4.87	0.0002

R-Square	Coeff Var	RMSE	R-square	Coeff.var	RMSE
0.86	4.61	0.61	0.87	5.64	0.77

Appendix 17: ANOVA table for rose flower head dry matter accumulation in flushes I and II

		Flush I				Flush II			
Source of variation	DF	Type III SS	Mean Square	F Value	Pr > F	Type I SS	Mean Square	F Value	Pr > F
Rep	2	0.88248222	0.44124111	2.33	0.1079	4.13682000	2.06841000	4.94	0.0112
Cover	2	1.73116222	0.86558111	4.58	0.0152	6.89874000	3.44937000	8.23	0.0008
Rep*Cover	4	1.04171111	0.26042778	1.38	0.2559	6.51992000	1.62998000	3.89	0.0082
Cultivar	1	7.40173444	7.40173444	39.14	<.0001	25.82449000	25.82449000	61.62	<.0001
Cover* Cultivar	2	11.82353556	5.91176778	31.26	<.0001	1.68940667	0.84470333	2.02	0.1444
Rep*Cover* Cultivar	6	2.37210000	0.39535000	2.09	0.0717	3.91939333	0.65323222	1.56	0.1799
Calcium	4	7.86285111	1.96571278	10.40	<.0001	29.19269556	7.29817389	17.41	<.0001
Cover*Calcium	8	6.33878222	0.79234778	4.19	0.0007	7.21740444	0.90217556	2.15	0.0485
Cultivar *Calcium	4	6.93154889	1.73288722	9.16	<.0001	2.22752667	0.55688167	1.33	0.2729
Cover* Cultivar *Ca	8	5.81256444	0.72657056	3.84	0.0015	7.99589333	0.99948667	2.38	0.0299

R-Square	Coeff Var	Root MSE	R-square	Coeff Var	RMSE
0.851872	6.390376	0.434851	0.826186	9.213707	0.647386

Appendix 18: ANOVA table for Cyanidin chloride quantification flushes I and II

		Flush I			Flush II		
Source of variation	DF	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F
Cover	2	7.0596164	11.0145	0.0019*	42.970131	18.8517	0.0002*
Cultivar	1	5.8818688	18.3538	0.0011*	19.941283	17.4971	0.0013*
Cover* Cultivar	2	0.0351902	0.0549	0.9468	0.214912	0.0943	0.9107
R-square	0.77				R-square	0.82	
RMSE	0.5661				RMSE	1.07	

Appendix 19: ANOVA table for Pelargonadin chloride quantification flushes I and II

		Flush I			Flush II		
Source of variation	DF	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F
Cover	2	21.506527	6.8071	0.0106*	2.0430941	10.6223	0.0022*
Cultivar	1	64.445743	40.7957	<.0001*	5.8106477	60.4207	<.0001*
Cover* Cultivar	2	2.259620	0.7152	0.5088	0.5278413	2.7443	0.1044
R-square	0.14				R-square	0.88	
RMSE	0.668				RMSE	0.310	

Appendix 20: ANOVA table for Lacl chloride quantification flushes I and II

		Flush I			Flush II		
Source of variation	DF	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F
Cover	2	2660.831	3.2448	0.0747	3515.907	1.7282	0.2190
Cultivar	1	30541.433	74.4895	<.0001*	70704.323	69.5057	<.0001*
Cover* Cultivar	2	1909.206	2.3282	0.1398	1337.632	0.6575	0.5359
R-square	0.88				R-square	0.86	
RMSE	20.25				RMSE	31.894	

Appendix 21: ANOVA table for kucl chloride quantification flushes I and II

Source of variation		Flush I			Flush II		
Source	DF	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F
Cover	2	119.23164	6.5224	0.0121*	342.73193	9.0554	0.0040*
Cultivar	1	380.97344	41.6811	<.0001*	191.21084	10.1040	0.0079*
Cover* Cultivar	2	15.29190	0.8365	0.4570	2.68156	0.0708	0.9320
R-square	0.82				R-square	0.70	
RMSE	3.023				RMSE	4.35	

Appendix 22: Effect of cover, cultivar and calcium foliar feed on the post- harvest fresh weight of rose cut flower on the third day of experimental set up flushes I and II

		Flush I			Flush II		
Source of variation	DF	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F
Cover	2	13.803042	9.6532	0.0002*	13.435909	9.9894	0.0002*
Cultivar	1	21.618801	30.2383	<.0001*	25.259804	37.5605	<.0001*
Calcium	4	29.648696	10.3675	<.0001*	30.183696	11.2205	<.0001*
Calcium*Cover	8	8.814624	1.5411	0.1624	8.250458	1.5335	0.1649
Cultivar *Calcium	4	3.222416	1.1268	0.3525	3.764184	1.3993	0.2451
Cover* Cultivar	2	3.348682	2.3419	0.1049	1.337909	0.9947	0.3758
Cover*Cultivar*Ca	8	2.428051	0.4245	0.9018	9.272969	1.7236	0.1114
R-square	0.66				R-square	0.69	
RMSE	0.8455				RMSE	0.8201	

Appendix 23: Effect of cover, cultivar and calcium foliar feed on the post- harvest fresh weight of rose cut flower on the sixth day of experimental set up flushes I and II

		Flush I			Flush II		
Source of variation	DF	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F
Cover	2	20.689576	11.8953	<.0001*	20.689576	11.9431	<.0001*
Cultivar	1	18.099218	20.8119	<.0001*	21.667840	25.0156	<.0001*
Calcium	4	40.209333	11.5590	<.0001*	39.415889	11.3764	<.0001*
Calcium*Cover	8	9.735480	1.3993	0.2155	5.778924	0.8340	0.5764
Cultivar *Calcium	4	5.892804	1.6940	0.1632	4.538982	1.3101	0.2766
Cover* Cultivar	2	3.806229	2.1884	0.1210	1.917207	1.1067	0.3373
Cover*Cultivar*Ca	8	4.400116	0.6325	0.7473	9.033204	1.3036	0.2592
R-Square	RMSE				R-Square	RMSE	
0.66	0.93				0.66	0.93	

Appendix 24: Effect of cover, cultivar and calcium foliar feed on the post- harvest fresh weight of rose cut flower on the nineth day of experimental set up flushes I and II

		Flush I			Flush II		
Source of variation	DF	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F
Cover	2	15.875047	10.0739	0.0002*	16.205602	9.8230	0.0002*
Cultivar	1	25.749551	32.6800	<.0001*	29.377960	35.6148	<.0001*
Calcium	4	37.853649	12.0105	<.0001*	31.012551	9.3991	<.0001*
Calcium*Cover	8	8.067364	1.2798	0.2712	6.583909	0.9977	0.4473
Cultivar *Calcium	4	5.223760	1.6574	0.1718	3.400951	1.0307	0.3989
Cover* Cultivar	2	5.745429	3.6459	0.0320*	3.641127	2.2071	0.1189
Cover*Cultivar*Ca	8	4.521960	0.7174	0.6753	10.449429	1.5835	0.1489
R-Square	RMSE				R-Square	RMSE	
0.69	0.88				0.67	0.91	

Appendix 26: Effect of cover, cultivar and calcium foliar feed on the post- harvest fresh weight of rose cut flower on the twelfth day of experimental set up flushes I and II

		Flush I			Flush II		
Source of variation	DF	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F
Cover	2	13.487582	9.5379	0.0003*	13.487582	8.8358	0.0004*
Cultivar	1	33.941921	48.0051	<.0001*	37.674810	49.3621	<.0001*
Calcium	4	30.438878	10.7626	<.0001*	23.909489	7.8316	<.0001*
Calcium*Cover	8	6.530096	1.1545	0.3415	4.114418	0.6738	0.7124
Cultivar *Calcium	4	4.586740	1.6218	0.1805	3.062529	1.0031	0.4131
Cover* Cultivar	2	4.660649	3.2958	0.0438*	2.950427	1.9328	0.1536
Cover*Cultivar*Ca	8	5.529540	0.9776	0.4623	10.605084	1.7369	0.1083
R-Square	RMSE				R-Square	RMSE	
0.70	0.84				0.68	0.87	

Appendix 27: ANOVA table for effect of poly film cover, cultivars and calcium foliar feed on bent necking occurrence during post- harvest flushes I and II

	Flush I				Flush II		
Source of variation	DF	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F
Cover	2	5.000000	8.0357	0.0008*	5.755556	8.3548	0.0006*
Cultivar	1	1.344444	4.3214	0.0419*	1.344444	3.9032	0.0528
Calcium	4	26.955556	21.6607	<.0001*	30.777778	22.3387	<.0001*
Calcium*Cover	2	0.155556	0.2500	0.7796	0.155556	0.2258	0.7985
Cultivar *Calcium	4	1.488889	1.1964	0.3217	1.711111	1.2419	0.3029
Cover* Cultivar	8	10.777778	4.3304	0.0004*	13.022222	4.7258	0.0002*
Cover*Cultivar*Ca	8	2.511111	1.0089	0.4391	2.622222	0.9516	0.4820
R-square	0.72				R-square	0.73	
RMSE	0.5578				RMSE	0.585	
P	<0.0001				P	<0.0001	

Appendix 28: ANOVA table for effect of cultivars on bloom size during post- harvest

		Flush I day 3			Flush II Day 12		
Source of variation	DF	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F
Cover	2	294.89355	3.4921	0.0368*	294.89355	3.4921	0.0368*
Cultivar	1	498.05954	11.7959	0.0011*	498.05954	11.7959	0.0011*
Calcium	4	289.79298	1.7158	0.1583	289.79298	1.7158	0.1583
Calcium*Cover	2	65.45433	0.7751	0.4652	65.45433	0.7751	0.4652
Cultivar *Calcium	4	102.16644	0.6049	0.6606	102.16644	0.6049	0.6606
Cover* Cultivar	8	421.83500	1.2488	0.2874	421.83500	1.2488	0.2874
Cover*Cultivar*Ca	8	389.43719	1.1529	0.3425	389.43719	1.1529	0.3425

Appendix 29: ANOVA table for effect of cultivars on leaf defoliation during post- harvest

	Flush II-day 9				Flush II Day 12		
Source of variation	DF	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F
Cover	2	0.8666667	0.7647	0.4700	4.822222	2.7821	0.0699
Cultivar	1	5.8777778	10.3725	0.0021*	4.900000	5.6538	0.0206*
Calcium	4	2.1555556	0.9510	0.4411	1.155556	0.3333	0.8545
Calcium*Cover	2	2.0222222	1.7843	0.1767	3.266667	1.8846	0.1608
Cultivar *Calcium	4	3.6222222	1.5980	0.1866	2.488889	0.7179	0.5830
Cover* Cultivar	8	4.2444444	0.9363	0.4938	6.177778	0.8910	0.5297
Cover*Cultivar*Ca	8	3.3111111	0.7304	0.6642	10.844444	1.5641	0.1550
R-squared	0.39				R-squared	0.33	
RMSE	0.94				RMSE	0.96	
P	0.002				P	0.021	

Appendix 30: ANOVA table for effect of calcium foliar feed on leaf defoliation during post- harvest

		Flush I			Flush II		
Source of variation	DF	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F
Cover	2	0.8666667	1.5600	0.2185	3.355556	3.7750	0.0286*
Cultivar	1	1.1111111	4.0000	0.0500	0.711111	1.6000	0.2108
Calcium	4	6.8222222	6.1400	0.0003*	18.044444	10.1500	<.0001*
Calcium*Cover	2	0.0222222	0.0400	0.9608	0.422222	0.4750	0.6242
Cultivar *Calcium	4	1.8888889	1.7000	0.1619	2.400000	1.3500	0.2621
Cover* Cultivar	8	2.5777778	1.1600	0.3382	2.422222	0.6813	0.7061
Cover*Cultivar*Ca	8	1.6444444	0.7400	0.6559	1.800000	0.5063	0.8469
R-squared	0.47				R-squared	0.52	
RMSE	0.527				RMSE	0.667	
P	0.0003				P	<0.0001	

Appendix 31 Published paper: Effect of light and air temperature through Poly Film Covers on Anthocyanin Content in Rose Cut Flowers

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Effect of Light and Temperature through Poly Film Covers on Anthocyanin Content in Rose Cut Flowers

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ABSTRACT

Quality is the most important attribute in rose cut flowers for both export and domestic market. Quality in cut flowers may be defined by many attributes however, among the most important is colour. A group of pigments commonly known as anthocyanins determine colour in plants. Anthocyanins play a significant role by ameliorate the effect of high irradiance in plants under stressful environment. They also play a key role in delaying senescence hence enhancing the cut flower vase life. Despite the advantages anthocyanins are affected by the preharvest conditions mainly light and temperature interfering with their stability. An experiment was set up to investigate the effect of light and temperature through selected coloured poly film covers on rose petal

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anthocyanin content. The greenhouse structure was covered by poly films of different colours that were compartmentalized i.e. UV-A clear, IR504 with yellow tint and UV-A 205/N with green tint replicated three times. Two rose cultivars Red calypso and Furiosa were established and maintained, upon maturity the flower heads were plucked and oven dried at 60℃ to constant weight. 5 g of the crushed petals was used in anthocyanin extraction. The anthocyanins were extracted and quantified in comparison with commercial standards using HPLC machine. The data obtained from the chromatogram as peak areas was subjected to analysis of variance (ANOVA) using SAS statistical package (SAS Inst., Inc., Cary, NC) at *P* = .05. Where there were treatment differences, mean separation was done using Tukey's procedure. Poly films significantly affected the quantity and quality of anthocyanin accumulation in rose petals. Cyanidin 3-0-glucoside was the most prevalent anthocyanin across all poly film covers and it was noted to be high under the UV-A 205/N (110.95±8.26 µg ‐ 5 g–1 DW) and IR504 (109.69±8.26 µg ‐ 5 g–1 DW) compared to UV-A clear (84.56± 8.26 µg ‐ 5 g–1 DW). The quantity of anthocyanins was low under the UV-A clear poly film that was characterized by high light transmission and day temperature. Combination of high irradiance and temperature affect the quality and quantity of anthocyanin in rose cut flowers.

Keywords: Rose cultivars; temperature; light; anthocyanin; poly film.

1. INTRODUCTION

Light affects productivity and quality of ornamental plants besides growth and development. Plants response to light is influenced by the fluctuating environmental conditions and more so light properties like duration, intensity and quality [1] Plant growth attributes such as height, leaf area and leaf length decrease in response to UV-B radiation [2]. Red and far red wave band influence the phytochrome pigment which initiates photomorphogenic changes in plants [3]. This wave band varies from one poly film to the other depending on colour and gauge. Previous studies have shown that absorption of the far-red light of the poly films increase with the concentration of the dye [4,5,2]. Intensity of light in the far-red region affects morphological plant responses [6] which may have positive or negative impact on the physiochemical processes of the plants. Depending on the quality of light transmitted plant quality may be jeopardized affecting the colour of the flowers.

Anthocyanin content in cut flowers is affected by pre- harvest conditions to which the plant is subjected. A wide range of colours is not only insured by substrates accumulation but also other factors such as co-pigments, vacuole pH and cell shape [7]. Environmental factors such as elevated temperature received during growth reduce anthocyanin content of flower petals [8]. In other studies, it has been argued that plant growth and development is determined by the genomic characteristics of the plant that affect fundamental components like the flower colour [9]. Depending on the changes that occur during biosynthesis different molecules are formed resulting to diverse types of anthocyanins.

Peonidin type of anthocyanins is biosynthesized from cyanidin, while petunidin and malvidin are biosynthesized from delphinidin type of anthocyanins [10]. Anthocyanin produced varies from one species to another depending on their genetic constitution. Anthocyanin stability and catabolism is quite dynamic and its concentration in plants is bound to vary from time to time [11]. Rose and carnation the major cut flowers for example are only able to produce anthocyanin based on pelargonidin and cyanidin. Cut flower consumers may prefer rose stem in which the leaves have deep green colour that blends well with other hues of the bloom. Therefore, it is important to study and maintain all factors that will enhance flower colour.

2. MATERIALS AND METHODS

2.1 Experimental Layout and Crop Establishment

The research was carried out under a split plot experiment laid down in a completely randomized block design. The main treatment involved poly film covers with different colours denoted as; G0 = UV-A clear (control), G1= IR 504 (green tint) and G2 = UV- A 205/N (Yellow tint) of similar gauge 200 microns. The greenhouse was divided into three sections of $44M^2$. Each section was covered with a different poly film cover as described above and replicated three times. Two rose cultivars; Red calypso and Furiosa were established and maintained till maturity for data collection.

2.2 Light Spectra: Poly Film Transmission Properties

The transmission and absorbance properties of the selected poly film covers were tested in the laboratory. Measurements were carried out on samples taken before the installation of the poly films on the greenhouse and subsequent years during the production period. A strip of the poly film was cut from the extra ends of greenhouse. The strip was carefully cut in a rectangular shape and locked in a jig before insertion in the cuvette holder to ensure an upright position is maintained perpendicular to the source of light. The poly films were then scanned at wavelengths (190-1000 nm) using UV- 1800 Shimadzu spectrophotometer. Care was taken to ensure that the light beam from the spectrophotometer entered through the outer surface of the poly film and left through the inner surface.

Relative humidity, temperature and photosynthetic active radiation (PAR) were monitored using watch dog mini data logger and weather station. The machine was plugged into the port on the watch dog while in the field for data collection under the ambient solar radiation. The machine was fixed firmly on a support, with the sensor head being at the level of the plant heads. Data was downloaded at the end of every flush using a specware soft ware. Although data was collected for the entire day, 0800 hr, 1200 hr and 1600 hr sampling times were used as a baseline for omparison of PAR among the treatments. Temperature and relative humidity were monitored both day and night and averaged over 7 day period.

2.3 Quantitative and Qualitative Anthocyanin Analysis

Five grams (5 g) of dry ground rose petals were weighed into 250 ml conical flasks and covered with aluminum foil paper. The ground petals were mixed with 50 ml methanol (MeOH) and formic acid at a ratio of (99:1v/v) and magnetically stirred at 900 rpm for 4 hours at room temperature. The resultant solution was filtered and evaporated to remove as much methanol as possible using a rotary evaporator (Buchi Rotavapour R-300, Switzerland) under reduced pressure at 35℃. The concentrated extract was dissolved in 10 ml distilled water and passed through a membrane filter 0.45 µM. Anthocyanin purification was then done by passing the extracts through reverse phase (RP) C18 solid phase extraction (SUPELCO, SPE) (Sigma–Aldrich, USA) catridge previously activated with 10% MeOH followed by 0.01% HCl v/v in distilled water. Anthocyanins get adsorbed onto the column while sugars, acids and other water-soluble compounds are washed out using 0.01% HCl in distilled water. Anthocyanins are recovered using acidified methanol (10% Formic acid v/v). The catridges were washed with ethyl acetate (Fischer Scientific, UK) to remove phenolic compounds other than anthocyanins. The purified extracts were stored at -10℃ until further analysis.

2.4 Quantification of Anthocyanins

The anthocyanins in the rose petals were characterised by HPLC using a Shimadzu LC 20 AT HPLC system fitted with a SIL 20A auto sampler and a SPD-20 UV–Visible detector with a class LC10 chromatography work station. UV detection was set at 520 nm using a Gemini C18 ODS (4.0 mm _ 4.6 mm i.d.) (Phenomenex Inc. Torrance CA, USA) fitted with a Gemini C6 ODS column (4.0 mm _ 3.0 mm i.d.) (PhenomenexInc. Torrance CA, USA) guard. The column temperature was at 35 ± 0.5℃. The eluents were mobile phase A water/acetonitrile/formic acid-87/3/10 v/v/v) and mobile phase B (100% HPLC grade Acetonitrile). The chromatographic conditions were: 3% B in A at the time of injection, at 45 min; 25% B in A, at 46 min; 30% B in A and at 47 min; 3% B in A (initial conditions). The flow rate of the mobile phase was1 ml/min and injection volume of 20 µl. The anthocyanin cyanidin chloride and pelargonidin chloride were used as standards for the identification and quantification of anthocyanin fractions in dried rose flower petals. Other types of anthocyanins (Cyanidin 3-0 glucoside, peonidin, delphinidin and Cyanidin 3-0 galactoside) were quantified using the calibrated standard curves in tea research Institute of Kenya Laboratories.

2.5 Data Collection and Analysis

Data collection involved quantification of the peak areas from the chromatograms. The collected data was subjected to analysis of variance (ANOVA) using SAS statistical package (SAS Inst., Inc., Cary, NC) at $P \leq 5\%$. Where there were treatment differences, mean separation was done using Tukey's procedure.

3. RESULTS AND DISCUSSION

3.1 Effect of Poly Film Covers on Greenhouse Microclimate and PAR

3.1.1 Light transmission through selected poly films

The poly films varied in their spectral properties including PAR transmission. It was observed that the quantity of PAR transmitted was dependent on sampling time and prevailing weather conditions. Average PAR values ranged from; 222 µmol m^{-2} s^{-1} at 0800 hrs, to 1613 µmol m^{-2} s^{-1} at 1200 hrs and 115 µmol m^{-2} s^{-1} at 1600 hrs for UV-A clear poly film cover. The intensity of transmission was significantly higher for UV-A clear poly film compared to IR-504 that transmitted, 245 µmol m^{-2} s-1, 1063 µmol m^{-2} s^{-1} and 40 µmol m^{-2} s^{-1} at 0800 hrs, 1200 hrs and 1600 hrs sampling time respectively. Comparison of the PAR values at 0800 hrs for UV-A clear and IR 504 poly film indicated low PAR values for UV-A clear. This could have been caused by the water condensate that formed on the UV-A clear poly film during the night hours thus hindering maximum transmission in the early morning hours. The quantity of PAR transmitted was noted to fluctuate from time to time in line with the prevailing natural environmental conditions (Figs. 1 and 2).

Transmission properties of the poly films were consistent in both flushes I and II. The least PAR but high temperature was recorded under UV-A 205 cover. In previous work, Copinet et al. [12]

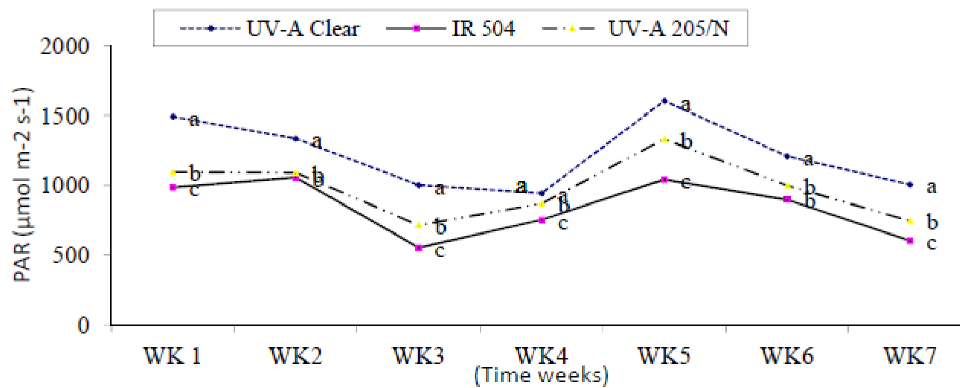

Fig. 1. Effect of selected poly film on transmission of photosynthetic active radiation (flush 1). Values presented are weekly averages (n=3) of PAR sampled at mid-day

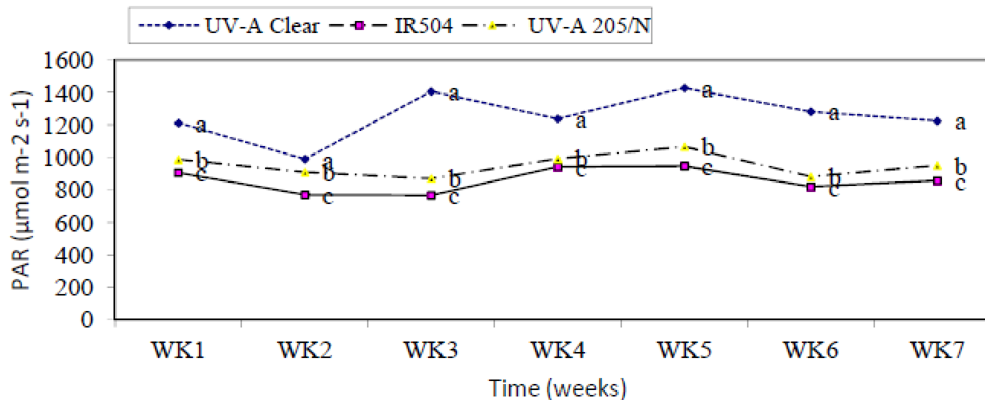

Fig. 2. Effect of selected poly film on photosynthetic active radiation during flush II The values presented are weekly averages (n=3) of PAR sampled at mid-day

studied the influence of temperature (30℃, 45℃ and 60℃) and relative humidity (RH) (30%, 50% and 100%) on the degradation of poly-lactic acid and established that degradation increased with increase in temperature and relative humidity. Copinet et al. [12] further examined effect of UV- at 315 nm wavelength in the same study and reported that UV- accelerated degradation process. This could be the reason as to why the UV-A clear poly film under high temperature and low relative humidity exhibited higher transmission. Part of the findings of this study state that increased humidity levels increase photo degradation of ester-based polymers through increased water absorption and hydrolytic reactions initiated by UV radiation, thus photo degradation is enhanced by presence of relative humidity. UV-A clear poly film allowed higher light transmission and higher relative humidity during the night hours which enhanced the degradation process.

3.1.2 Poly film covers, greenhouse temperature and relative humidity

Relative humidity was higher under UV-A clear poly film at 0400 hrs. The lowest values were recorded under the same cover during at 1200 hrs. Relative humidity was noted to be inversely proportional to the temperature with the lowest values being recorded at 1200 hrs. Relative humidity of 69.2%, 76.1% and 71% with temperatures of 31.2℃, 31.1℃ and 30.9℃ was recorded under UV-A 205/N, UV-A clear and IR504 poly films respectively at 1200 hrs. Higher values of relative humidity 93.1%, 96.5% and 93.3% and low temperature of 8.5℃, 8.9℃ and 9.2℃ was recorded at 0400 hrs under the UV-A 205/N, UV-A clear and IR504 respectively (Figs. 3 and 4).

The IR treated poly film showed tendency of forming condensate during night hours. This could be attributed to the fact that the IR treated poly film was slightly warmer during the night hours as opposed to the cool air outside the greenhouse. Temperature fluctuation between 1600hrs and 0400 hrs was similar across all covers. The highest difference in temperature was noted at 1200 hrs where by the UV-A clear recorded 2.5℃ and 1.3℃ higher than UV-A 205/N and IR504 respectively. The poly film covers constituted different additives which had potential impact on microclimate. Conventionally, under conditions of elevated temperatures farmers have adopted whitening of the greenhouse roof to reflect excess light and reduce temperature within the structure. Influence of whitening of greenhouse microclimate was studied and observed to reduce the transmission coefficient of solar radiation from 0.62 to 0.31 as a result changing air temperature drastically [13]. Apparently, the UV-A clear poly film that was permissive to high transmission accumulated higher temperature during the day although it could not be retained at night. Holcman and Sentelhas, [14] evaluated microclimate under different shading screens among them being red, blue and black. The reflective shade screen used as the control

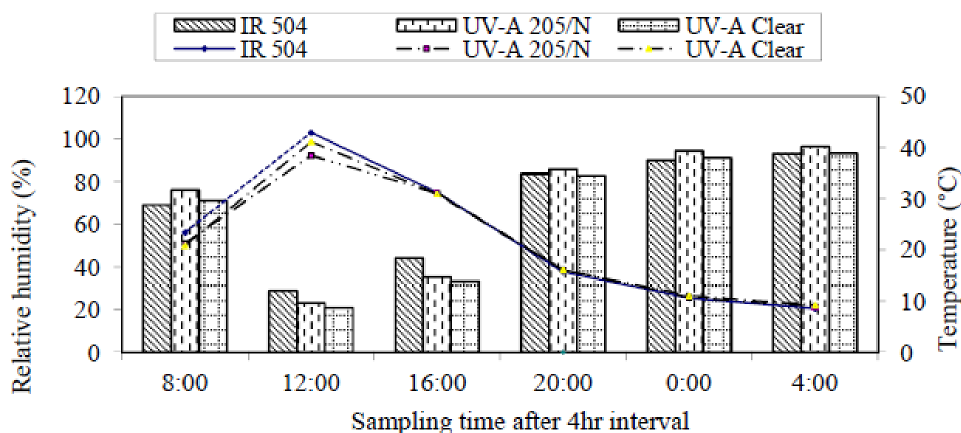

Fig. 3. Effect of selected poly film covers on percent relative humidity and air temperature (flush 1). Bar graphs and line graphs represent relative humidity and temperature respectively. Values presented are means over a growth period of 42 days at different sampling time; 0800 hrs, 1200 hr and 1600 hr

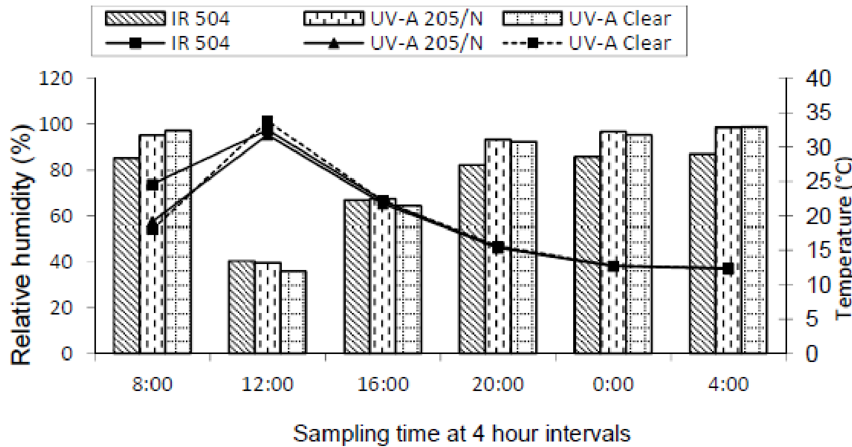

Fig. 4. Effect of selected poly film covers on percent relative humidity and air temperature (Flush 1I). Bar graphs and line graphs represent relative humidity and temperature respectively Values presented are means over a growth period of 42 days at different sampling time; 0800hrs, 1200hrs and 1600hrs

transmitted 56.3% light followed by the red screen which transmitted 27%, while black recorded the least transmission of 10.4%. Temperature also varied drastically, with the blue screen recording 1.3℃ higher temperature than the external conditions. The colour of the greenhouse cover therefore, effects greenhouse microclimate and the extent vary among different colours as observed in this study.

The structures in the current study had higher temperature than normal outdoor temperature, which could be attributed to the small size of the structure. The bigger the greenhouse the better the air flow thus size greatly affect air temperature within a structure. According AlHelal and Abdel-Ghany, [15] they observed that greenhouse air temperature is affected by more than one single factor including; solar radiation, level of greenhouse venting and the size of the greenhouse. Structural units used in his experiment were relatively small precisely 0.0048Ha in size and about two-thirds the height of normal greenhouse (6 M) as a result high temperature was recorded consistently. Holcman and Sentelhas, [14] pointed out that besides climate, greenhouse design, size and height affects the internal microclimate. Greater height of the greenhouse structure enhanced air circulation minimizing heat buildup within the greenhouse. Conventionally, reduction in air temperature is a key objective of greenhouse use in the tropics and as observed here it may be limited by height of the structure. The smaller the greenhouse size the higher the temperature and the bigger the greenhouse in width and height the lower the temperature.

Several modifications have been adopted in the plastic industry, including use of shade screens, cladding of the poly films with different colours and treatment with UV- and IR additives among others. According to AlHelal and Abdel-Ghany, [15] colourful screens have the capacity to alter the spectral properties which in turn influence the microclimate by lowering temperature. In the current study, it was observed that the UV-A clear poly film transmitted more light compared to the coloured ones. The findings in this study concurs with the work done by Shahak, [16] who reported that the darker the colour of the poly film the less the amount of light transmitted. This explains why the control poly film (UV-A clear) recorded the highest light transmission and the highest temperature at 1200 hrs compared to IR504 and UV-A 205/N.

The influence of the poly films on microclimate was consistent in both flushes I and II although the levels varied with the prevailing weather conditions at the time of the experiment. The poly films had significant effect on relative humidity and corresponding temperature within the greenhouses structures which also varied with the specific sampling time. Greenhouse relative humidity directly influences the plant water relations and indirectly affect plant growth and development. High temperature, as was

the case under UV-A clear cover translated to low relative humidity at 1200 hrs which demonstrates that relative humidity is highly affected by temperature. Although the experiment was conducted under same natural conditions, the results showed variations in temperature in addition to relative humidity. Mean temperature values of 41℃, 35.2℃ and 32.8℃ were recorded at 1200 hrs during the hottest part of the day and 12.3℃, 13.1℃ and 12.1℃ at 0400 hrs during the coolest part of the day under UV-A clear, IR-504 and UV-A205/N covers respectively. It is evident that during the day UV-A clear recorded the highest temperature compared to UV-A 205/N, while the mean night temperatures between the two poly films were not significantly different. However, it is also important to note that, while IR504 poly film recorded the lowest mean day temperature than the control it was significantly higher than the UV-A 205/N. IR504 had the highest mean night temperature (13.1℃) being 0.8℃ and 1.0℃ higher than UV-A clear and UV-A 205/N respectively. It is argued that poly film colour is an integral part of greenhouse microclimate. For example, Kitta et al. [1] maintains that microclimate under different shading screens is dependent on the colour of the greenhouse cover material used. It is apparent therefore, that colour of the covering material affects the microclimate under the growth structures.

3.1.3 Poly films and flower petal anthocyanin content

Poly films significantly affected the quantity and quality of anthocyanin accumulation in rose petals. Cyanidin 3-0-glucoside was the most prevalent anthocyanin across all poly film covers and it was noted to be high under the UV-A 205/N (110.95±8.26) and IR504 (109.69±8.26) compared to UV-A clear (84.56± 8.26) that exhibited high irradiance. The quantity of cyanidin 3-0-glactoside and cyanidin chloride were statistically similar under the IR504 and UV-A205/N poly films compared to UV-A clear. Interestingly pelargonidin chloride was relatively stable across all the poly film treatments. Peonidin chloride was relative low under the UV-A clear poly film compared to IR504 and UV-A205/N (Tables 1 and 2). The anthocyanin quantities were higher in the cultivar Red calypso compared to Furiosa.

Cyanidin 3-0- glucoside and cyanidin 3-0-galactoside were high in Red calypso and lower in Furiosa implying that species of same plant accumulate different quantities of similar anthocyanins. Apparently, the quantities of delphinidin chloride and pelargonidin chloride were not significantly different among the cultivars used in the study. The major anthocyanins were cyanidin 3-0-glucoside and cyanidin 3-0-galactoside while the minor anthocyanins were the chlorides of peonidin and pelargonidin. Previous works have established that the amount of coloured material in leaves or petals depend on cultivars, plant habitat and the climatic conditions encountered during the growing season [17].

Anthocyanin accumulation was substantially high in flush II compared to flush I which probably reflects influence of changes in the environmental conditions. Flush I was characterized by high irradiance consequently low anthocyanin content was observed in rose petals whereas low irradiance was recorded in flush II where petals were noted to accumulate more anthocyanins. Further differences were observed in specific anthocyanins, whereby peonidin, cyanidin and pelargonidin based

Table 1. Effect of cover and cultivar on rose petal anthocyanin content flush I

Flush 1	Anthocyanins [(mean ± SE) (µg - 5 g–1 DW)]				
Poly film	**Cya 3-0-glu**	**Cya-3 -0-gla**	**Cya-cl**	**Pela-cl**	**Peo-cl**
UV-A clear	84.56 ±8.26b[1]	14.88±1.23b	2.99±0.23b	5.49±0.51b	0.97±0.17b
IR504	109.69±8.26a	20.58±1.23a	4.50±0.23a	7.89±0.51a	1.90±0.17a
UV-A205/N	110.95±8.26a	20.07±1.23ab	3.98±0.23a	7.72±0.51a	2.09±0.17a
Red calypso	142.93± 6.74a	23.11±1.0a	4.40±0.18a	8.92±0.41a	2.18±0.14a
Furiosa	60.54±6.74b	13.91±1.0b	3.26±0.18b	5.14±0.41b	1.13±0.14b

[1]Means followed by different letter(s) along the column for or poly film cover and cultivars are significantly different at 5% level of significance according to Tukeys' HSD procedure.

Where; letters 'a' and 'b' denote mean separation. While; Cya 3-0-glu, Cya 3-0-gla, Cya-cl, Pela-cl and Peo-cl are Cyanidin-3-0-glucoside, Cyanidin 3-0-galactoside, Cyanidin chloride, Pelargonadin chloride and Peonidin chloride respectively

Table 2. Effect of cover and cultivar on rose petal anthocyanin content flush II

Flush II	Anthocyanins	[(mean ± SE) (µg - 5g–1 DW)]			
Poly film cover	Cya 3-0-glu	Cya-3 -0-gla	Cya-cl	Pela-cl	peo-cl
UV-A clear	110.16 ±13.02b[1]	19.84±1.77b	4.32±0.43b	6.76±1.40b	5.85±0.72a
IR504	140.07±13.02a	28.44±1.77a	7.60±0.43a	9.82±1.4a	4.39±0.72b
UV-A205/N	139.54±13.02a	29.65±1.77a	7.58±0.43a	9.59±1.4a	4.84±0.72b
Red calypso	192.60± 10.63a	29.23±1.45a	7.56±0.35a	9.01±1.14a	4.11±0.59a
Furiosa	67.25± 10.63b	22.72±1.45b	5.45±0.35b	8.46±1.14a	4.61±0.59a

[1]Means followed by different letter(s) along the column for or poly film cover and cultivars are significantly different at 5% level of significance according to Tukeys' HSD procedure.

Where; letters 'a' and 'b' denote mean separation. While; Cya 3-0-glu, Cya 3-0-gla, Cya-cl, Pela-cl and Peo-cl are Cyanidin-3-0-glucoside, Cyanidin 3-0-galactoside, Cyanidin chloride, Pelargonadin chloride and Peonidin chloride respectively

anthocyanin increased with decrease in temperature. 30.3% more cyanidin-3-0-glucoside was recorded in flush II compared to flush I while cyanidin-3-0-galactoside increased by 33.3% in flush II compared to flush I. This observation supports the finding of Laleh et al. [18] who studied the effect of temperature on anthocyanin among other factors and established a positive relationship between temperature and anthocyanin. What is not clear from this study is the effect of other microclimatic factors like relative humidity on the anthocyanin since it was difficult to quantify their effect singly under field conditions.

A wide range of flavonoids and phenolic compounds are produced in plants in response to UV- radiation related stresses. Plants produce and accumulate these secondary products to protect themselves against UV light damage [19]. Other studies have shown that where both wavelengths UV-A and UV-B are excluded anthocyanin reduced drastically implying that both UV-A and UV-B are essential in anthocyanin photo-induction. This observation could also explain the reason why there were less anthocyanins under the UV-A clear poly film cover that had high irradiance with substantial amount of UV-A and UV-B radiation transmitted.

Temperature may impact negatively or positively on anthocyanin production. Dela et al. [8], demonstrated that anthocyanins are induced by low temperatures and reduced by high temperatures. Several authors have observed and reported that the expression of genes for anthocyanin biosynthesis pathway increased at low temperature [20,21,22]. Furthermore, Ban et al. [23] studied the effect of temperature on apples and established that high temperature caused a decline in accumulation of cyanidin the major anthocyanin in Rosaceae family causing fluctuations in skin colour. When the same apples were subjected to cooler temperatures there was renewed synthesis and improvement in colour [22]. Low temperature cause high levels of ABA that act as a signal to influence the expression of the main genes involved in the anthocyanin biosynthesis pathway, resulting in an increase in anthocyanin levels [24]. This explains why we had more anthocyanin accumulation in flush II compared to flush I. Anthocyanins are highly influenced by temperature and have been observed to be stable at 0℃ whether in light or darkness, implying that temperature has greater influence on anthocyanin stability both in the absence and presence of light. The combination of light and temperature therefore could have accelerated the anthocyanin degradation process. The effect of temperature could further be attributed to the hydroxylation of 3- glycoside structure which confers protection to the unstable anthocyanin structure. low temperatures on the other hand increase both anthocyanin content and the expression of genes of the anthocyanin biosynthetic pathway while high temperature affect the activity of the phenylpropanoid pathway responsible for anthocyanin biosynthesis [20,22] resulting in less accumulation.

4. CONCLUSION

Anthocyanin quality and quantity is highly dependent on the environmental conditions. Low temperatures enhanced anthocyanin accumulation in rose flower petals. Clear poly films therefore, may not be the best in areas with high irradiance as they exhibit high temperature jeopardizing quality. Growers should therefore adopt appropriate poly films in relation to the

environmental conditions to obtain cut flowers with best quality for export.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Appendix 32 Published paper: Pre- harvest Calcium Treatment under selected Poly Films Improves Leaf Chlorophyll Content in Rose Cut Flowers

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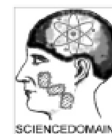

Pre Harvest Calcium Treatment under Selected Poly Films Improves Leaf Chlorophyll Content in Rose Cut Flower

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Authors' contributions

This work was carried out in collaboration between all authors. Author GOA designed the study, wrote the protocol and wrote the first draft of the manuscript. Author JNA managed the literature searches, analyses of the study performed the spectroscopy analysis and author GL managed the experimental process and identified the species of plant. All authors read and approved the final manuscript.

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ABSTRACT

Quality is a key attribute in marketing of cut flowers. A number of factors may determine quality in cut flowers, among them being the pre-harvest conditions under which the plant was grown. This experiment was designed to investigate the effect of light transmission through selected poly films, rose cultivar and calcium foliar feed on leaf chlorophyll content. The study site was at an elevation of 2238 m above sea level with average maximum and minimum temperature ranges from 19℃ to 22℃ and 5℃ to 8℃ respectively. The area receives a t otal annual rainfall ranging from 1200 to 1400 mm. The experimental design was split split plot laid down in a Randomized Complete Block Design (RCBD) with poly films forming the main plot treatments. Data collection involved use of Watch dog data logger mini weather station to determine Photosynthetically active radiation among

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other spectrum properties. Light transmission was evaluated using UV-1800 Shimadzu spectrophotometer. It was observed that leaf chlorophyll content increased with increase in calcium concentration in the foliar feed. Interactive effect was observed between the calcium foliar feeds and the poly film covers on stem firmness. The obtained results were consistent in both trials however the stem firmness was varied from one flush to the other. Effect of temperature caused by varying light intensity on chlorophyll content is also discussed. The results obtained show impact of environmental and nutritional factors on rose cut flower quality.

Keywords: Calcium; temperature; rose cultivar; chlorophyll content.

1. INTRODUCTION

Calcium plays a crucial role as a regulator of growth and development in plants [1,2] however, its movement through the phloem vessels is very limited [3]. It is known to strengthen the plant cell wall [4,5] and improve plant structural strength where it accumulates as calcium pectate and binds the cells together. In addition the mechanical strength of the stems is highly correlated with the content of the secondary cell wall components such as cellulose, hemi-cellulose and lignin [6]. The mode of application of calcium as a fertilizer determines its availability in the plants, together with the prevailing weather conditions.

Generally, the soil may have sufficient quantities of calcium yet the plant shows deficiency mainly in young tissues with new growth [3] a special case implicated to poor translocation of this nutrient. Different ways have been tested to mitigate the problem and has been shown that application of foliar fertilizer significantly affect plant growth and quality [7,8]. Fertilizer utilization is 10 times efficient when applied as a foliar feed compared to similar a mounts applied directly to the soil [9,10]. Foliar application increases availability of nutrients to the plant and stimulates biological activities resulting to positive changes in plant growth and development [11]. Plant nutrient availability is influenced by the inherent characteristics for example different plant cultivars have different demand for calcium in their developmental processes [12] making it difficult to establish general optimal levels of calcium in plants. Furthermore, the environmental and edaphic factors play a crucial role, for example deviation of soil temperature from normal and soil pH from the neutral, affects availability of nutrients [13].

Rose cut flower is mainly a greenhouse enterprise and the use of coloured poly films has very profound influence on spectrum properties, which affect light transmission in turn influencing plant growth and development. Light transmission through such poly films depends on the concentration of the cladding and other additives during the treatment process. Concentration of the dye for example increases far red light absorption of the poly film [14,15]. These differences in light transmission bring about variations in greenhouse microclimate mainly temperature and relative humidity which impacts on nutrient absorption and general plant morphogenesis.

Leaf chlorophyll content is influenced by a number of factors mainly temperature, light and cultivar which is inherent. The effect of these environmental factors on pigment levels may occur either singly or synergistically [16]. Anthocyanin concentration increases under low and decreases at elevated temperatures, implying that temperature affects plant pigmentation [17]. The influence of air temperature on carotenoid concentration in spinach was studied and reported to decrease with an increase in air temperature [18]. Low temperature (20℃) resulted in significantly higher anthocyanins than higher temperature of 30℃ [19]. Chlorophyll one of the main plant anthocyanins is equally affected by temperature. Therefore, the objective of this study was to evaluate the effect of selected poly films and pre harvest calcium application on leaf chlorophyll content of rose flower cultivars.

2. MATERIALS AND METHODS

2.1 Planting Material, Experimental Design and Treatment Application

Top grafted plants of two rose cultivars were purchased from the commercial propagator (Stokman Rozen limited) in Naivasha. The research was carried out under a split- split plot experiment laid down in a completely randomized block design. The main treatment involved poly film covers with different colours denoted as; G_0 = UV-A clear (control), G_1= IR

504 (green tint) and G_2 = UV- A 205/N (Yellow tint) of similar gauge 200 microns. The greenhouse was divided into three sections of $44M^2$. Each section was covered with a different poly film cover as described above and replicated three times. The sub-plot treatment included two rose cultivars; Red calypso and Furiosa. Calcium was applied to the sub-sub plots and the application rate was based on the manufacturer's specification (2.5 ml/L) as shown: the blank T_0 = Distilled water, T_1 = CalMax® 1.25 ml/L (half the recommended rate),, T_2 = CalMax® 2.5 ml/L (recommended rate), T_3 = CalMax® at the rate of 3.75 ml/L (50% higher) and T_4= CalMax® at the rate of 5.00 ml/L (double the recommended rate).

Soil treatment was done following land preparation using Sodium methylaminomethanedithioate at the rate of 0.12 ml/m^2 (The application rates as per the product specification) was applied through drip lines and plots were left undisturbed for 21 days. Plots were aerated after three weeks and germination test carried out to a certain depletion of chemical residues before planting. Planting was done 14 days later after achieving 95% germination in all main plot treatments. The plants were grown in double rows spaced at 30 cm x 20 cm to accommodate 10 plants per square meter. Management activities involved hosing, fertigation, bending, weeding, de-suckering and general plant cleaning.

2.2 Data Collection and Analysis

The transmission and absorbance properties of the selected poly film covers were evaluated in the laboratory. Measurements were carried out on samples taken before and after the installation of the poly films on the greenhouse structure. A strip of the poly film was cut from the extra ends of greenhouse. The strip was carefully cut in a rectangular shape and locked in a jig before insertion in the cuvette holder to ensure an upright position perpendicular to the source of light. Care was taken to ensure that the light beam from the spectrophotometer entered through the outer surface of the poly film and left through the inner surface. Transmission of active radiation was evaluated using UV-1800 Shimadzu spectrophotometer.

Non destructive measurement of foliar chlorophyll was done using Chlorophyll content meter (CCM- 200 plus; Opti-Sciences, Tyngsboro, MA). Where by 3 readings were taken from the 3rd, 4th and 5th developed leaves. Mean value were determined and recorded. The collected data was subjected to analysis of variance (ANOVA) using JMP and SAS statistical package (SAS Inst., Inc., Cary, NC) at $P \leq 5\%$. Where there were treatment differences, mean separation was done using Tukey's procedure.

Split-split plot mathematical model was fitted for analysis as shown below:

$$X_{ijk} = \mu \ldots + B_i + G_j + (BG)_{ij} + C_k + GC_{jk} + (GC)_{jk} + T_l + GT_{jl} + CT_{kl} + GCT_{jkl} + (GCT)_{jkl} + e_{ijkl}$$

Where;

Xijk	= Response of rose plants
μ	= the overall experiment mean
G_i	= the main plot treatment effect
B_j	= the block effect
$(BG)_{ij}$	= the main plot error (error a)
C_k	= the subplot treatment effect
GC_{ik}	= Interaction effect of the jth poly film type and the kth rose cultivar effect
$(GC)_{jk}$	= the sub-plot error (error b)
T_l	= calcium foliar feed treatment effect
$(GT)_{jl}$	= the treatment interaction effect of poly flim and calcium foliar feed
(CT)kl	= the treatment interaction effect of varieties and calcium foliar feed
(GCT)ikl	= the treatment interaction effect of jth poly film, the kth rose varieties and l^{th} calcium foliar feed
eijk	= the sub subplot error (error c)
i, k, l	= a particular treatment
j	= a particular block

3. RESULTS AND DISCUSSION

3.1 Micro Climate under the Greenhouse Poly Film Covers

Poly film type had significant effect on the transmission of photosynthetically active radiation (PAR). There was significantly high transmission under the UV-A clear poly film compared to UV-A 205/N and IR504. Generally, light transmission through the different poly films increased with increase in wavelength. At low wavelength of 400 nm, the transmission under UV-A clear poly film was 35% compared to 54.8% recorded at 680 nm. Similarly for poly films UV-A 205/N and IR 504 7.9% and 8.1% light transmission was recorded at 400 nm, while at 680 nm the percent light transmission was 33% and 31% respectively (Fig. 1). Mean

temperature values of 41℃, 35.2℃ and 32.8℃ were recorded at 1200 hrs of the day and 12.3℃, 13.1℃ and 12.1℃ at 1600 hrs under UV-A clear, IR-504 and UV-A205/N covers. During the day UV-A clear recorded the highest temperature compared to UV-A 205/N, while the mean night temperatures between the two poly films were not significantly different. Although, IR504 poly film recorded the lowest mean day temperature it had the highest mean night temperature (13.1℃) of 0.8℃ and 1.0℃ greater than UV-A clear and UV-A 205/N respectively.

3.2 Leaf Chlorophyll Content

Generally, high temperature under UV-A clear as characterized by high transmittance impacted negatively on the total leaf chlorophyll content (Fig. 2). Chlorophyll content was significantly high under IR504 poly film cover compared to UV-A 205/N and UV-A clear. High temperature has been reported in other studies to impact on plant growth and development. According to Hutin et al. [20] they observed that when the photosynthetic complexes absorb excess light, reactive oxygen species were generated in the chloroplasts causing damage in the systems of photosynthetic pigments. Comparison made on the effect of chlorophyll by temperature and nutrition in another study showed that chlorophyll content especially chlorophyll a is affected more by variations in season compared to the strength of the nutrient solution [21] implying that the effect of temperature on chlorophyll supersedes plant nutrition at any given time. It is possible that the plants developed mitigation strategies to protect the destruction of the photosynthetic apparatus under this high temperature. Several changes occur within the plant cell under high temperature conditions, among them being the closure of the stomata. Once the stomata close the photosynthetic activities are affected and assimilation of CO_2 is also affected hence reduction in the chlorophyll content among other physiological processes.

Chlorophyll content was also observed to vary with the prevailing weather condition, it was high in trial II compared to trial I. Generally, the overall mean day temperature was low in trial II compared to trial I, showing the relationship between temperature and leaf chlorophyll. A study done by Gilmore and Marilyn [22] to establish which type of chlorophyll is sensitive to which type of environmental conditions established that Chl-b was lower than Chl-a at low temperature indicating the sensitivity of Chl-b to environmental changes. The effect of greenhouse microclimate on Chl-a and Chl-b may vary in magnitude depending on the environmental factor that dominates. Clear observation noted here is that chlorophyll was more affected by high solar radiation as opposed to temperature alone. This observation is in agreement with Lopez et al. [23] who observed low chlorophyll content in *Amaranthus hypochondriacus* leaves under full sunlight. Chlorophyll reduction under full sunlight in this particular study was thought to be a mechanism to reduce photo-induced damage.

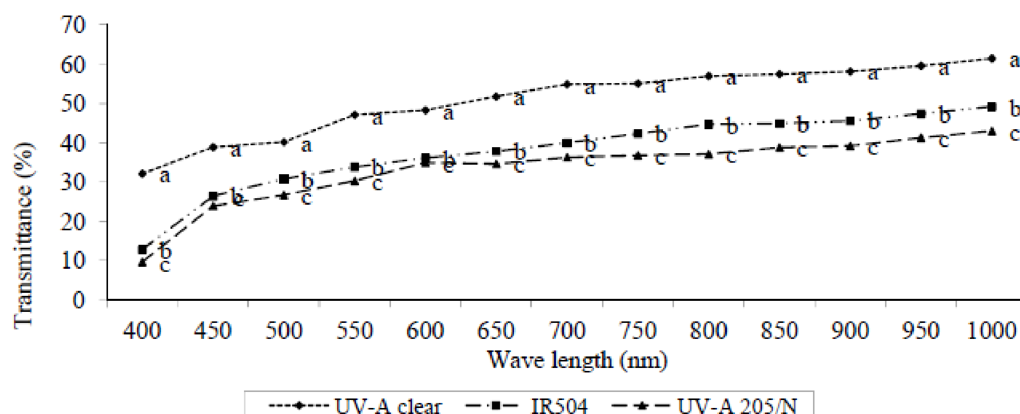

Fig. 1. Effect of selected poly films on light transmission in the PAR and infra red wavelength regions before installation

Where a, b and c denotes mean separation with a being significantly higher than b and c

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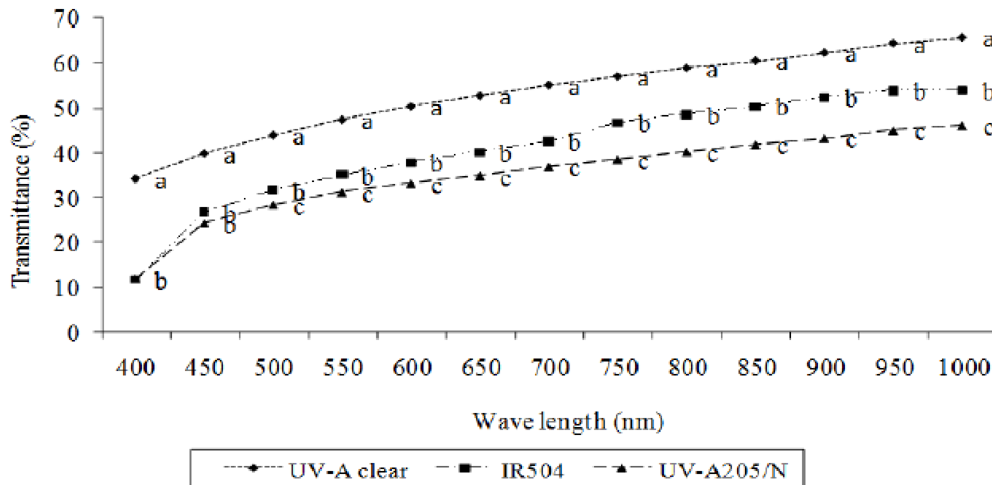

Fig. 2. Effect of selected poly films on light transmission in the PAR and infra red wavelength region after installation

Values presented are averages over 24 months period. Where a, b and c denotes mean separation with a being significantly higher than b and c

The amount of solar radiation absorbed by a leaf is basically a function of the foliar concentration of photosynthetic pigments [24] which may differ from one plant species to the other. Chlorophyll content determines the photosynthetic potential of the plant and can drastically affect crop yield. Different authors present varying arguments about the effect of temperature on leaf chlorophyll, with most findings describing that chlorophyll increases with increase in temperature as observed in tomatoes Gupta et al. [25] and grasses [22]. Leaf chlorophyll content in the current study was observed to be low under the control poly film cover that exhibited very high solar radiation and temperature. This could be attributed to the mechanism to survive the excess temperature by reducing the capabilities of leaf absorbance of solar radiation.

3.3 Calcium Foliar Feed and Leaf Chlorophyll Content

Chlorophyll content was significantly affected by the application of calcium foliar feed. Chlorophyll content under the experimental plots treated with 2.5 ml/L, 3.75 ml/L and 5 ml/L of calcium foliar feed was statistically high compared to 1.25 ml/L and the control (Fig. 3). The results of trial II were inconsistent to trial I, leaf chlorophyll content was high under T4 which was statistically similar to T3. In trial II unlike trial I calcium foliar feed level T4 was higher than T2, T1 and the control experiment (T0). Leaf chlorophyll content under T1 treatment was significantly higher than the T0 but lower than the preceding treatments with higher calcium concentration in both trials I and II.

Low and high calcium concentration have been reported to influence plant growth negatively [7]. In addition according to Gupta et al. [25] low and high calcium concentrations were observed to decrease plant biomass production and fruit yield of bitter gourd. According to this study of Gupta et al. [25] they established that calcium concentration influenced chlorophyll a and chlorophyll b separately. They also established that Chl a and Chl b were high (1.67 and 0.81 mg g-1 fresh weight) at 4.0 mM of calcium than 8.0 mM (1.19 and 0.497 mg g-1 fresh weight) respectively. This observation is in consonance with the results of the current study where higher calcium concentration denoted as T3 and T4 had no significant effect on chlorophyll concentration compared to T2. However, lower concentration of T1 and the control had significantly lower chlorophyll content (48.67 and 45.31 respectively). In another study to evaluate the effect of season verses nitrogen nutrition on pigment dynamics in tomato it was observed that chlorophylls were affected more by the season and less by the strength of the nutrient solution [21]. The role of calcium in cell

division and expansion in addition to its role in gaseous exchange through regulation of stomata could be implicated in this study. Consequently, where calcium concentration was low, leaf chlorophyll content was also observed to be low.

Combined treatment of red calypso and calcium foliar feed at an application rate of 3.75 ml/L had the highest leaf chlorophyll content. Low chlorophyll content (40.18f) was recorded under the cultivar Furiosa with the control experiment that had no calcium compared to Red calypso under similar treatment (Table 1). There was significant variation in the chlorophyll content indicating plant difference in response to growth conditions. The difference in chlorophyll content observed amongst the different cultivars is due to genetic variability in plants. This finding supports Daymond and Hardley, [26] who observed differences in chlorophyll content among different cacao clones. This observation could in part be attributed to the potential of different cultivars to acclimate to greenhouse growth factors at specific levels, with every cultivar having its own optimal level.

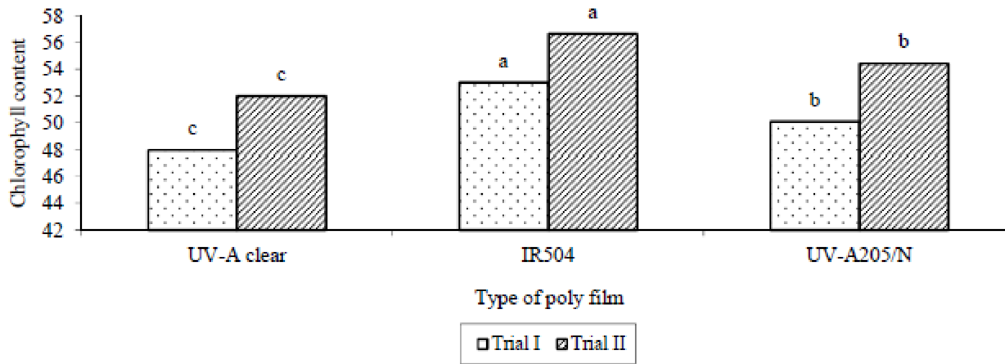

Fig. 3. Effect of greenhouse cover on the rose leaf chlorophyll content (trial I&II)
Where a, b and c denotes mean separation with a being significantly higher than b and c

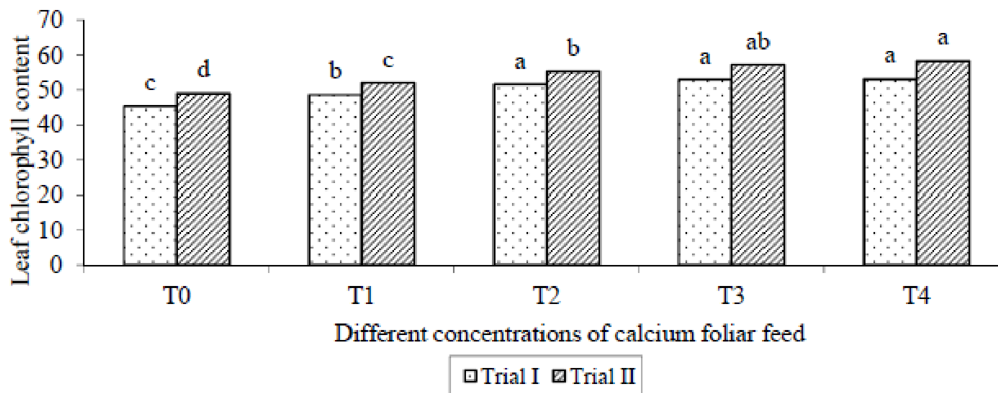

Fig. 4. Effect of calcium levels on leaf chlorophyll content (Trial 1 & 2)
Where T0 is a control experiment and T1, T2, T3 and T4 are calcium treatments at the rate of 1.25 ml/L, 2.5 ml/L, 3.75 ml/L and 5 ml/L respectively

Table 1. Effect of calcium foliar feed and rose cultivar interaction on leaf chlorophyll content

Calcium/Variety	Trial 1				
	T0	T1	T2	T3	T4
C1	49.81cd[1]	51.79bc	55.89a	56.23a	54.43ab
C2	40.81f	45.56e	47.51de	50.12cd	51.57bc
	Trial 2				
C1	52.52 de[1]	54.59bcd	57.26ab	60.59a	57.89ab
C2	45.39f	49.56f	53.32cd	55.96bcd	56.6bc

[1]Means followed by the same letter(s) along the column for different calcium foliar feed levels by cultivars are not significantly different at 5% level of significance according to Tukey's Studentized Range (HSD) Test. Where T0 is a control experiment and T1, T2, T3 and T4 are calcium treatments at the rate of 1.25 ml/L, 2.5 ml/L, 3.75 ml/L and 5 ml/L respectively. C1 and C2 are rose cultivars Red calypso and Furiosa

Table 2. Effect of cover and calcium interaction on the leaf chlorophyll content

Cover and calcium	Trial I			Trial II		
	UV-A clear	IR504	UV-205/N	UV-A clear	IR504	UV-205/N
0	41.68f[1]	48.38de	45.87ef	46.2f[1]	51.62de	49.05ef
T1	45.92ef	52.00abcd	48.13de	49.88ef	55.43abcd	50.90def
T2	49.62cde	54.90ab	50.58bcd	52.08cde	58.97ab	54.82bcd
T3	51.20bcd	55.98a	52.35abcd	56.53abc	59.63a	58.65ab
T4	51.37bcd	53.95abc	53.68abd	55.35abcd	57.67ab	58.75ab

[1]Values in the column followed by different letter (s) are significantly different at 5% level of significance for selected poly film covers and along the rows for calcium foliar feed according to Tukey's Studentized Range (HSD) Test. Values are the means of the treatments (n = 3) Where T0 is a control experiment and T1, T2, T3 and T4 are calcium treatments at the rate of 1.25 ml/L, 2.5 ml/L, 3.75 ml/L and 5 ml/L respectively

Leaf chlorophyll content was high under the IR504 poly film and calcium at concentration 3.75 ml/L. Under the control experiment of poly film UV-A clear and no calcium foliar feed, leaf chlorophyll content was significantly low compared to IR504 cover. Generally the leaf chlorophyll content was low during trial I compared to trial II. This could be due to temperature differences which was the only varying factor in the study. Leaf chlorophyll content was statistically similar in all calcium treated experimental units under the IR504 poly film. There was little variation in temperature recorded under the IR504 poly film hence the chlorophyll content did not vary. Under the UV-A clear and UV-A 205/N poly films chlorophyll was high under T2, T3 and T4 while T1 and T0 were statistically lower. This implies that under lower temperature low concentrations of calcium had effect compared to similar concentrations under high temperature. High temperature under the UV-A clear poly film is likely to have initiated stomata closure leading to reduced photosynthesis activities and consequently low chlorophyll content.

4. CONCLUSION

Cut flower quality is affected by growth environment under the poly film greenhouse covers. High chlorophyll content was observed under the IR504 poly film, implying that the coloured poly films in this study served the role of reducing greenhouse temperature and had better quality cut flowers compared to the clear poly film. Leaf chlorophyll content varied with the prevailing weather condition, it increased with decrease in temperature during the second trial and decreased with increase in temperature during the first trial. Red calypso and calcium foliar feed at an application rate T3 (3.75 ml/L) recorded the highest leaf chlorophyll content in both trials I and II. Treatment combination of calcium foliar feed at application rate 3.75 ml/L and IR504 poly film cover significantly improved leaf chlorophyll content negating the use of higher calcium concentration. In this study it can be concluded that, leaf chlorophyll content vary with temperature, cultivar and calcium concentration.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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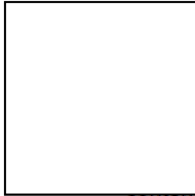

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