DETERMINANTS OF COLOUR AND FRAGRANCE CHARACTERISTICS OF *POLIANTHES TUBEROSA* LINN. FLOWER

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Determinants of colour and fragrance characteristics of *Polianthes tuberosa* Linn. Flower

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DECLARATION

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

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DEDICATION

This thesis is dedicated to my granddaughter Ashley Khalayi

ACKNOWLEDGEMENT

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ACRONYMS AND ABBREVIATIONS

ε	Molar absorbance
A	Absorbance
AAS	Atomic Absorption Spectrophotometer
ANOVA	Analysis of Variance
ASAL	Arid and Semi-Arid Lands
ASDS	Agricultural Sector Development Strategy
CAADP	Comprehensive Africa Agricultural Development Programme
CIELAB	Commission Internationale d'Eclairage (CIE) L*, a*, b* space coordinates
DIC	Days in chamber
DC	Developing Country
DF	Dilution factor
ERS	Economic Recovery Strategy
EU	European Union
FID	Flame ionization detector
GDP	Gross Domestic Product
GoK	Government of Kenya
GC-MS	Gas chromatography mass spectrometry
HCDA	Horticultural Crops Development Authority
icipe	International Centre for Insect Physiology and Environment
JICA	Japan International Cooperation Agency
KARI	Kenya Agricultural Research Institute

L   Cell path length

Meq   Milli-equivalent

MoA   Ministry of Agriculture

MW   Molecular weight

OM   Organic matter

RHSCC   Royal Horticultural Society Colour Chart

SPAD   Soil and Plant Analyzer Development

SRA   Strategy for Revitalizing Agriculture

V   Final volume

Wm^{-2}   watts per square metre

Wt   Sample weight

ABSTRACT

Tuberose (*Polianthes tuberosa* Linn.) is one of the summer flowers grown by smallholder farmers in Kenya, for export. It is a high value perennial crop grown in the field with no plant support structures or shading. A survey in 2007 provided the baseline information on distribution and production indicating that rejection of cut flowers was attributed to poor colour, pest damage and stem length. High rejections of up to 27.5 % lead to low export volumes and low returns to investment since the rejected flowers fetched very low prices on the local market or cannot be marketed at all. Also flowers produced out of the export schedule are marketed at a loss on the local market. Colour was a factor considered for this study and when tuberose are not sold on the export market, to extract essential oil for export would contribute to increased productivity, commercialization and competitiveness therefore its abundance was considered one of the factors to be investigated. Volatiles are synthesised from amino acids, membrane lipids and carbohydrates and their formation is dependent on availability of C2-C8 acids and alcohol which is similar for essential for pigment synthesis. A nutritional study was set up to investigate the effect of soil applied magnesium, nitrogen and light quality on colour development. This was followed by investigating the influence of floral applied magnesium on colour development and stability under transient temperatures in a controlled environment at temperature regimes of day/night temperature of 19ºC/9ºC, 25ºC/15ºC, and 32ºC/22ºC. Controls of fragrance in tuberose florets were studied by determining the composition of the floral volatiles under varied environmental conditions using a portable volatile collection technique *in situ*. The volatiles were

analyzed and the chemical compounds identified using gas chromatography and mass spectrometry.The nutritional study showed that soil pH was not significantly linked to anthocyanin accumulation and colour development in the florets. Neither did magnesiumappear to have increased in the tissues, but there was an increase in accumulation of anthocyanins by 2.61% and colour intensity by up to 53 radians. Florets with no net cover had the highest colour intensity but were not significantly different from those under red shade net. The red shade net cover or growing in the open with no net cover gave the best colour intensity of 53.1 radians; the measure of redness found on the petals significantly decreased when flowers without magnesium treatment were moved from 19ºC to 32ºC while those with magnesium remained the same. The two major chemical components identified in the tuberose floral volatile were methyl benzoate and 1,8 - cineole which accounted for 69–84% of the total fraction. These volatile components are used for the industrial manufacture of cosmetics, pharmaceuticals and confectionery. The number and type of volatiles emitted were location dependant: Juja at an altitude of 1350 m at 18–21°C emitted 21 compounds; Sagana at altitude 1214 m at 17–17.5°C emitted 27 with Cyclopentanone, 2-cyclopentylidene specific to this location; and Meru at altitude 2060 m at 7–9°C emitted 26 compounds with Muurola-4(14),5-diene<trans> and 2-Pyrrolidinone, 1-methyl-1specific to the location.When soil amendments were applied, the ammonium sulphate and magnesium nitrate lowered the soil pH but that lightness, chroma, hue, and anthocyanins in the florets were not significantly linked to the soil pH, there was no increase in magnesium in the plant tissues but an in accumulation of anthocyanins.

Investigations of various light conditions on colour of the florets confirmed the full sun conditions enhanced colour development more that all the other light conditions. For temperature and magnesium concentration, it was determined that magnesium was not required for colour to develop at low temperature but when temperatures increased magnesium appeared to contribute to the maintenance of the developed colour. The number and type of volatiles emitted were different according to altitude and temperature, thus the effect of altitude and temperature have to be taken into account when growing tuberose for the volatiles. Thus further studies are recommended on nutritional studies incorporating different rates of magnesium as a soil amendment and /or floral spray. Value addition applications for industrial purposes have a great potential for cottage industry and also medium and large enterprises.

CHAPTER ONE

1.0   INTRODUCTION

Tuberose cut flower is grown commercially in several countries including in China, Egypt, France, India, Italy, Japan, Mexico, New Zealand, Rwanda, South Africa, Taiwan and North Carolina, USA (http://www.cbi.eu/marketinfo/cbi/2013; Barba-Gonzalez, *et al*., 2012). Kenya is located at the equator and endowed with ideal natural conditions for floriculture. It has become a leading producer of a wide range of top-quality cut flowers and supplies 90% of its produce to the EU and with a market share in of approximately 35% of cut flowers. Europe is a major market for cut flowers and foliage and Kenya is the largest developing country (DC) supplier of cut flowers to the EU with the main cut flowers sold being roses, spray chrysanthemum, tulipa, gerbera and lilium. The rapid growth of Kenya in the floriculture industry is attributed to the warm temperatures, limited days without sunshine or rain and availability of water for irrigation, making ideal growing conditions for a wide range of flowers all year round without the need for greenhouses (Anon, 2001). Tuberose is one of the flowers grown by smallholder farmers in Kenya. The cut flower is valued for its sweet fragrance, and the main export destination is the Netherlands with negligible amounts to other countries (HCDA, 2002, 2007, 2012; Muthoka and Muriithi, 2008; Muriithi *et al*., 2011). In Rwanda, tuberose is grown as a cut flower and the flowers whose grade makes them non-exportable, are to be used for extracting organic essential oil that fetch higher prices (Turner, 2001). The profitability of the essential oil is higher if it is certified to be organic. Usman and Ashfaq (2013) using gross margins for small, medium and large scale farmers show that tuberose is a very

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profitable crop in India especially for the smallholder. In Kenya, tuberose is not so profitable compared to other summer flowers (Muthoka and Muriithi, 2008; Muriithi *et al*., 2011) but if tuberose that is not sold on the export market were used to extract essential oil for export, it would contribute to increased productivity, commercialization and competitiveness of this crop and to the agricultural sector as outlined in the Agricultural Sector Development Strategy 2010–2020 (ASDS) (GOK, 2008a).

Vision 2030 rightly identified agriculture as a key sector to deliver economic growth, to achieve this growth smallholder agriculture has to be transformed to an innovative and commercially oriented way of farming (GOK, 2008b). The agricultural sector contributes 26% of the gross domestic product directly and 25% indirectly. It also accounts for 65% of Kenya's total exports and provides more than 18% formal employment. Of the six subsectors in agriculture, horticulture has recorded a remarkable export–driven growth and is the largest subsector contributing 33% of the agricultural gross domestic product (GOK 2008a).Smallholders are confronted with new opportunities and constraints due to globalized markets of agricultural goods. In East Africa high potential mountain areas have long been integrated in global value chains for the production of coffee and tea. A rather new development is the production of horticulture crops (fruits, flowers, vegetables) for export, which has pushed into areas under more arid conditions that are therefore more prone to resource challenges. The expansion of horticultural agro-businesses puts pressure on limited natural resources, especially water, which can aggravate the situation for ecosystems and downstream users. At the same time, it offers new economic opportunities. In Kenya, horticultural production has been the second most

important foreign exchange earner in the agricultural sector, after tea, over the past decade. The growing market for horticulture products has been proposed as a pro-poor strategy due to the sector's high labour requirements and the opportunities it offers to commercialize small-scale farms (Weinberger and Lumpkin, 2007; Laibuni *et al*., 2012).

Tuberose in Kenya is produced over a wide range of agro-ecological zones from Upper Highlands (UH2) sub humid areas with temperatures ranging between 10 -15ºC with seasonal night frosts to Lower Midlands (LM4) transitional areas with temperatures in the range of 21 -24ºC and above that are transitional (Muthoka and Muriithi, 2008). Muriithi *et al*., (2011), carried out a survey in 2007 to find out the reasons for the drastic decline in tuberose production.. The total area under tuberose production in 2006 in Kenya was estimated to be over 67 acres. The crop is grown in diverse agro ecological zones in Kenya and each zone may face unique situations and trends that affect production, flower quality and fragrance attributes. The major findings of the survey were: current tuberose production is concentrated in Kiambu county; farmers and supply most of the planting materials directly or indirectly through exporters. Though the Kenya Agricultural Research Institute supplied 15% of the tuberose planting material, they were the main disseminators of production technologies for tuberose production. The farmers had adopted a plant density of 25 plants $m^{-2}$and fertilizing with both organic manure and chemical fertilizers at planting and top dressing. Challenges weremajor pests identified asnematodes, pale colouredflowers, visible pest damage and short stem lengththat led to rejection of cut flowers. The low export volumes could be explained partly by the high cut flower rejections and when sold on the domestic market fetches low prices. Lack of profits

from tuberose growing explains the high abandonment by switching to more profitable flower crops leading to low acreages under production and low volumes of the cut flower (Muthoka and Muriithi, 2008; Muriithi *et al*., 2011). Tuberose export volumes and values have been declining since 1997 to date (HCDA, 2012).

1.1   Statement of the problem

Tuberose export volumes and values had been declined since 1997. A purposive surveyin 2007 (Muriithi *et al*., 2011), found that rejection of tuberose cut flowers was because they were pale, damaged by pestsor had short stems. But flower colour was noted by more than two thirds of the respondents as the reason for rejection. Colour of all flowers plays a dominant role in purchase preference. Tuberose varieties cultivated were single or double with white petals. In India where tuberose is a very important flower for cultural festivities colour diversity (blue, red, scarlet, rose to yellow) was obtained by colouring agents such as: ammonium purpurate, eosin yellow, bromocresol blue, bromocresol green and phenol red (Sambandamurthi, and Appavu, 1980). Other efforts to get new flower coloursby mutation using gamma rays only produced tuberose plants with leaf chimeras (Abraham and Desai, 1976). However, in the 1980's breeding studies on tuberose started in Taiwan with the cultivated single tuberose by Shen and co-workers(1987; 2002). In Kenya it was reported that tuberose cut flowers are for export and exporters prefer the vivid reddish pink as opposed to pale flowers (Muriithi *et al*., 2011). The cut flower rejections at grading lead to low export volumes and thus limited profits from tuberose production in comparison to other summer flowers. The farmers opted for other flower crops which were more

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profitable (Muthoka and Muriithi, 2008). The colour of tuberose florets is one of the major aesthetic values that contribute to its quality. Tuberose cut flower consumers prefer vivid reddish pink flowers (Muriithi *et al*., 2011). Farmers often produce tuberose flowers with pale pink to greyish pink, which are rejected on the export market (Huang *et al*., 2001; Muriithi *et al*., 2011).

1.2   Justification

Tuberose is an important cut flower that can be easily grown by smallholder growers. It requires fewer inputs and technical outputs to produce. Smallholders supply statice, leather leaf fern, gladiolus, strelitzia and tuberose, among other flowers. Tuberose is grown by smallholders one double variety"Pearl" is known brought in by European settlers (Muriithi *et al*., 2011). The export of tuberose cut flower contributes to the much-needed foreign exchange (GoK, 2008b). Kenya is a leading foreign supplier cut flower and foliage to the European Union (EU) with a comparative advantage despite having a very limited product range. This is because its products in these markets are of relatively higher quality compared with her major competitors, thus assuring better prices (Laibuni *et al*., 2012). Increased consumption of tuberose products on the market would spur the industry's growth by creating job opportunities. Tuberose is versatile in that apart from being a cut flower, the essential oil is a high-quality perfume base, a resource that remains untapped at 2 ml retailing for USD 55.00 (http://artisanessentialoils.com/shop/absolutes-new/organic-tuberose-enfleurage/). Most of the fragrant oil comes from India and is one of the most ex-pensive of the fragrant oils used in perfumes, valued more than USD 2,000.00 per pound (Hodges 2010). Value addition by extracting

the essential oil for the cosmetic, pharmaceutical and pesticides industry would provide more income to the grower. Tuberose is grown in the open field and there its production tenable even to the rural poor and can be used to contribute to the country's move towards wealth creation and employment as envisioned in key policy documents such as Kenya's Vision 2030 and the ASDS. One such programme is the CAADP initiative which aims to boost agricultural research and ensure that results are disseminated and put into use in the field and out scaling and up scaling workable options that can improve the farmers' income (http://www.nepad.org/system/files/caadp.pdf). Having farmers producing high-value crops for urban and export markets, is an important strategy in raising rural incomes through wealth distribution thus reducing poverty and increasing economic access to food and household food security. Integrating smallholders into high-value global markets represents a unique opportunity to effect large-scale poverty reduction in the countryside (Bolo, 2010). Such an intervention strategy is critical to maintain export competitiveness for labour-intensive floriculture crops that require careful husbandry.

1.3 Objectives

1.3.1 Broad objective

To determine factors that influence tuberose flower attributes for improved incomes and livelihoods leading to food security.

1.3.2 Specific objectives

1.0 To determine the effect of nutritional factors on the colour development of *Polianthes tuberosa* Linn.

2.0   To establish the effect of modified light on colour development of *Polianthes tuberosa* Linn.

3.0   To establish the effect of temperature and magnesium on colour development of *Polianthes tuberosa* Linn.

4.0   To determine the influence of location on composition of biochemical compounds of tuberose floral volatiles.

1.4   Research questions

The research questions were (i) what are the nutritional conditions that enhance colour development? (ii) what environmental conditions enhance colour development?(iii) whatis the interaction of a foliar applied element and temperature on tuberose floret colour development and retention?and (iv) what are the biochemical components of the floral volatiles of tuberose and how is their composition affected by location?

1.5   Scope

The study was to cover alltuberose growingareas in Kenya and understand the issues faced in the different tuberose growing locations. The guidelines used for tuberose production were those available from the Ministry of Agriculture and HCDA JICA manual of 2001 and the KARI Commercial production of tuberose manual of 2004.

1.6 Limitations

Tuberose in Kenya is produced over a wide range of agro-ecological zones from Upper Highlands (UH2) sub humid areas with temperatures ranging between 10 -15ºC with seasonal night frosts to Lower Midlands (LM4) transitional areas with temperatures in the range of 21 -24ºC and above. In this study the following limitations were:

1. It was not possible to use all the localities and replicate all thetuberose growing conditions in Kenya.

2. Tuberose is a perennial crop, the same crop planted in 2007 was used in subsequent years and no replanting was donefor the consecutive studies.

3. Availability of validated secondary data at the Ministry of Agriculture and HCDA was a challenge and assumptions were that the data available was validated

CHAPTER TWO

2.0   LITERATURE REVIEW

Tuberose was discovered in the 16[th]century by the early Spaniards who had traveled to Mexico to document plants of medicinal interest. Trueblood (1973) reviewed the ethno botanical data in the Aztec Nahuatl language. Tuberose was referred to as "omixochitl", meaning bone white flower. The Spaniards described it as "pleasing odor, fragrant, sweet" (Trueblood, 1973). Fifteen species of *Polianthes* have been discovered in Mexico with flower colour in white, orange-red, red or striped (Barba-Gonzalez, *et al*., 2012). (Sheela, 2008). The development of new cultivars has not been very successful. There are three types tuberose: single semi − double and double but only the single and double are cultivated, both of them have white flowers (Sheela, 2008). These two varieties single and double haveflowers with white petals. In India where it an important flower for cultural festivities a variety of colours are obtained by use of colouring agents to induce colourssuch as red, scarlet, rose to yellow (Sambandamurthi, and Appavu, 1980). Other efforts to diversify tuberose colours were by irradiation but no colour mutation of flower has been possible (Abraham and Desai, 1976). The flower is used by florists in bouquets as accents, corsages and boutonnieres. It is an important flower whose fresh petals when processed produce an essential oil that is used in the preparation of high-grade perfumes and cosmetics (Trueblood,1973; Asif *et al.,* 2001; Rakwarton, 2011).

Tuberose is grown in the tropical and subtropical areas as a cut flower and for fragrance (Benschop, 1993; Huang *et al*, 2001). The crop is a day neutral bulbous

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perennial and grows well in the field at a temperature range of 20 - 30 °C with no shading or plant support (Anon, 2001; 2004; Watako, 2005). Tuberose is vegetatively propagated and bulbs are commercially used with tissue culture being experimented within Kenya (Hutchinson *et al*., 2004). Flowering performance of tuberose has been demonstrated to vary according to the temperature regime (Watako, 2005). Nutrition studies show that 42.5 kg N Ha^{-1} was optimum for good quality cut flowers (Ngamau, 1992). The quality of tuberose cut flowers grown in Kenya by smallholder farmers remains low (Muriithi*et al*., 2011). "Double" tuberose varieties acquire a reddish pink colour on the outer florets preferred by the consumers (Huang *et al*., 2001a & b).

2.1 Conceptual framework

The conceptual framework (Figure 2.1) shows how the various components are linked together to address the study objectives.

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                 ┌─────────────────────────────────────────┐
                 │   Quality and Value Added Aspects of     │
                 │          Tuberose Cut Flower             │
                 └─────────────────────────────────────────┘
                      │                          │
                      ▼                          ▼
         ┌──────────────────────┐   ┌──────────────────────┐
         │ Tuberose plant fresh │   │  Tuberose by -       │
         │      produce         │   │     products         │
         └──────────────────────┘   └──────────────────────┘
                      │                          │
                      ▼                          ▼
         ┌──────────────────────┐   ┌──────────────────────┐
         │  Colour development  │   │    Value addition    │
         └──────────────────────┘   └──────────────────────┘
                      │                          │
                      ▼                          ▼
  ┌────────────────────────────────┐  ┌──────────────────────┐
  │ Tuberose grower survey,        │  │  Floral volatile     │
  │ Nutrition, Light,              │  │    components        │
  └────────────────────────────────┘  └──────────────────────┘
       │      │      │      │                    │
       ▼      ▼      ▼      ▼                    ▼
  ┌──────┐┌──────┐┌──────┐┌──────┐  ┌──────────────────────┐
  │Agro- ││Nutri-││Effect││Effect│  │ Identification and   │
  │nomic ││tional││of    ││of    │  │   Quantification     │
  │and   ││amend-││light ││tran- │  └──────────────────────┘
  │mar-  ││ment  ││      ││sient │              │
  │keting││of    ││      ││      │              ▼
  └──────┘│soil  │└──────┘└──────┘  ┌──────────────────────┐
       │  └──────┘    │      │      │ Potential utilization│
       ▼      ▼       ▼      ▼      │    for livelihood    │
  ┌────────────────────────────────┐└──────────────────────┘
  │ Cut flower quality             │             │
  │      determination             │             │
  └────────────────────────────────┘             │
                      │                           │
                      ▼                           ▼
         ┌──────────────────────────────────────────┐
         │     Documentation and dissemination       │
         └──────────────────────────────────────────┘
                  │                        │
                  ▼                        ▼
  ┌──────────────────────┐   ┌──────────────────────┐
  │ Enhanced tuberose    │   │  Increased income    │
  │    productivity      │   │                      │
  └──────────────────────┘   └──────────────────────┘
              │                        │
              ▼                        ▼
         ┌──────────────────────────────────────────┐
         │ Improved food security and livelihoods    │
         └──────────────────────────────────────────┘
```

Figure 2.1   Conceptual framework

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Data collected and information generated from the studies will be interpreted and used to package technologies that can be applied the smallholder farmer. Thedissemination and use of appropriate agronomic technologies generated would lead to increased income from the tuberose value chain and make it an economically attractive enterprise. Biochemical components identified in tuberose floral volatiles will be documented as new information and made available for industrial applications which can be translated into potential utilization for livelihood. The wealth created and distributed in the rural areas growing tuberose cut flowers will translate in improved livelihoods and food security for the smallholder farmer.

2.2   Botanical classification

Tuberose is classified in the Agavaceae family the genus *Polianthes* (Hutchinson, 1959;Trueblood, 1973; Barba-Gonzalez *et al*., 2012). However some taxonomists (Shah and Gopal, 1970) placed it in the Amaryllidaceae family supported with cytological studies of shared characteristics in vegetative and floral organs. The Agavaceae family is characterized by a rosette of basal leaves, flowers on a raceme or panicle with bracts along its length subtending the flowers. The petals are nearly free, generally being joined at the base (Hutchinson, 1959; Barba-Gonzalez *et al*., 2012). *Polianthes tuberosa* Linn. is the only species cultivated as an ornamental cut flower in tropical and subtropical areas. The flower colour of all known cultivars of *P. tuberosa* is white; however, many attempts have been made to introduce colours from related species (*Polianthes* has 15 species all from Mexico). Most of the species have white flowers except *P. geminiflora* with reddish orangeand*P.howardi* purple and P. *densiflora* yellow (Rocha *et al*., 2006; Barba-

Gonzalez *et al*., 2012). *Polianthes* is characterized by twin flowers, which arise from a single bract along the flower spike (Fig. 2.2).
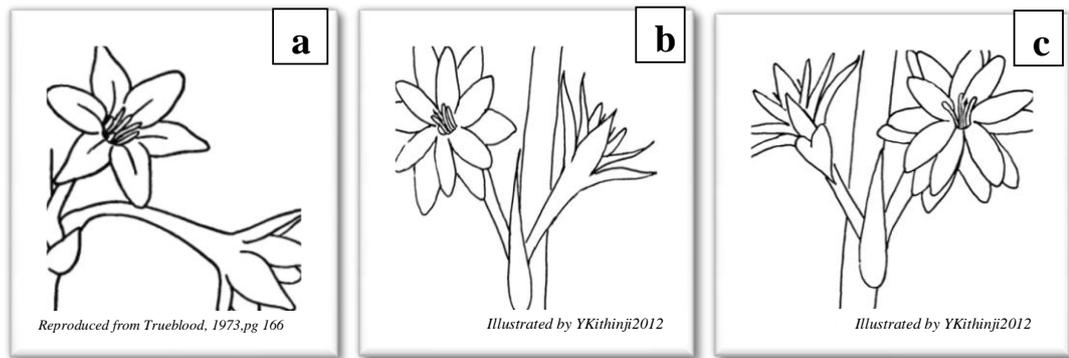
Figure 2.2　　Illustrations of *Polianthes tuberosa* L. showing twin florets with bract: a) Single, b) semi double and c) double flowers
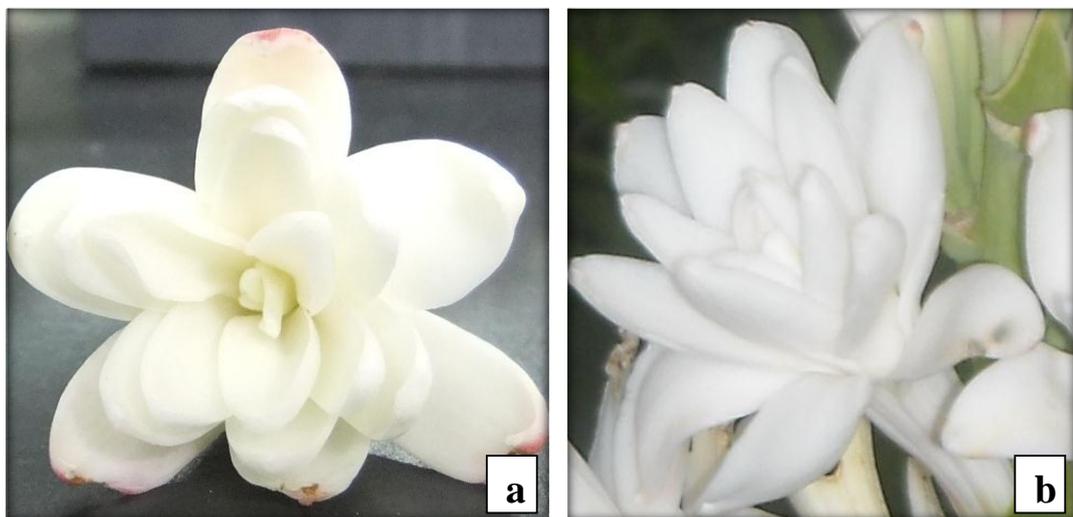
Figure 2.3　　*Polianthes tuberosa* L. showing the rows of petals for a) semi double and b) double florets "Pearl"

There are three types of tuberose: single, semi–double and double. The 'single' have one row of corolla segments shown in Fig. 2.2a;'semi double' with two to three rows of corolla segments and refers to a flower having the outermost stamens converted into petals (Fig. 2.3 a); while 'double' which is also referred to as the 'Pearl Double', has four rows of corolla, the inner ones remain perfect, while double-flower forms often arise when some or all of the stamens in a flower are replaced by petals (Fig. 2.3b) (Barba-Gonzalez, *et al*., 2012). This mainly arises from mutations, where one organ in a developing organism is replaced with another, generally known as homeotic mutations. In Kenya, the double and semi double varieties are grown without market preference (Muriithi *et al*., 2011).

2.3   Tuberose cultivation

Tuberose is a bulbous perennial that grows well in open fields with temperatures ranging from 20–30 °C (Huang *et al*., 2001);it requires neither shading nor plant support structures. Tuberose does well in altitude between 1200 and 1800 metres above sea level. The major production areas in Kenya are Limuru and Maragua (Anon, 200; Muriithi *et al*., 2011). Tuberose grows well in loam and sandy loam with a soil pH range of 6.5 to 7.5 (Asif *et al.,* 2001; Singh, 2006). Commercial propagation is mainly by bulbs (Singh, 2006). However, efforts to propagate using tissue culture have been undertaken extensively using various explants such as: the bulb scale (Nazneen *et al*., 2003; Mishra *et al*., 2006), stem disc (Gajbhiye *et al*., 2011), shoot tips (Hutchinson *et al*, 2004), and rhizome (Sangavai and Chellapandi, 2008).

The stage for harvest is related to the number of opened florets showing sufficient accumulation of carbohydrates that enhance petal opening after harvesting (Varu and Barad, 2010). Cut flowers are graded based on length of the spike, the longer the spike the longer the vase life (Anon 2001*)*. Tuberose evolves minimum endogenous ethylene and the use of ethylene synthesis and receptor inhibitors such silver thiosulphate (STS) do not delay flower senescence of *P. tuberosa* (Naidu and Reid 1989; Waithaka *et al.*, 2001; Abbasi and Hassanpour, 2011). However, preservative solutions consisting of gibberellic acid (GA$_3$)at40 ppm (Abbasi and Hassanpour, 2011) or citric acid in the form of hydroxy quinine citrate(8-HQC)at 250ppm and 2% sucrose (Waithaka *et al.*, 2001) have been shown to increase vase life. Tuberose florets were not sensitive to ethylene exposure. From the survey, tuberose growers harvest and transport immediately to the market, they do not use preservatives (Muriithi *et al.*, 2011).Harvesting of tuberose bulbs is done when the older leaves dry, plant growth ceases and bulbs are almost dry. Bulbs are harvested18–24 months after planting. Planting varies according to temperatures experienced during the growth period (Anon, 2001*)*.The distribution, production and quality characteristics of tuberose in Kenya were established in this study. Areas of concern in production include: provision of good quality, adequate and affordable planting material; employing appropriate crop production management practices such as the use of recommended inputs, control of pests and diseases; postharvest handling and access to markets. The survey looked at all links in the tuberose value chain thus these findings bring out the gaps that need intervention. For example, it was clear that smallholder growers lack a system for producing and bulking high-quality planting material to satisfy the needs of commercialised farmers. There was

also inability by smallholder farmers to access high-quality planting material because of high costs a fact which led to most farmers accessing them from their neighbours. Currently, there is need for certification and a regulatory provision for tuberose bulbs to curb widespread distribution of poor quality material which contribute to the dissemination of pests. Further, there is need for germplasm conservation and production of certified clean planting material which is the basis for good quality cut flowers (Muriithi *et al*., 2011). Other interventions require building capacity of farmers in sorting and grading, packaging, postharvest handling, and marketing. Some of the data adduced from this study may be used towards the formulation of the floriculture policy. The cause of decline in tuberose export volumes was due to high rejections of poor quality cut flowers. Low productivity can be attributed to poor quality bulbs infested with nematodes. Abandonment of tuberose enterprises was in areas with other flower choices caused in the reduction of tuberose acreage (Muriithi *et al*., 2011).

2.4   Flower attributes

The colour of flowers is key to consumer appeal. However, tuberose varieties that were available had white petals. In India where tuberose is an important flower for cultural festivities its colour was diversified by use ofcolouring agents.Blue, red, scarlet, rose to yellow flowers were achieved using ammonium purpurate, eosin yellow, bromocresol blue, bromocresol green and phenol red (Sambandamurthi, and Appavu, 1980). Mutation has never produced coloured tuberose (Abraham and Desai, 1976). Shen *et al*., (1987; 2002) have been breeding tuberose in Taiwan using the single variety.

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2.4.1 Flower colour

The colour of tuberose florets is one of the major aesthetic values that contribute to its consumer appeal. The final colour of a flower is attributed to a number of factors including pH of the vacuolar contents, concentration of anthocyanins, chelation of anthocyanins by metal ions, and number of glycosidic bonds (Grotewold, 2006, Gould and Lister, 2006, Tanaka *et al*., 2008). An understanding of these factors is important in regulating colour in tuberose.

2.4.2 Anthocyanins

Anthocyanins are a group of plant pigments responsible for colours ranging from red to violet and blue (Koes *et al*., 2005). These pigments accumulate in the vacuoles of epidermal cells, and both their chroma and hue are dependent on external conditions, as well as on the pH in the vacuoles (Gould and Lister, 2006, Tanaka *et al*., 2008). Anthocyanins are water-soluble pigments produced via the flavonoid pathway in the cytoplasm of the coloured plant cell. The attachment of the sugar molecule makes them particularly soluble in the sap of the vacuole, where these molecules are stored Fig. 2.4 (Taiz and Zeiger, 2010). These are responsible for the pink-red colours of most flower petals, of most red fruits (like apples) and almost all red leaves during the autumn. Anthocyanins absorb light in the blue-green wavelengths, allowing the red wavelengths to be scattered by the plant tissues to make these organs visible to us as red (Ellestad, 2006).

Anthocyanins are able to accumulate in epidermal vacuoles and blend with the plastid pigments to give various hues that vary with light exposure, and night and

day temperatures (Ellestad, 2006; Gould and Lister, 2006). Anthocyanins are important for attracting pollinators and for seed dispersal (Dela *et al*., 2003).In most flowers, anthocyanin synthesis occurs during petal growth and is under developmental control and this would be the appropriate period to apply treatments. Investigations on flower anthocyanin pigmentation using reddish-purple tuberose established that 80%-100% of the anthocyanin pigment in tuberose was cyanidin (Haung *et al*., 2001c).
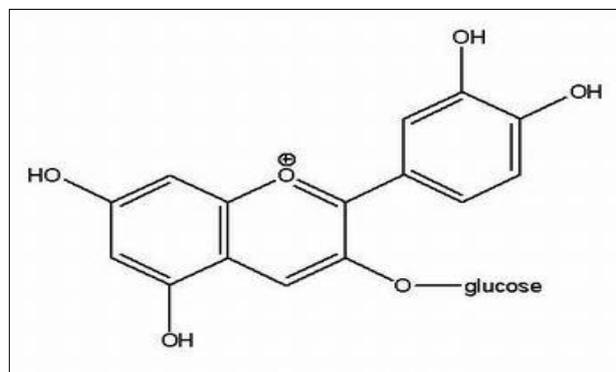
Figure 2.4   Anthocyanin with sugar molecule (after Gould and Lister, 2006; Taiz and Zeiger, 2010)
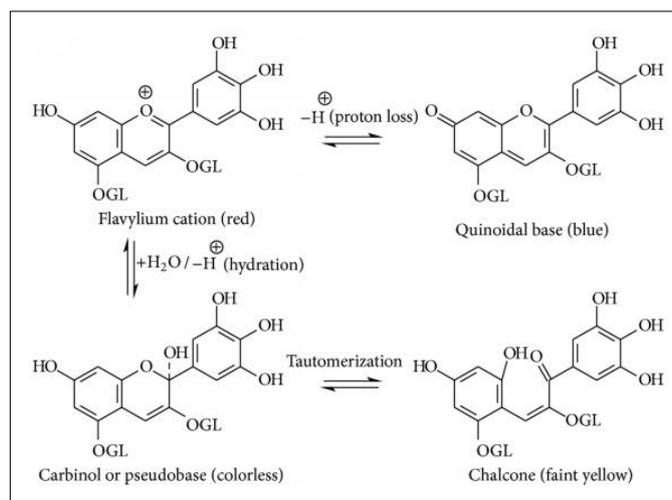
Figure 2.5   Structure change of anthocyanin with pH in aqueous solution (Cavalcanti *et al*., 2011)

Anthocyanins show great susceptibility toward pH variation, being more stable in acidic media at low pH values than in alkaline solutions at high pH value (Wang et al., 2014). The ionic nature of anthocyanins enables the changes of the molecule structures according to the prevailing pH values and results in different colours and hues at different pH values. In acidic aqueous solution, anthocyanins exist in the form of four main equilibrium species: red flavylium cation, blue quinoidal base, colourless carbinol or pseudobase, and yellowish chalcone (Wang et al., 2014). Under acidic conditions (pH < 2.0), the anthocyanins exist primarily in the form of red flavylium cation. Increasing the pH value causes fast loss of the proton and produces quinoidal base forms, blue or violet. At the same time hydration of flavylium cation occurs and the carbinol or pseudobase is generated, which slowly reaches equilibrium and produces the chalcone in faint yellow. The relative amounts of above four forms of anthocyanins at the equilibrium condition vary according to pH values (Wang et al., 2014).

2.5   Temperature effect on anthocyanin

Temperature has a major effect on anthocyanin accumulation shown by various workers on different crops (petunia –Shvarts *et al*., 1997; rose - Dela *et al*., 2003; grape–; Mori *et al*., 2007). Low temperature favoured higher accumulation of anthocyanins in apples compared to high (Ubi *et al*., 2006; Wang *et al*., 2011). Ban *et al*., (2009) reported that high temperatures prevent the accumulation of cyanidin and uridine diphosphate- sugars (UDP-sugars) which are precursors of anthocyanin synthesis. Anthocyanin synthesis has also been reported to resume after resumption

of a low temperatures regime (Yamane *et al*., 2006). A new line of tuberose was reddish-purple at 20ºC and white at 30ºC (Huang *et al*., 2001c). Cell cultures have been used to illustrate anthocyanin responses to temperature with a fourfold increase at 20ºC compared to 30ºC (Zheng and Wang, 2003).

Therefore temperature affects anthocyanin by increasedaccumulation when it is low, and decreased anthocyanin accumulation at elevated temperatures (Shaked-Sachray *et al*., 2002).In addition, temperature may also affect the stability of anthocyanins. Therefore, the decrease in anthocyanin concentration at elevated temperatures may result from both a decrease in synthesis and an increase in degradation. Anthocyanins are formed when corresponding anthocyanidins are stabilized by the addition of a sugar residue (Gould and Lister, 2006) demonstrating the link between anthocyanin accumulation, sucrose and light. The poor reddish pink colour was associated with the dry season by growers in Kenya could be the result of rapid growth during the dry hot months and less pigments accumulated giving a pale pink colour (Muriithi *et al*., 2011).

2.6   Metal complexes with anthocyanins

Metals have been commonly used to stabilize the colour of cyanidin, delphinidin, and petunidin, which have more than one free hydroxyl group in the chromane ring and are capable of metal chelation (Castañeda-Ovando, et al., 2009). The most common metals in anthocyanin complexes are tin (Sn), copper (Cu), iron (Fe), aluminum (Al), and magnesium (Mg) Cavalcanti et al., 2011). The main characteristic of anthocyanins and anthocyanindins with o-di-hydroxyl groups in the

B ring is their ability to form metal-anthocyanin complexes (Castañeda-Ovando, et al., 2009)

Several studies have examined the effect of different metals on anthocyanin stability and hue in solutions (Shaked-Sachray et $al.$, 2002; Yoshida et $al.$, 2009). Tin, copper, and aluminum ions were capable of forming stable complexes with anthocyanins (Yoshida et $al.$, 2009). Stable ternary complexes containing anthocyanins, unidentified colourless compounds, and magnesium and/or magnesium plus ferric ion or aluminum were described. The main known effect of metals on anthocyanins in flowers is a change in hue of the flower colour (Shaked-Sachray et $al.$, 2002). A low soil pH favors availability of Mg^{2+}, Fe^{3+} and Al^{3+}cations for plant uptake (Taiz and Zeiger, 2010). The red colour in many fruits and flowers has been found to be due to anthocyanins with a common aglycone (cyanidin) bound to different sugars, thereby producing different cyanidin glycosides (Montefiori et $al.$, 2005).

Stability of anthocyanin colour can be improved by co -pigmentation, where covalently linked anthocyanin gives brighter, stronger and more stable colours than what would be expressed by an intact anthocyanin molecule. Magnesium treatment of the cells reported dramatic results in cell suspension anthocyanin concentration by 2.5 to 4.5 times increase and inhibited the degradation of anthocyanins (Sinilal et $al.$, 2011). Metals have been shown to stabilize the colour of different flowers (Shaked-Shakray et $al.$, 2002).

2.7   Light quality and flower colour

Studies on different crops at low and high altitudes show overwhelmingly that light and temperature play an important role in anthocyanin accumulation and degradation (Huang *et al*., 2009). In colour development studies, bagging is used to reduce light reaching the target organ resulting in bleaching it. On re – exposure to light, there is a rapid increase in colour development (Huang *et al*., 2009). Bleaching has used been used to study anthocyanin accumulation by bagging the target organ to block light and limit anthocyanin production. Anthocyanin production is dependent on light intensity and quality (Shahak *et al*., 2004). Modified light manipulation by use of coloured shade nets has been used in various studies to influence colour intensity of various flowers (Oren-Shamir *et al*., 2001; Rajapakse and Shahak 2007) bearing in mind that anthocyanin accumulation is also phenologically controlled and influenced by biotic and abiotic factors such as nutrients (nitrogen and phosphate), sucrose, pathogen infection, water stress, UV, visible and far-red light (Iglesias *et al*., 2008: Huang *et al*., 2009: Jakopic *et al*., 2009).

The relationship between colour development and light intensity is analogous; low light intensity leads to a reduction in flower colour of many plants at as reported in many plants among them petunia, pears and apples and it is limited to tissue exposed to light (Albert *et al*., 2009; Huang *et al*., 2009; Carvalho *et al*., 2011).Rose and petunia flowers were paler when plants are grown in low light conditions (Oren-Shamir *et al*., 2001; Rajapakse and Shahak 2007).

Manipulation of the light environment is an old practice in horticultural production to enhance desired traits of produce. Supplementary lighting with specific filters that filter out specific wavelengths through absorption or reflection (Rajapakse

and Shahak 2007; Shahak *et al*., 2004). Black nets are the most common for shading of ornamental crops and nurseries at 40-80% shading. Coloured nets combine the physical protection and differential filtration of the solar radiation for light to freely pass unchanged in quality through the holes while the fraction hitting the threads is modified. This provides varying mixtures of natural, unmodified light, together with the spectrally modified and diffused light (Shahak *et al*., 2004). The spectral properties for the black net were found to be: transmits light to the same extent throughout 300 – 850 nm, red nets exhibit spectral cut off below 580 nm while the pearl was 380 nm. The shade percentage reduces the total amount of light by 50%. The coloured nets also enhanced the intensity of scattered light under them relative to the natural light (Shahak *et al*., 2004).

Direct measurements of the light component through red, yellow and pearl nets show that that the light is spectrally modified and also fully red, yellow and pearl (Oren-Shamir *et al*., 2001). The light under the coloured nets is more scattered compared to natural light or light filtered through the black net which does not scatter at all (Shahak *et al*, 2004). However, different species and cultivars had varied responses to colour nets.

Other biochemical components in plants that are influenced by ecological factors are the vol*atile aromatic compounds* which give plants their fragrances. The volatile compounds accumulate in all types of vegetative organs or specialized storage (Maccioni *et al*., 2007).Volatiles are synthesised from amino acids, membrane lipids and carbohydrates and their formation is dependent on availability of C2-C8 acids and alcohol also essential for pigment synthesis (Grotewold, 2006). Tuberose is cultivated in many countries for commercial production as a cut flower,

pot plant and for fragrance production (Anon, 2001). In Kenya it is planted in March during the rainy season and flowering coincides with the peak export period from September through to March. However flowers produced during the low export period of May to August do not command a competitive price (HCDA, 2007; Muthoka and Muriithi 2008). In Rwanda, where tuberose is also grown as a cut flower, agribusiness development technical experts have recommended processing of non-export grade flowers for organic essential oil in order to command higher profits (Turner, 2001).

2.8   Floral volatiles

Plant volatiles accumulate in all types of plant organs e.g. flowers, pollen and leaves (Maccioni *et al*., 2007). Floral volatiles have a role in pollinator attraction (Raguso, 2009) and assumed to be its primary function. However, pollinator attraction and visitation is also linked to flower morphology and colour, though dynamic patterns in floral scent emission can be related to pollinator behaviour (Raguso, 2004). Chemical compounds in the floral scent show close association with the pollinators attracted. The strong, sweet fragrances of moth-pollinated flowers are characterized by specific classes of volatile compounds commonly used in perfumery (Knudsen and Tollsten, 1993). However, tuberose extracts have been shown to have anti –fungal (Nidiry and Babu, 2005) and ant-bacterial activity (Lodhia *et al*., 2007).

Floral fragrance may be composed of one or more than 100 compounds varying from pico-grams to micrograms emitted per hour. The composition varies spatially in different parts of the flower (Maccioni *et al*., 2007) and based on the circadian rhythm or external stimuli such as light or temperature. An increase in

temperature leads to increase in emission (MacTavish, *et al*., 2000; Picone *et al*., 2004). In other studies increase temperature and rainfall in the field increased emission of volatiles in apples (Vallat, *et al*., 2005). The monoterpene pinene was three times higher at 30°C compared to 20°C in *Pinus halepensis* and *Quercus ilex* (Llusia and Penuelas, 2000).

Tuberose volatiles are very unstable and show variable polarity, solubility, volatility, pH, and concentration. They are easily oxidized by contact with air or degraded by heat (Guenther, 1948). It is extracted using organic solvents giving a residue known as a concrete. The main components of the tuberose concrete are geraniol, nerol, benzyl alcohol, methyl benzoate, methyl silicate, ethanol, benzyl benzoate and methyl anthranilate (Sheela, 2008). The push and pull collection device is a novel method for volatile collection where only the desired portion of the plant is enclosed in a chamber (Tholl, *et* al., 2006). Air is pushed into the chamber regulated by a flow meter and pulled through a collection trap by a vacuum pump regulated by another flow meter (Knudsen and Gershenzon, 2006). The process has the advantage of not trapping non-volatile compounds, therefore yielding a fragrance that should be closer to the flower's true natural odor and is therefore more accurate than other sample extraction methods.

Therefore, knowledge and extraction of volatiles for fragrance characterization, the cosmetic, pharmaceutical and food industry would potentially provide more income to the grower. The objectives of the study were analyze and identify the chemicals compounds present in the volatiles emitted *in vivo* by tuberose flowers grown in Kenya and also determine the effect of the environment on emission of tuberose flower volatiles.

2.9 Summary

Tuberose is an ancient flower revered in some societies for cultural festivities. The focus of the research work done includes production technologies to improve the flower attributes of colour and biochemical compounds that enhance its use for aesthetic purposes and as an essential oil. Breeding of tuberose has been exclusively been carried in China and Japan while postharvest in the USA and Africa. In Kenya, findings of a tuberose survey carried out in 2007 have the shown challenges in adoption of technology such as planting material, topdressing and irrigation The tuberose farmers were concentrated in Kiambu county agro ecological zone LH1; causes of cut flowers rejection, prices and distance to airport were recorded and analyzed. Investigation of colour in the "double" tuberose grown in Kenya is important. Therefore agronomic means under which the colour can develop are to be determined to increase the value of the flower and better income for the farmer. The reddish-purple is attributed to anthocyanin containing tuberoses and pale purple flowers are less popular (Huang, *et al.*, 2000). Lack of profits from tuberose growing as reported by 49% of the respondents explains the high abandonment by switching to more profitable flower crops leading to low acreages under production and low volumes of the tuberose cut flower (Muriithi *et al*., 2011).

2.10 Research gaps

The colour of tuberose florets is one of the major aesthetic values that contribute to its quality during grading. The final colour of a flower is attributed to a number of factors including pH of the vacuolar contents, concentration of

anthocyanins, chelation of anthocyanins by metal ions, and number of glycosidic bonds (Grotewold, 2006, Gould and Lister, 2006, Tanaka *et al*., 2008). An understanding of these factors is important in regulating colour in tuberose. Tuberose flower colour is determined principally by anthocyanins (Huang, et al., 2000). and carotenoids. The former pigments give rise to orange, pink, red, purple, and blue, whereas the latter are principally responsible for a range of yellow and orange colours (Taiz and Zeiger, 2010). Tuberose varieties single and double are white petalled flower and in India where it an important flower for cultural festivities. To achieve a the reddish purple colour through agronomic means the following factors will be examined: low pH to enhance magnesium uptake by topdressing with an acidic magnesium source to enhance the uptake of magnesium and formation of metal complexes and increase the accumulation of anthocyanins in tuberose flowers leading to a vivid reddish pink colour;modified light and pigmentation of flowers by combining shading and coloured nets to enhance development of the reddish pink colour on tuberose florets. The effect of magnesium on colour development and stability in tuberose flowers has never been investigated in tuberose. The influence of varied temperature and magnesium concentration on the development and stability of colour on tuberose florets can be investigated.

Extraction of volatiles for fragrance characterization, the cosmetic, pharmaceutical and food industry would potentially provide more income to the grower. The volatiles of tuberose in Kenya when analyzed and identified, the chemicals compounds present in the volatiles emitted *in vivo* by tuberose flowers grown in Kenya will be the effect of the environment on emission of tuberose flower volatiles.

Once these gaps are addressed, it will enhance competitiveness of tuberose enterprises and encourage farmers to grow more and better quality tuberose flowers for export and domestic markets. The higher volumes will provide raw materials for the pharmaceutical, cosmetic and confectionery industry.

CHAPTER THREE

3.0   MATERIALS AND METHODS

3.1   The effect of nutritional factors on the colour development *Polianthes tuberosa*Linn.

The Kenya Agricultural Research Institute (KARI) station in Thika was the main field forexperimentsand also the source of material for *ex situ* experiments. The field station is located within the Research Centre in Thika sub county, Central Kenya. Itis situated at 0^o 59'South and 37^o 04' East at an altitudeof 1548 metres. The weather data for the period March to September, 2007 was obtained from the weather station, which was500 metres from the experimental plot. Temperatures range at KARI -from 15 °C at night to 28 °C on hot days. The plot usedfor the experiments had been fallow for the previous two years. The plants were grown using guidelines from KARIand Ministry of Agriculture (Anon, 2004; 2001). Tuberose bulbs were planted in March 2007 on raised 1-metre beds witha spacing of 20cm between plants and 20cm between the rows.Irrigation was done when the need aroseusing overhead sprinklers.

3.1.1   Tuberose flower spike development

At 78 days after planting there was the first flush (approximately 10% flowering) and flowering continued throughout the experiment. The tuberose flower spike emerged with a flower head enclosed in bract leaves. Flower stages were determined for anthocyanin extraction based on morphology. Fig. 3.2.1 shows

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photographs of some of the stages and Table 3.1 gives a description of the floret developmental stages.

Table 3.1   Showing a detailed description of flower stages of tuberose spike development

Stage	Name	Description
1	*Tight bud	A bud that is tightly enclosed by the bract leaves terminating in a pointed end
2	Swollen	A bud with expanded florets under tight bracts giving it a more elliptical shape (blunt end)
3	*Cabbage head	A bud with expanded florets and loosened bracts giving a flat head resembling a cabbage
4	Bud break	The bracts are completely loosened exposing green florets
5	Elongation	Elongated spike with 1^{st} internode clearly visible
6	*Colouration	More than two internodes clearly visible, with a pinkish tinge appearing on florets
7	Spike elongation	More than 3 internodes visible
8	Floret expansion	Swollen basal florets
9	Harvest for export	The flower spike is fully elongated, basal florets are enlarged and white, partially open
10	*Harvest-local market	The basal floret on the flower spike is in bloom

* Shown in Figure3.1
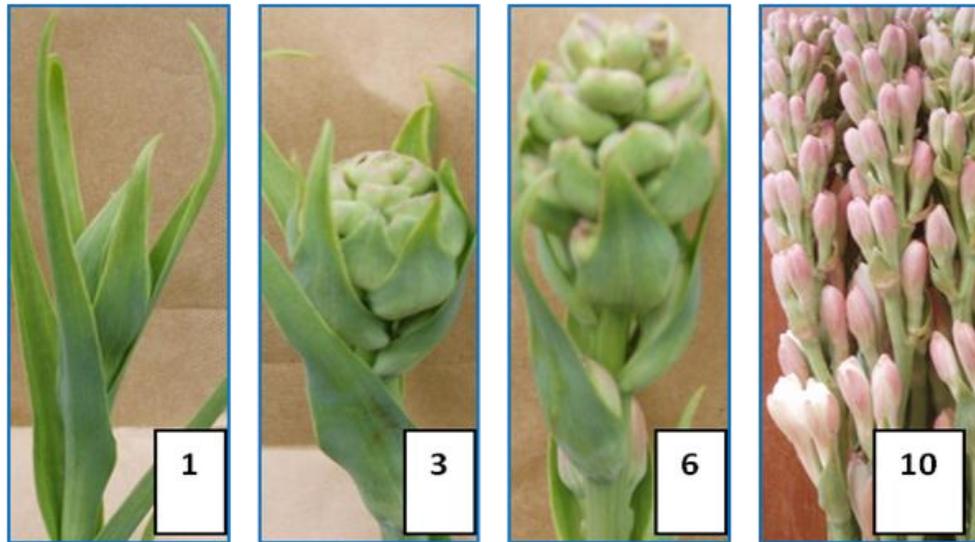
Figure 3.1   Showing some of the selected tuberose flower development stages as described in Table 3.1 above

3.1.2   Treatments and experimental layout for the effect of nutritional factors on the colour development of *Polianthes tuberosa* Linn.

Three chemical fertilizers, calcium ammonium nitrate (CAN), ammonium sulphate (AS) and magnesium nitrate ($Mg(NO_3)_2$), were used as soil amendments. Application of CAN is a routine practice by farmers and hence was used as a control in this study. The fertilizers have different chemical compositions and different pH. The fertilizers were crushed and the pH measured using a pH meter. Each fertilizer was applied at a dose of 13 g N per 2-m^2plot. The experimental layout was a completely randomized block design with four blocks and was replicated three times.

3.1.3   Soil chemical analysis

A baseline of the experimental field was established before planting. This was used to establish and select a suitable experimental site. Twelve samples were

taken using a soil auger at a depth of 10–12 cm using the transect method with a random starting point and equal distance between the sampling points. Three samples were mixed to get 4 composite samples that were analyzed. Thereafter, 3 soil samples were taken per experimental plot from the 12 plots. Subsequent samples were taken a week before fertilizer application and thereafter every 2 weeks until the 18th week after fertilizer application. This was to monitor the changes in nutrient levels.

The samples were air dried and analyzed. The parameters were determined as follows: soil pH was measured using a pH meter with a glass electrode at a soil solution ratio of 1:2:5 (Okalebo *et al.*, 1985); total soil and plant tissue N, P, K, S, Zn were determined using the Kjeldahl digestion method (AOAC, 1995).and the atomic absorption spectrophotometer (AAS) method (AOAC, 1995).was used to determine Fe, Ca and Mg content (AOAC, 1995).

3.1.4 Chlorophyll measurements

Five plants per plot were randomly selected and a recently full expanded leaf was used to measure leaf chlorophyll, leaf area, tissue nutrient analysis and dry weight. Chlorophyll was measured using a hand held Minolta™ SPAD-502 meter. SPAD refers to soil and plant analyzer development used for determining the chlorophyll content given as a SPAD value (an index of relative chlorophyll content of plant leaves). Three chlorophyll measurements were taken on each leaf along the midrib, then averaged and recorded. These same leaves were detached for leaf area measurement using a LICOR™ 3100 leaf area meter. The leaves were oven dried and dry weight determined using a laboratory weighing balance. The dried leaves were then used to analyse plant tissue.

3.1.5 Colour measurements

Four mature flower spikes at stage 10 were selected and harvested from each plot. This was carried out for all the replications. Outer petals from each flower spike were detached and placed flat on a white piece of paper. The petals were laid closely together to avoid any spaces between them to fit the 8-mm diameter head of the portable tristimulus colour analyzer (Chromameter II; Minolta™) used as described by Gonnet (1998). Flower colour was expressed in CIELAB colour-space coordinates. The meter was calibrated using the manufacturer's standard white tile. The CIELAB coordinates scale measuredL*(Lightness; Black–White where 0=black, 100=white)a* (Green–Red, where negative value=more green, positive value=more red) and b* (Blue–Yellow; where negative value=more blue, positive value=more yellow);h (Hue angle; degree of brownness where the higher the value, the more brown the colour), and C* (Chroma; colour intensity, where the higher the value the more intense). The chroma and hue values were calculated from the space coordinates as: $C* = (a*^2 + b*^2)^{0.5}$; $h_{ab} = \tan g^{-1} (b*/a*)$.

3.1.6 Chlorophyll a, b and carotenoid extraction and determination in petals

Petals used in colour measurements were wrapped in foil and stored in a freezer for extraction. Frozen petals (0.5 to 1.0 g) were ground with a pestle and mortar and mixed with chilled 80% acetone. The residue was re-extracted with 5mL aliquots of 80% acetone until the acetone remained clear. The combined extracts were adjusted to 20 mL with 80% acetone and centrifuged at 5000xg(gravities) for l0 minutes. Absorbance was measured at 645 for chlorophyll a and 663nmfor

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chlorophyll b, and at 480 nm for carotenoids. Chlorophyll and carotenoid content were calculated from the data using the equations according to Ross (1974).

3.1.7 Total anthocyanin content

Anthocyanins reversibly change colour with pH thus the differential pH method is for measuring total pigment concentration. Samples are diluted with aqueous pH1.0 and 4.5 buffers and absorbance measurements are taken at the wavelength of maximum absorbance of the pH1.0 solution. The difference in absorbance between the two buffer solutions is due to the monomeric anthocyanin pigments (Wrolstad, *et al.*, 2005). To determine total anthocyanin content, outer flower petals used in colour determination were weighed and crushed in acetone The extracts were filtered through Whatman filter paper and the filtrates were made up to a final volume of 5 mL with distilled water. The total anthocyanin content of the acetone extract was measured using a pH differential method (Meyers *et al.*, 2003). A UV mini 1240 Shimadzu spectrophotometer was used to measure absorbance at 510 and 700 nm in buffers at pH 1.0 and 4.5. Absorbance readings were converted to total mg of cyanidin 3-glucoside per 100 g fresh weight of tuberose petals using the molar extinction coefficient of 26,900 and absorbance of A = [(A510 - A700)pH1.0 - (A510 - A700)pH4.5]. The total anthocyanin in the sample was calculated as:

$$\% \text{v/v} = \frac{A}{\varepsilon L} \times MW \times DF \times \frac{V}{Wt} \times 100\%$$

where A = Absorbance, ε = Cyd-3-glu molar absorbance (26,900), MW = anthocyanin, molecular weight (449.2), DF = dilution factor, V = final volume (mL),

Wt = sample weight (mg), L = cell path length (1 cm). Data are reported as means ± SD for three replicates.

3.1.8 Measurements of magnesium levels in flower petals

Magnesium analysis was carried out as described by Okalebo (1985). Florets (2.5 g) were dried to 70°C for 48 hours. 0.30 g of dry material was digested in 4.4 ml of digestion mixture at 360°C for 2 hours. The digest was made up to 50 ml with distilled water. Magnesium concentration in the digest solutions was determined using an atomic absorption spectrophotometer at wavelength 285.2.

The concentration of magnesium in the tissues was expressed in percentage and calculated as follows:

$$Mg\% = \frac{(a - b) \times v \times f \times 100}{1000 \times w \times 1000}$$

Where a = concentration of Mg in the digest, b = concentration of Mg in the blank, w = weight of sample, v = volume of the digest solution, and f = dilution factor.

3.2 The effect of light quality on colour development of *Polianthes tuberosa*Linn.

3.2.1 Study sites

Two locations were chosen to study the effect of modified light on colour development. Modified light was achieved by the use of shade nets in red, black and beige compared to natural direct solar radiation referred to as open. KARI- Thika station in was chosen for sunny and warm conditions while Meru represented the overcast and cool conditions. Below are details for the Meru study site.

Gregory Gitonga Farm—Meru

This experimental site was provided by a farmer,Mr. Gitonga.It lies at longitude 37'34'' and latitude 0'00'' andat altitude 2911 meters. The temperatures ranged from 05 °C at night, to 29 °C on hot days; December and July were the coldest months and September the hottest month. The long rains were in October to December while short rains were during the months of March to April. The tuberose plants were grown followingprocedures for tuberose growing guidelines;the plants were regularly irrigated using overhead sprinklers.

3.2.2 Treatments for the effect of light quality on colour development of *Polianthes tuberosa* Linn.

Coloured nets providing 50% shading were obtained from Shadenet Ltd, Thika, Kenya. They were black, red, and beige colours. Spectra of solar radiation in

the open and under the nets were measured by a Lux meter. Emerging flower spikes at cabbage stage 3 (Fig.3.2a) were identified at cabbage stage; they were bagged with a brown bag for 4 days. The brown bag covering the inflorescence was large enough to allow for air circulation. A waterproof cover was put on the brown bag to avoid moisture infiltration (Fig. 3.2.b). Four flower spikes were left uncovered in open field conditions as a control. The brown bags were removed from the inflorescences after 4 days and the inflorescence covered with: no net, red, beige, and black nets (Fig. 3.2c) for 10 days. Each experimental cycle took 14 days using 16 tuberose plants. The inflorescences were harvested for colour determination in the laboratory.
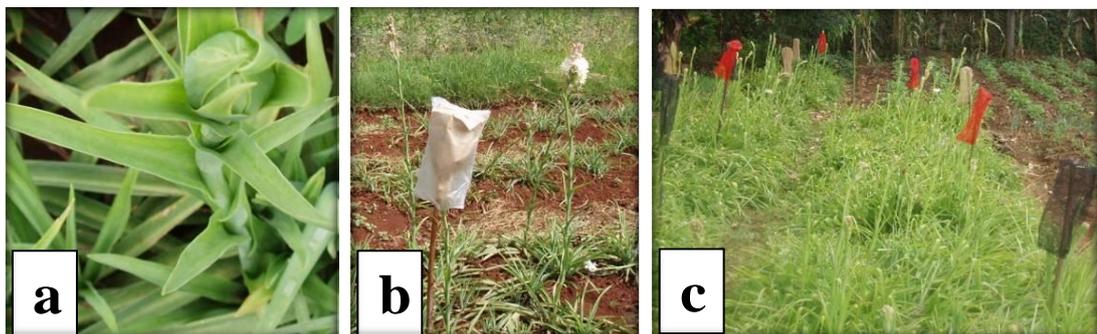
Figure 3.2   a) Tuberose flower at cabbage head stage b) bagging and c) coloured nets

3.3 The influence of magnesium on colour development of the florets of *Polianthes tuberosa*Linn. Under transient temperature

3.3.1 Experimental Design

Three chambersfor holding florets were set at the following temperatures: 32ºC (high), control of 25ºC (normal field) and 19ºC (cool) with a diurnal range of approximately 10ºC (Fig. 3.1).The light levels in all the chambers were (300 μmol m^{-1} s$^{-1)}$.Neutral day regimes were achieved by turning the lights off in the chamber after 12 hours of light. The growth chambers were maintained at day/night temperature of 19ºC/9ºC, 25ºC/15ºC, and 32ºC/22ºC.

Flower spikes at Stage 3 with expanded florets and loosened bracts (Fig. 3.4a) were selected from field grown plants, bagged with brown bags and detached after 4 days. The flower spikes were held in an upright position by Styrofoam board in a plastic container containing 5% sucrose solution at a pH 6.9 (Fig 3.4b). Thespikes were sprayed using a spray bottle with water (control), 0.0 and 1.2 and 2.4nM$Mg(NO_3)_2$(Fig 3.4c)and placed in the Styrofoam and covered loosely with plastic film to enable gas exchange. The four containers were placed on one shelf in a completely randomized design. Each treatment had3spikes and was replicated4 times.

3.3.2 Transient temperature

Three sets of treated flower spikes were put in each chamber for 3 days and then moved to a subsequent temperature for the next 3 days as shown in the Table 3.2.

Figure 3.3   Showing the cool white fluorescent bulbs on all 4 sides to give uniform lighting in the growth chamber.
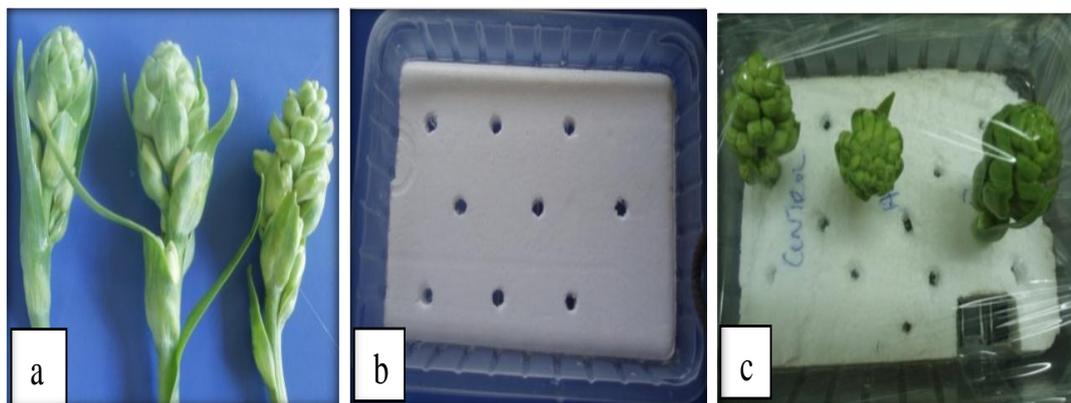
Figure 3.4 Showing the a) flower spikes 0 days after treatment (dat). b) plastic container and Styrofoam c) plastic film-covered spikes showing control (C) 0.0 nM, (A) 1.2 nM and (B) 2.4nM $Mg(NO_3)_2$.

Table 3.2 Table showing the range of temperature treatments

1st temp	2nd temp	DIC (3:3)	1st temp	2nd temp	DIC (3:3)	1st temp	2nd temp	DIC (3:3)
19 ℃	32 ℃	**(19:32)**	19 ℃	25 ℃	**(19:25)**	19 ℃	19 ℃	**6 dat**
25 ℃	19 ℃	**(25:19)**	25 ℃	32 ℃	**(25:32)**	25 ℃	25 ℃	**6 dat**
32 ℃	25 ℃	**(32:25)**	32 ℃	19 ℃	**(32:19)**	32 ℃	32 ℃	**6dat**

DIC – days in chamber
(3:3) – three days in each chamber at temperature in column

After three days in the chamber, flower heads removed photographed. One flower head was removed from the container and used for colour measurement. Flower heads were prepared for colour measurements by detaching outermost petals and placing them flat on a white piece of paper. They were laid closely together to avoid any spaces between them to fit the 8-mm diameter head of a portable tristimulus colour analyzer (Chromameter II; Minolta™) used. Flower colour was expressed in CIELABc olour-space coordinates.

3.3.3 Data analysis

The Statistical Package SAS 9.1 was used to analyze the data and means were separated using $LSD_{0.05}$. The ANOVA test was also used to determine whether differences between colours of samples were statistically significant. Values of $p < 0.05$ were considered to be significantly different. The differences between means and the relationship between the: coloured nets and the colour and colour parameters and the temperatures were determined using $LSD_{0.05}$.

3.4 The influence of location on composition of biochemical compounds of tuberose floral volatiles

3.4.1 Study sites

The treatment was the experimental site. There were four sites with different altitudes (Table 3.4). Plants for the studies were grown by commercial flower farmers in Sagana and Meru. In Tigoni and Juja plants were grown in institutional plots provided for the research work. All the plants were also grown according to the tuberose growing guidelines (Anon, 2001). The study was carried out 15-30[th] October 2009.

John Kahindi Farm—Sagana

This experimental site was provided Mr. Kahindi, a commercial flower farmer who had grown tuberose since 2002. John Kahindi's farm is situated between longitude 40' 26.148" and latitude 13' 35", and lies at altitude 1214 metres.

Temperatures here range from 12 °C at night to 29 °C on hot days. Irrigation was done regularly using overhead sprinklers.

JKUAT Horticulture Farm —Juja

JKUAT farm is in Juja at a latitude; 1° 10' South and longitude; 37° 7' East at an altitude of 1416 m. This experimental site was provided by the Department of Horticulture, JKUAT. The tuberose plants were grown using procedures according to the tuberose growing guidelines and irrigation was done regularly using overhead sprinklers.

KARI -Tigoni Floriculture —Tigoni

The KARI-Tigoni station is located 40 km North-west of Nairobi city centre, at an altitude of 2131 masl, latitude of 1°15' South and longitude 23° 46' E. This experimental site was provided by the Centre Director, KARI-Tigoni. The tuberose plants were grown using procedures according to the tuberose growing guidelines and irrigation was done regularly using overhead sprinklers.

Table 3.3   Showing volatilesampling times and weather conditions in Sagana, Juja, Tigoni and Meru.

Location	Altitude(m)	Sampling time (p.m.)	Ambient temp. (°C) and rainfall (mm)
Sagana	1214	5.45–6.45 7.03 -8.03	17.5 17.5–17.0 Rain ceased ½ hr- wet soils
Juja	1520	5.30-6.30 6.46 -7.46	21–19.0 19.0–18.0Dry
Tigoni	1850	5.52–6.52 7.14 -8.14	10.5 10.5–10.3light drizzle during sampling
Meru	2068	6.00 -7.00 7.20 -8.20	9.0–8.0 8.0–7.0Dry

3.4.2   Volatile collection technique and pollinator monitoring

The floral volatiles of tuberose were collected using the push and pull technique. The portable pump for collecting volatile emissions in vivo is illustrated in Figure 3.5A. The pump was connected to flow meters controlling the air flow into and out of the chamber and a Super Q volatile trap was used to collect the volatile by vacuum suction. With this method volatiles are conveniently collected *in situ* from enclosed plant organs to avoid additional emission of VOCs due to wounding effects (Tholl, *et* al., 2006). The volatile trap was made of a glass cartridge (7 mm internal diameter (id)) filled with 100 mg of Super Q (Alltech Associates, State College, Pennsylvania, USA) adsorbent. A double pump (Figure 3.5. B) was used in this study and two samples were collected simultaneously *(a* and *b),* a makeshift tent for pump was used during rain and drizzle (Fig. 3.6. A). The collection chamber was made of a

polyvinyl acetate bag (406 x 444 mm) sealed with a plastic twist tie (Fig.3.6B). The sampling was conducted from 1730 to 2100 hours, when a high volatile emission was expected. Tuberose flowers exhibit nocturnal maxima (Dudareva *et al*., 2006). At each site a control (blank no inflorescence enclosed to determine extraneous substances within the chamber) and three samples were taken. Each sampling included three samples from individual inflorescence. Three open flowers on each inflorescence were sampled. The 1st hour of sampling was referred to as *Time1* and the 2nd hour as *Time2* at all sites. Sampling time was exactly one hour. Pollinators visiting the flowers were monitored through observation during the time of volatile collection. After collection, the filter was eluted with 3 mL of dichloromethane and the extracts were stored at $-18°C$.
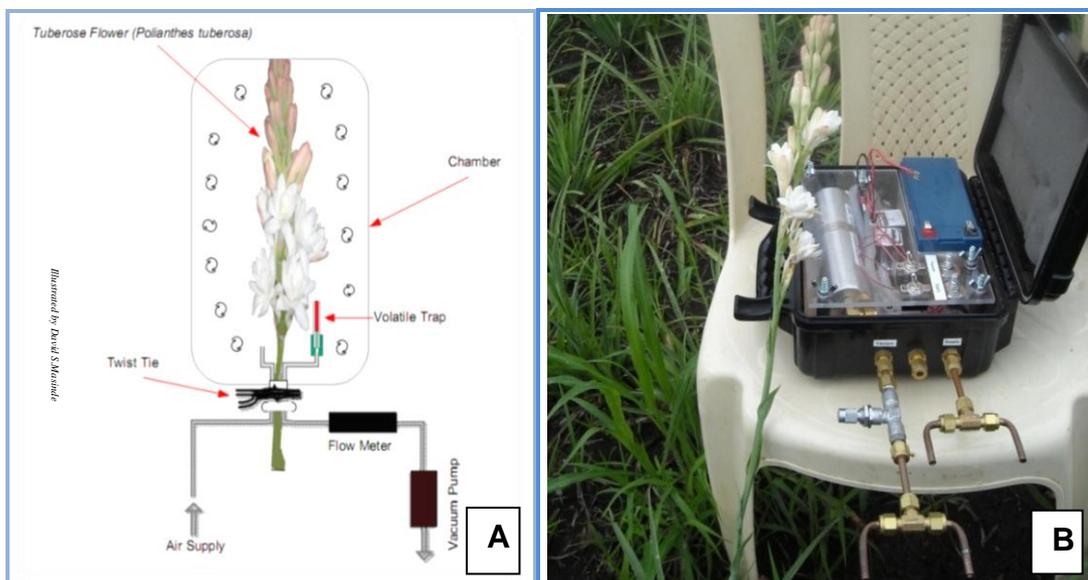
Figure 3.5   A) Push and pull device showing the inflorescence in the chamber. B) VOC pump set up for in vivo volatile collection

Figure 3.6 A) Makeshift tents for pump when there was drizzle in the field.B) The polyvinylacetatebag inflates and forms the chamber when air is pumped in

3.4.3 Gas chromatography mass spectrometry (GC–MS) analysis of floral volatiles

Volatiles were analyzed using GC HP 5890 series II GC equipped with a flame ionization detector (FID) and an HP-5 column (30× 0.25 mm internal diameter (ID)×0.25 μm film thickness). Nitrogen was used as carrier gas, with a column pressure of 46 psi and injection temperature of 250°C. One μL of sample was injected in the splitless mode, with the oven temperature programmed from 60°C for 5 minutes to 280°C at 10°C/min, and held at this temperature for 15 minutes. GC-MS analysis was carried on an Agilent Technologies 7890A GC equipped with an HP-5 MS capillary column (30×0.25 mm ID×0.25 μm film thickness) coupled to 5795C MS. One microliter of each sample was injected in the splitless mode, and helium was used as carrier gas at 1.0 ml min^{-1}. The oven temperature was from 35°C for 5 min, increased to 280°C at 10°C min^{-1} and then held at this temperature for 15 minutes. Spectra were recorded at 70 eV in the electron impact (EI) ionization mode.

Compounds were identified by comparing mass spectra and retention times with those of reference compounds as well as with mass spectra in different computer libraries.

3.4.4 Data analysis

Volatile components were analysed after the control values were subtracted from the sample values. The means were presented with the standard deviation. Variation in data and percentages of the peak area were determined using Excel Analysis Toolpak, 2007 for Windows.

CHAPTER FOUR

4.0   RESULTS

4.1   Effect of nutritional factors on the colour development of *Polianthes tuberosa* Linn

The monthly average maximum temperatures ranged from 23°C to 28°C and minimum temperatures from 13°C to 16 °C. Daily temperature ranged from 17°C to 29°C during the day and as low as 10°C at night to 22°C (data not shown).Rainfall was heavy during establishment of the plants but minimal during top dressing (June–September 2007) hence irrigation was carried out; solar radiation was low between June and September (2007) and relative humidity was low during the month of March and June.
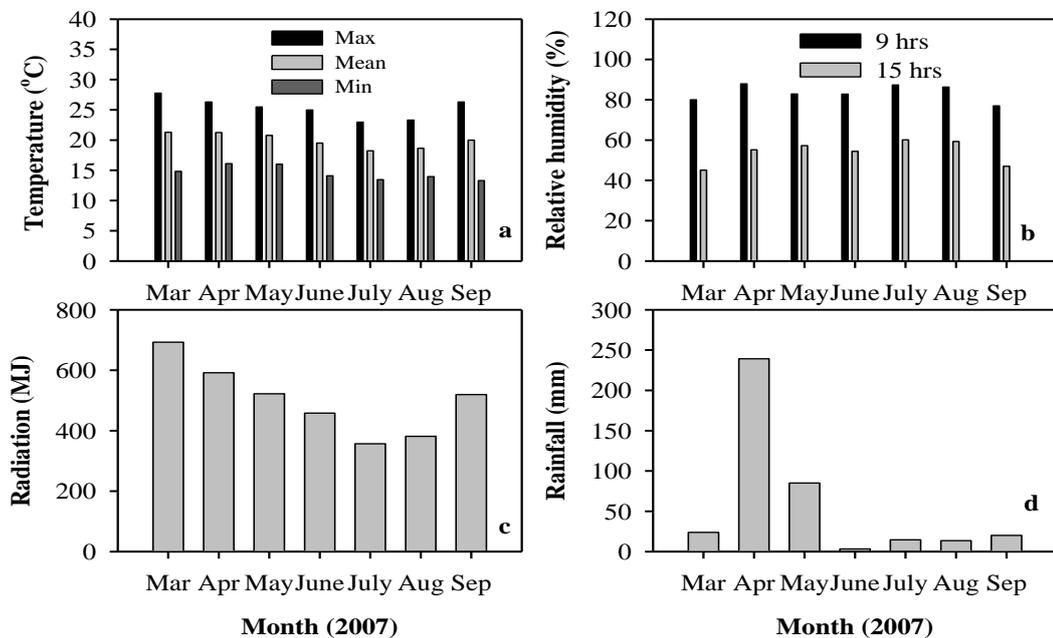
Figure 4.1 Pattern of key weather elements a) temperature, b) relative humidity, c) radiation and d) rainfall during the experimental period at KARI-Thika.

Table 4.1 Chemical and physical characteristics of the applied amendments used as treatments for nutrition studies at KARI -Thika (January–September 2007)

Treatment	pH (measured)	Commercial Nutrient content	Weight of fertilizer (g) 13g N per 2-m^2 plot
Calcium ammonium nitrate (CAN; farmer's practice =control)	5.3	26% N 26.5% CaO	50
Ammonium sulphate(NH_4SO_4; AS)	2.7	21% N 24% S	62
Magnesium nitrate ($Mg(NO_3)_2$)	3.7	15% Mg 11% N	118

Table 4.2 Soil characteristics prior to and after tuberose establishment in the experimental field at KARI -Thika (January–September 2007)

Prior to planting (fallow 2 years)										
pH	%N	% OM[a]	%P	K Meq[b]	Ca Meq	Mg Meq	Zn ppm)	Fe ppm)	Mn (ppm)	Cu (ppm)
5.9	0.4	11.0	0.0046	8.1	3.3	4.2	1.6	27.2	208.4	2.1
After planting (before top dressing)										

| 6.6 | 0.058 | | 0.094 | 5.6 | 0.82 | 4.06 | 14.6 | 76.6 | 563.6 | 1.7 |

[a]Meq –milli-equivalent
[b]OM –0rganic matter

Table 4.3   Soil pH in the experimental plots during the nutritional studies of tuberose at KARI- Thika (January–September 2007)

Amendments	Weeks after treatments			
	0	12	16	18
CAN	6.8	5.1a	5.3a	5.3a
NH_4SO_4	6.8	5.1a	4.8a	4.6a
$Mg(NO_3)_2$	6.8	6.5b	6.5b	6.8b
$LSD_{0.05}$	-	0.5	0.5	0.6

Means followed by the same letter down the column are not significantly different at (p ≤5%) using SAS 9.1 statistical package

Leaf growth was similar between the fertilizer types in terms of leaf area and leaf dry weight (Fig. 4.2). The fertilizers AS and $Mg(NO_3)_2$ were acidic relative to CAN (Table 4.2). The soils were generally of a weak acidic pH ranging from 5.9 to 6.6 before the fertilizer additions (Table 4.2). After application of fertilizers, the soil pH was significantly lower in soils treated with CAN and AS (Table 4.3).
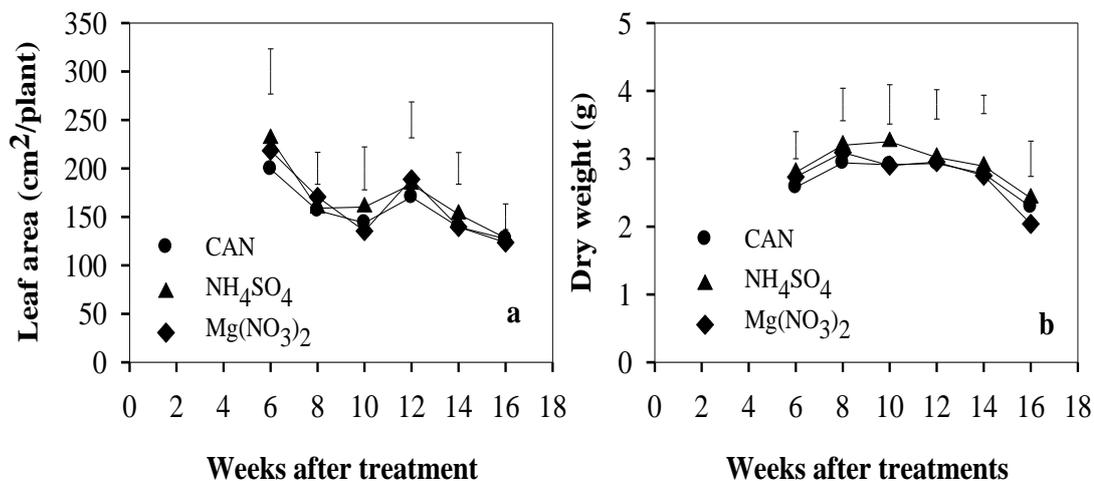
Figure 4.2 Comparison of vegetative growth during application of amendments a) leaf area and b) dry weight at KARI -Thika (January–September 2007). Vertical bars show LSD$_{0.05}$.
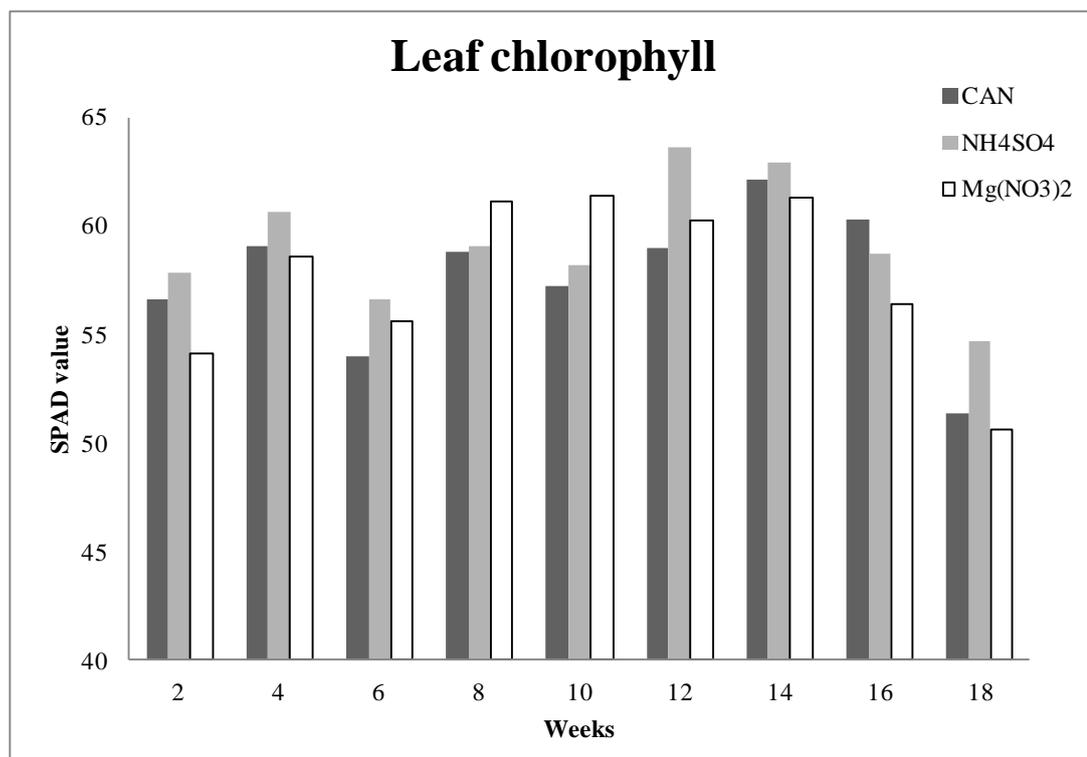
Figure 4.3 Foliar SPAD values (chlorophyll) of tuberose plants during application of amendments at KARI -Thika (January–September 2007). Vertical bars show LSD$_{0.05}$.

SPAD values (chlorophyll) did not show significant differences between the fertilizers applied except at 10 weeks after application when the $Mg(NO_3)_2$ treatment had significantly higher values, with values reaching higher levels that ranged from 61–64 between 8 and 14 weeks. By the 18th week, the values had declined to 51–55(Fig. 4.3).

The type of fertilizer applied had no significant effect on the L* values. However, L* values in plants where NH_4SO_4 was applied increased gradually up to the 16th week before declining (Fig. 4.4a). L* values were generally in the range of 55–70. The a* values were significantly higher in plants supplied with CAN at 16 weeks, but the other sampling dates showed no clear trends (Fig. 4.4b). Fertilizer types had no significant effect on b* values, but generally plants supplied with CAN and $Mg(NO_3)_2$ saw a decline in b* values over time. In contrast,　plants supplied with NH_4SO_4showed a general increase in b* values before a decline at the 14th week (Fig. 4.4c).
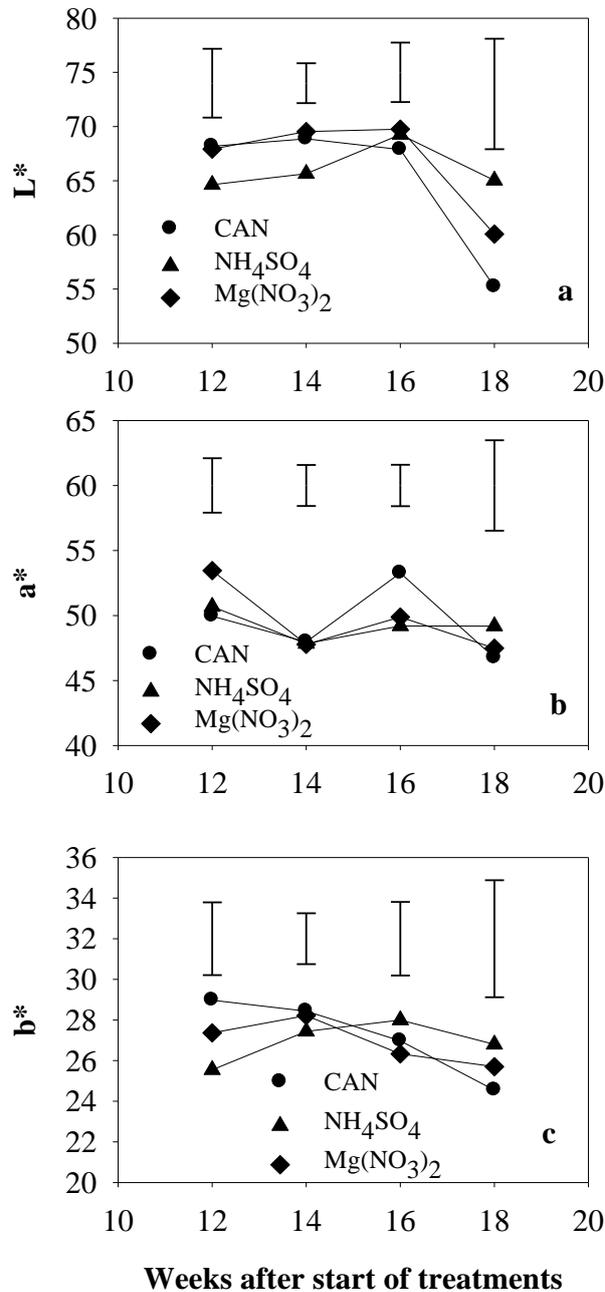
Figure 4.4 The CIELAB space coordinates of tuberose petals during application of amendments a) L* Lightness; Black–White; b) a* Green-Red; and c). b* Blue-Yellow. Vertical bars show LSD$_{0.05}$.

Chroma was significantly higher in petals of plants supplied with CAN at 16 weeks (Fig. 4.5a). However, there were no clear trends in chroma levels among the sampling dates. The values generally ranged from 53 to 60 radians. Fertilizer types

had no significant effect on hue values, but generally plants supplied with CAN and Mg(NO$_3$)$_2$showed a decline in hue values over time, while plant supplied with AS had a general increase before a decline at 14[th] week (Fig. 4.5b). The hue values were generally in the range of 27 to 30.
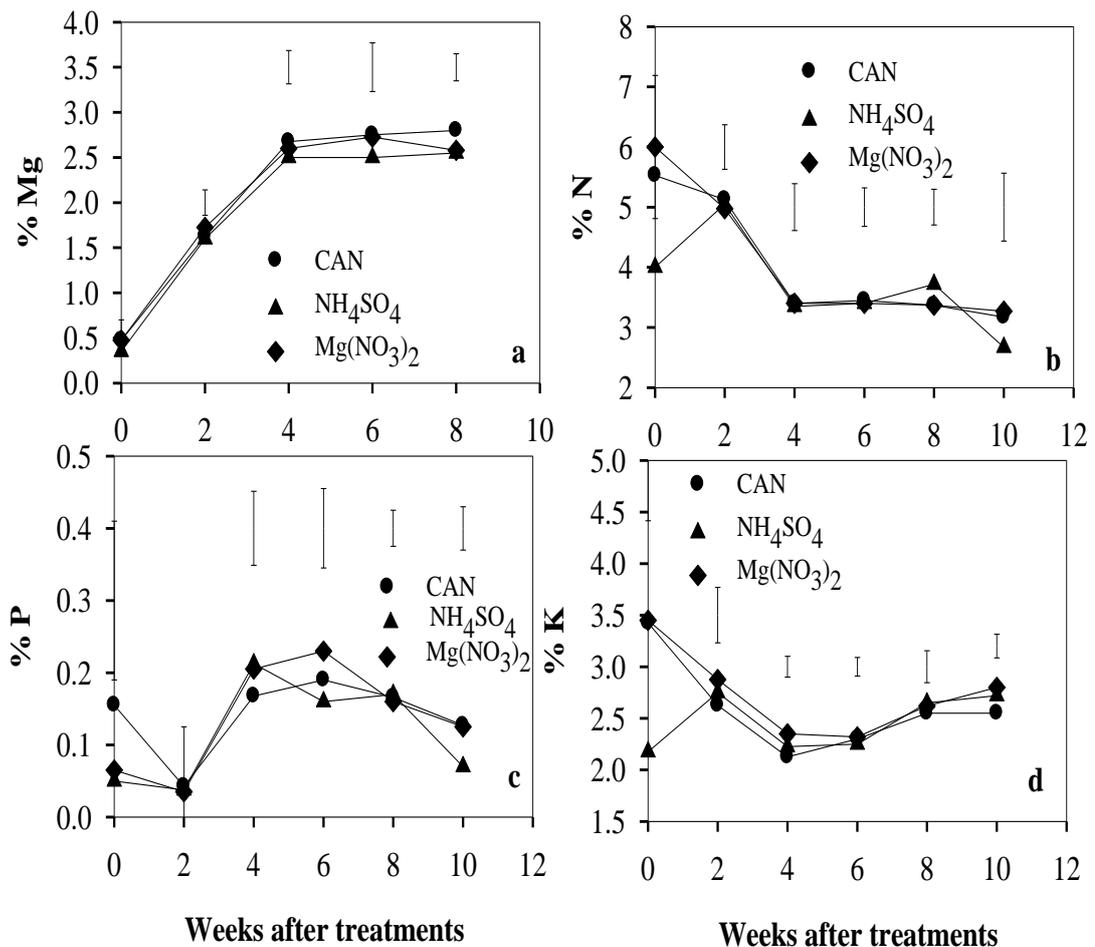
Figure 4.5 Levels of chemical elements in tuberose leaf tissue on dry weight basis: a) % Mg; b) % N; c) % Pd) % K grown at KARI-Thika (March–September 2007). Vertical bars show LSD$_{0.05}$.

Table 4.4   Effect of soil applied amendments on chlorophyll, anthocyanin and Mg contents of tuberose petals at week 18 at KARI -Thika (January–September 2007)

Amendment	pH (measured)	Chlorophyll (µg/g)	Anthocyanin (% w/w)	Mg (%)
CAN	5.3	2.36	0.88	0.46
NH_4SO_4	2.7	1.69	2.64	0.49
$Mg(NO_3)_2$	3.7	1.67	2.61	0.47

n=12

The tissues had similar levels of Mg, N, P, and K irrespective of the type of fertilizer applied (Fig. 4.6). Mg content increased up to the 4[th] week and peaked at levels of 2.5% to 2.8%. Nitrogen showed a general decline from high levels of 6% to about 3% in the 10[th] week. P increased to peak levels at 4–6 weeks ranging from 0.16% to 0.23% before declining to 0.07% then to 0.13% by the 10[th] week. K was generally in the range of 2.1–3.4%.

CAN gave the highest chlorophyll content and the lowest anthocyanin content relative to AS and $Mg(NO_3)_2$ (Table 4.4). Mg content was marginally higher for AS and $Mg(NO_3)_2$ treatments. The AS and $Mg(NO_3)_2$had the highest anthocyanin content and the lowest chlorophyll content (Table 4.4).

4.2   Effect of light quality on colour formation of *Polianthes tuberosa* L. florets

Table 4.5   Solar radiation (Wm^{-2}) under open and various shade treatments at KARI – Thika (2008)

Shading	8.00am	12.00 noon	3.00pm	Average
Open	980 a	1717a	705a	1194
Beige net	540c	732d	431c	571
Black net	303e	531c	218d	351
Red net	362d	682d	331d	391
Brown bag	109f	145e	45e	99
LSD	20	95	29	

Means followed by the different letters down the column are significantly different

The shade percentage reduces the total amount of light by 50%. The coloured nets had enhanced shading significantly different among them. The irradiance through the beige net was 540 and red 362 Wm^{-2} while black was 303Wm^{-2} at 8.00 AM in the morning. On average, the brown bag had the lowest 99Wm^{-2} and the open had the highest irradiance 1194Wm^{-2}.

Table 4.6 The colour of tuberose florets from different regions and treatments in comparison with the RHSCC standards and the instant visual from calculator

	Colour name	L*	a*	b*	Chroma	Hue	Visual
			RHSCC				
RHS CODE	White plate	100	0.0	0.0	0.0	360	
4R 411	Pink	71.5	69.5	22.5	73.05	17.94	
4R 412	Dull pink	57.7	63.3	19.8	66.32	17.37	
10R 1002	Pale yellowish pink	85.1	56.1	20.2	59.63	19.80	
10R 1003	Yellowish pink	79.3	72.8	35.8	81.13	26.17	
10R 1004	Strong yellowish pink	75	80.8	43.6	91.81	28.35	
			FARMERS				
1	Meru	62.1	55.9	23.4	60.6	22.7	
2	Thika	68.6	41.8	31.8	52.6	37.4	
			SHADING				
1	Brown bag	65.6	38.1	31.5	49.5	39.6	
2	Black 50%	78.0	35.0	30.9	46.8	41.4	
3	Red 50%	74.7	40.7	30.9	51.2	37.3	
4	Beige 50%	77.0	33.1	34.7	47.9	46.4	
5	open	72.9	54.6	22.9	59.2	22.7	

Visual obtained using online color calculator from www.easyrgb.com

In Meru, the redness values differed significantly between treatments. At Thika, the florets left open had the highest redness at 54.6 radians, while florets under black and red nets had the lowest at 35.0–40.7 radians (Fig.4.7a). The redness values were statistically similar, with open at 43.3 radians and black at 42.9 radians (Fig.4.7). Similarly, in Meru open florets had the highest redness value at 43.3 radians while under black and red nets redness ranged between 38.4 radians and 43.0 radians, although there were no significant differences (Fig.4.7).

Figure 4.6   The L* value for tuberose under different light shading at Thika and Meru. Bagging was used as control for both regions. Vertical bar shows $LSD_{0.05}$.
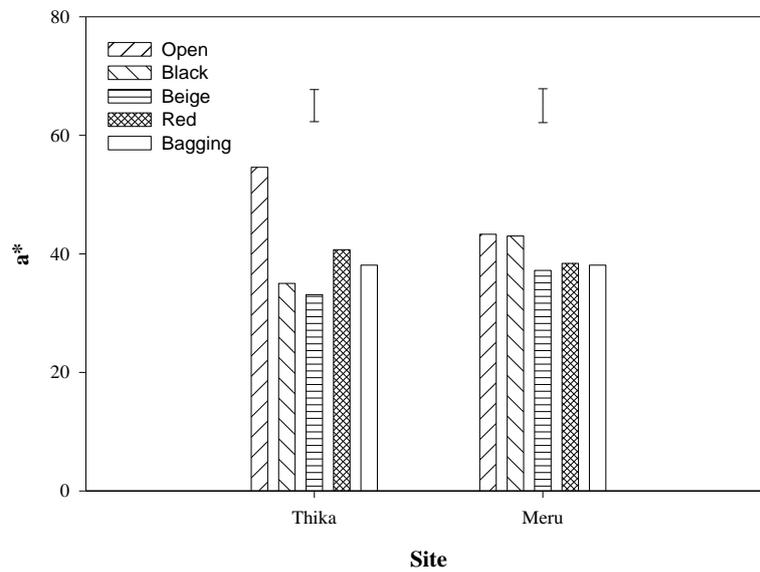
Figure4.7   The redness value for tuberose under different colour shading at Thika and Meru. Bagging was used as control for both regions. Vertical bar shows $LSD_{0.05}$.

Red and black netting resulted in significantly higher yellowness value at 30.9 while open had the lowest at 22.9 radians during the first sampling (Fig. 4.9).

On the second sampling, open also had the lowest at 26.4 radians while black had a higher value of 27.6 radians (Fig. 4.9). At Meru, there were no significant differences. However, red and black netting had higher yellowness values at 27.6–33.4 radians while open had the lowest at 26.4 radians (Fig. 4.9).

Chroma C* showed significant differences between treatments at Thika with the open having the highest at 59.2 radians and black having the lowest at 46.8 radians on the first sampling (Fig.4.10). On the second sampling, there were no significant differences with chroma ranging between 50.7 and 51.1 radians (Fig. 4.10). In Meru, there were no significant differences and no clear trend (Fig.4.10).

Hue h^{ab} was significantly different between treatments at Thika. Red and black netting had the highest hue at between 37.3 and 41.4 radians while open had the lowest at 22.7 radians during the first sampling (Fig. 4.11). On the second date, black had higher hue at 32.6 radians but was not significantly different from open at 31.3 radians (Fig. 4.11). At Meru, there were no significant differences, but red and black had higher hue values of between 32.6 and 39.3 radians while the open had the lowest at 31.3 radians (Fig. 4.11).

The flowers harvested under modified light conditions appeared visually different especially under open and beige net, while those under red net were similar to the open when colour measurements were done with the tristimulus colorimeter (Fig. 4.12).
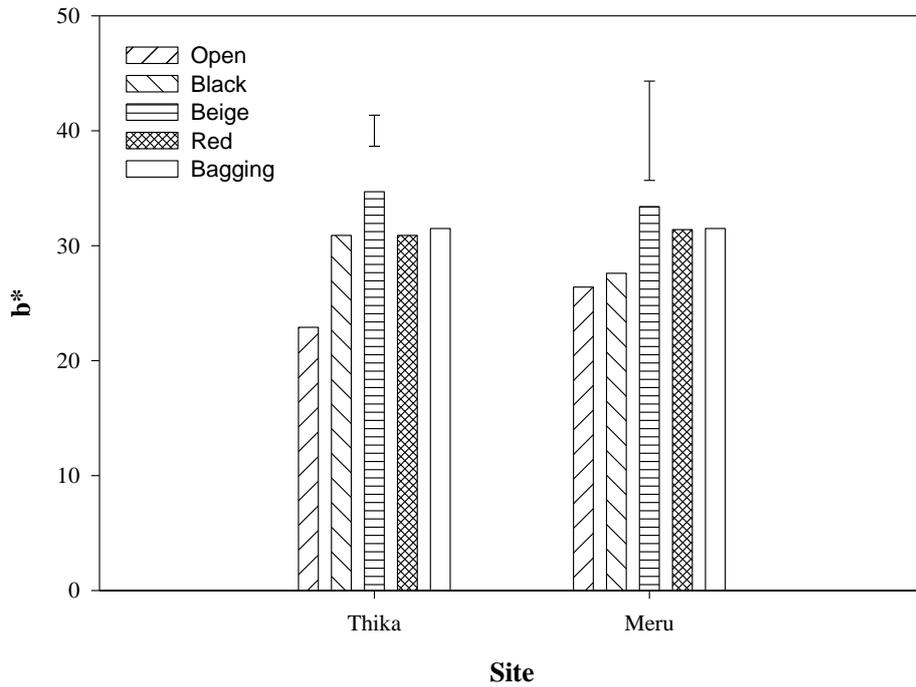
Figure 4.8   The yellowness value for tuberose under different colour shading at Thika and Meru. Bagging was used as control for both regions. Vertical bar shows LSD0.05.
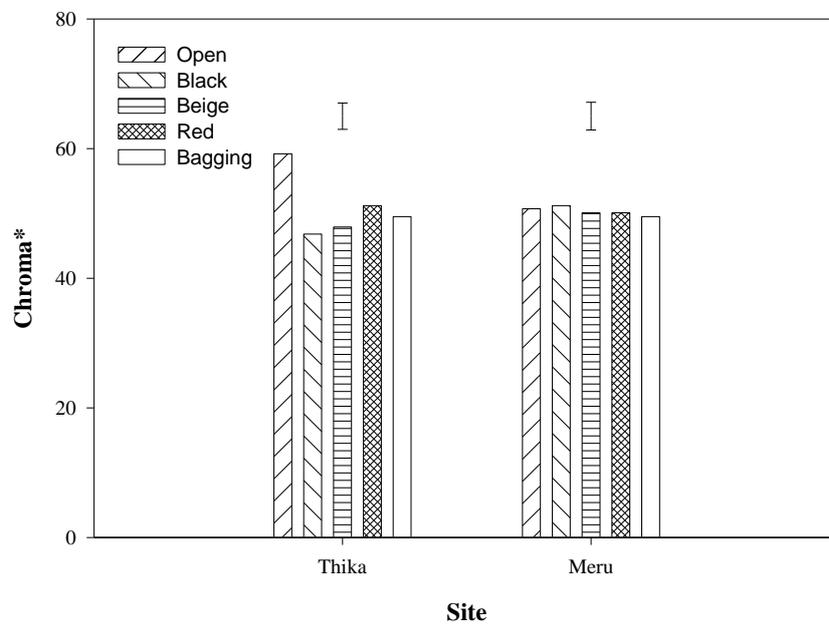
Figure 4.9   The chroma value for tuberose under different colour shading at Thika and Meru. Bagging was used as control for both regions. Vertical bar shows $LSD_{0.05}$.
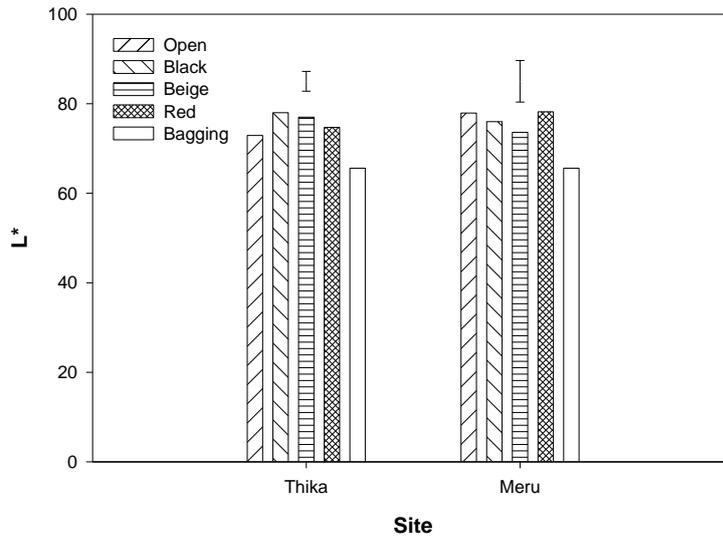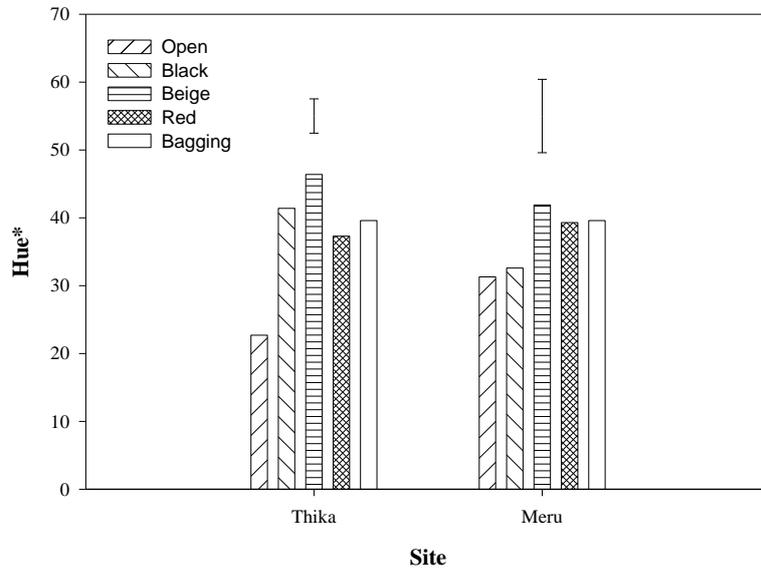
Figure 4.10   The hue value for tuberose under different colour shading at Thika and Meru. Bagging was used as control for both regions. Vertical bar shows $LSD_{0.05}$.
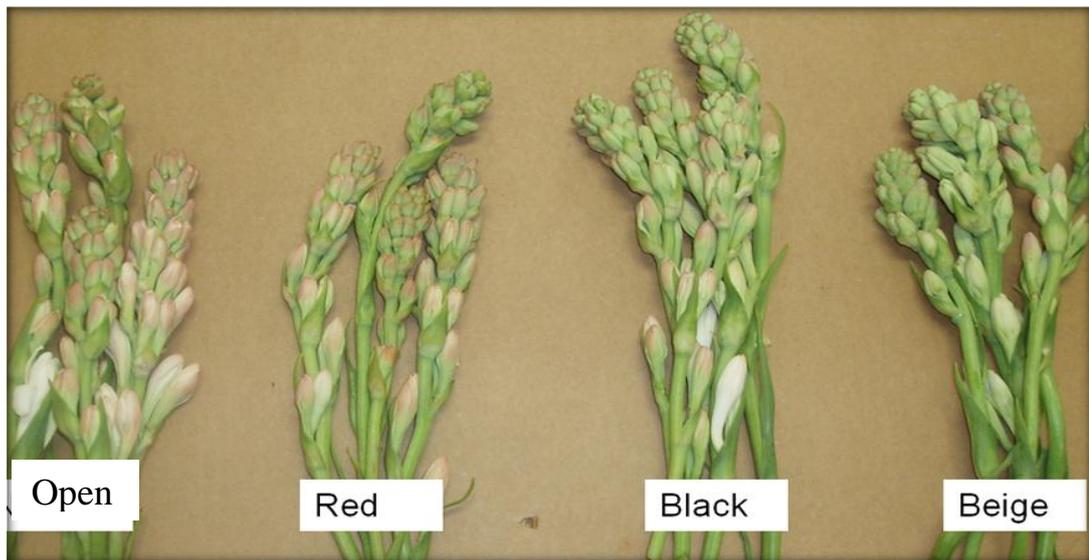

Figure 4.11   Flower spikes 10 days after light conditions were modified by coloured shade nets.

4.3   The influence of magnesium on colour development of the florets of

   Polianthes tuberosa **Linn.under transient temperature**

Colour developed at transient temperatures: at 19:25ºC, 25:19ºC, 25:32ºC, 32:25ºC and 32:19ºC did not appear to be different for all the parameters measured. However, hue and a* were significantly different for changes after 3 days at 19ºC to 32ºC for 3 days, and from 32ºC after 3 days to 19ºC for 3 days (Table 4.7). The higher Mg concentration had a significantly lower hue and higher redness

Various intensities of colour developed are shown in Fig. 4.13 on the flower spike at different temperatures and different days after treatment. At 19/9ºC,colour developed on the florets with or without Mg treatment. Fig. 4.13 (i) shows that there was a blush on the exposed petals. The close-up reveals that in Fig. 4.13 (ii) the petals treated with Mg developed the reddish-pink colour as well as the bract subtending the twin florets. Fig. 4.13(iii) shows that with 2.4nM Mg treatment produced a more intense reddish pink colour than Fig 4.13 (i and ii). There was a difference in colour development at 19ºC after 3 days compared to colour development at 32ºC after 3 days. At 19ºC the petals appeared to have a more intense reddish pink colour than those at 32ºC which appeared faded or bleached (Fig. 4.13 iv). Comparison of colour developed at 3:3 days 19:32ºC, and at 19ºC for 6 days showed the colour of petals of the florets moved from19ºC to 32ºC did not appear to be as intense as those at 19ºC for the entire 6 days (Fig. 4.13 v).

Table 4.7 The influence of Mg solution applied on the florets on colour development at transient temperatures

Temperature (3:3 d)	nM Mg	Hue	a*
19/32	0.0	52.50 [c]	28.63 [c]
19/32	1.2	53.70 [bc]	27.87 [c]
19/32	2.4	47.49 [d]	32.07 [a]

Means followed by the different letter down the column are significantly different

Fig. 4.13(vi) shows that with 2.4nM Mg at 32ºCbleaching and effects of scorching was observed. After 6 days, there was no colour development at 32ºC, however, floret petals were severely scorched, especially those that had not been treated with Mg (Fig. 4.13vii).

At 19ºC the yellowness decreased and was significantly different from values at 3 days (Fig. 4.14 iii). However, at 25ºC the Fig. 4.14 (ii) shows the redness decreased but was not significantly different from the values at 3 days while the yellowness decreased but was not significantly different from values at 3 days (Fig. 4.14 iv).

Figure 4.12   Flower heads showing colour development with Mg^{++}: treatments i) 0.0 nM 6d (19ºC); ; ii)1.2 mM (19ºC); iii) 2.4 mM (19ºC); iv) 3 d trts left 32ºC&19ºCright; v) 3d (19ºC) left compared to transient right -3d (19ºC): 3d (32ºC) vi) 3d 2.4 nM (32ºC); vii) 6d 0.0 nM (32ºC)
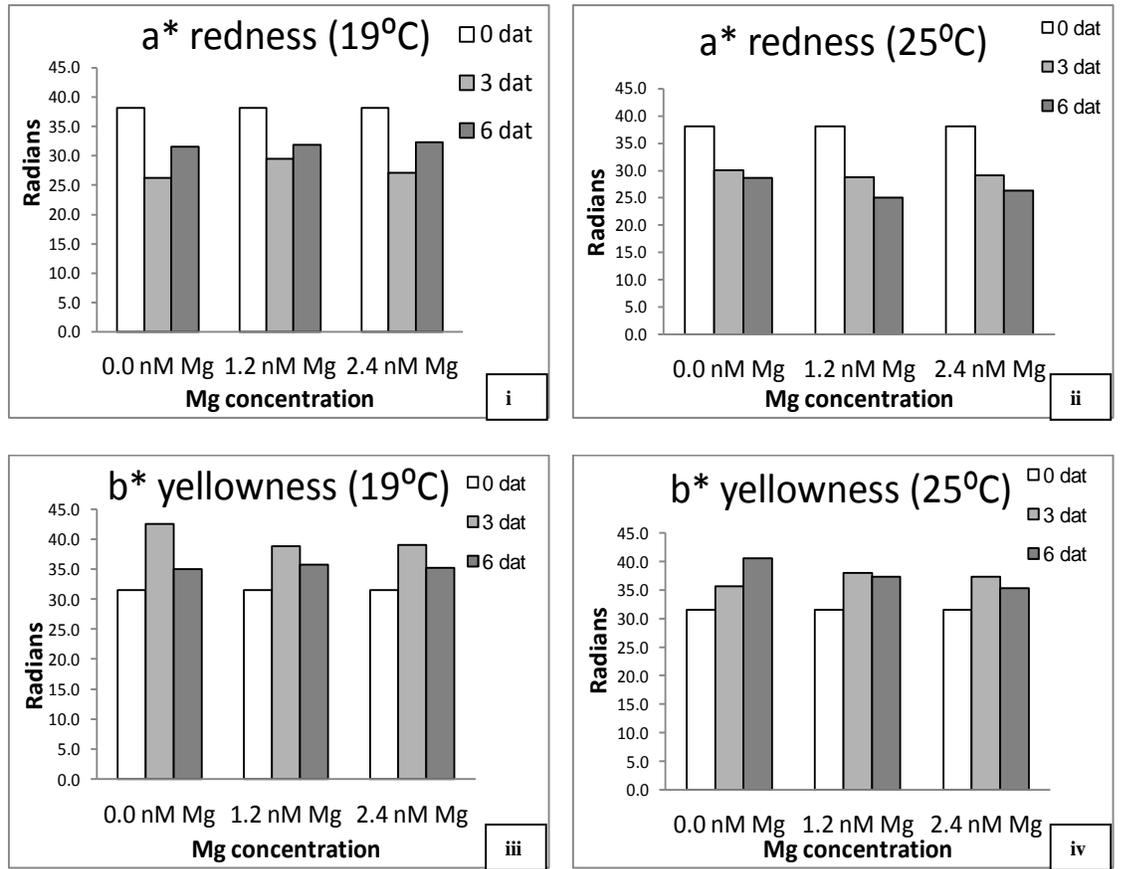
Figure 4.13   Space coordinates for i) a* redness at 19ºC and ii) 25ºC and the iii) b* yellowness at 19ºC and iv) 25ºC sprayed with 0.0, 1.2 and 2.4 nM Mg at 0, 3 and 6 days after treatment (dat)
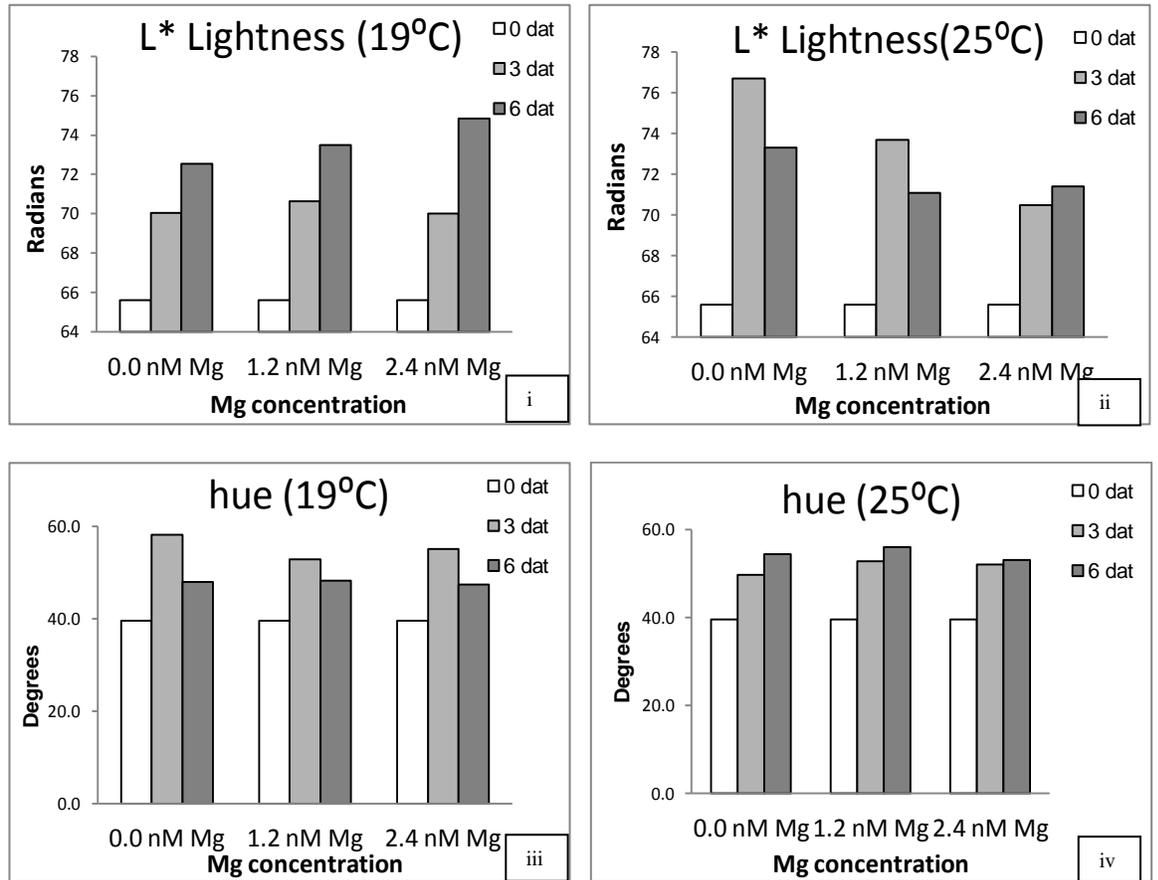
Figure 4.14　The relationship between the space coordinates L* at i) 19ºC and ii) at 25ºC and the calculated hue iii) at 19ºC and iv) at 25ºC sprayed with 0.0, 1.2 and 2.4 nM Mg at 0, 3 and 6 days after treatment (dat)

At 19ºC Lightness increased over time and was significantly different with application of 2.4nM Mg (Fig. 4.15 i). At 25ºC the Lightness decreases without Mg and at 1.2 nM Mg but increases with 2.4nM Mg (Fig. 4.15 ii). The reduction in hue at 19ºC was significantly different over time (Fig. 4.15 iii) but did not appear to have been influenced by the Mg treatment. However at25ºC there was no difference (Fig. 4.15 iv).

4.4   The composition of the floral fragrance of *Polianthes tuberosa* Linn.flower grown in Kenya
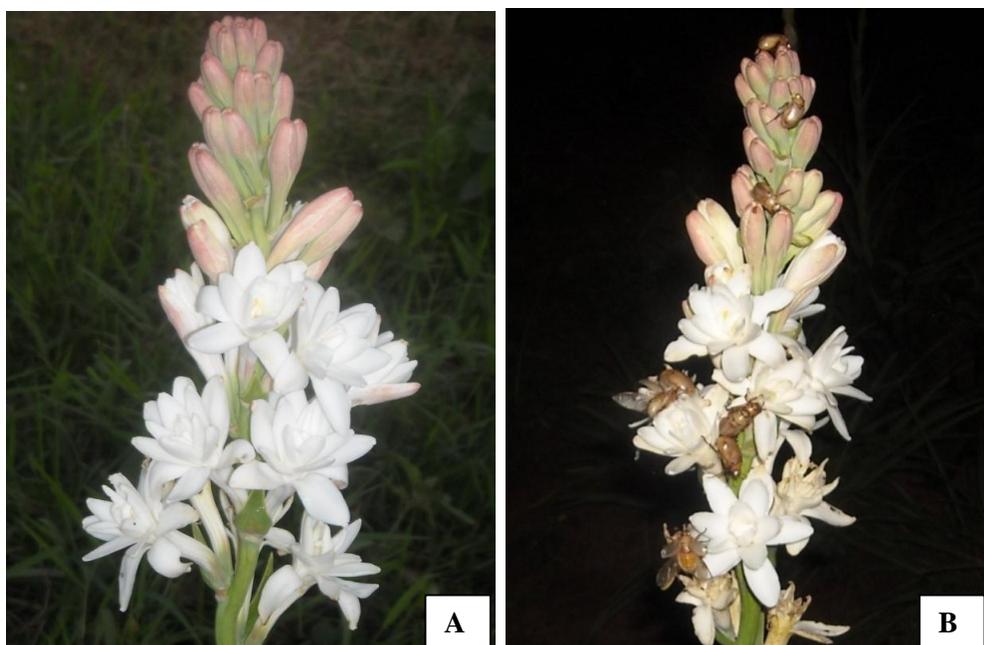
Figure 4.15   Visitation of beetles driven by scent emission: A) 1714 hrs no beetles, and B) 1817 hrs with beetles

GC-MS analysis revealed more than 28 compounds from the *in vivo* emission of tuberose flowers (Table 4.6). Twenty-one compounds were found in sufficient abundance to allow quantification in all flowers at all altitudes. Most compounds could be classified according to terpenoid biosynthetic pathways. The major compounds were methyl benzoate with average abundance of 47%, 1,8-cineole with 29% average abundance, alpha terpineol with 5%, and methyl anthranilate with 3%. Of the volatiles

from tuberose, 14 were oxygenated compounds, nine were monoterpenes, three were sesquiterpenes, and one was an aromatic compound (Table 4.7).

Table 4.8   Mean percentages of peak areas of 28 identified chemicals emitted in vivo from tuberose flowers in different tuberose growing locations

	Chemical identified	Mean percentage area under peaks of chemicals				Classification
No	Compound	Sagana	Meru	Juja	Tigoni	Functional group
1	Thujene<alpha->	0.08±0.1	0.00±0.0	0.11±0.1	0.00±0.0	Monoterpene
2	Pinene<alpha->	1.19±0.0	0.82±0.3	1.68±0.5	0.88±0.0	Monoterpene
3	Benzaldehyde	1.51±1.3	1.97±2.2	1.22±1.5	1.69±1.7	Aromatic aldehyde
4	Sabinene	1.97±0.1	1.40±0.4	2.76±0.5	1.56±0.1	Monoterpene
5	Myrcene	0.34±0.0	0.28±0.1	0.51±0.1	0.33±0.0	Monoterpene
6	Cineole <1,8->	27.24±2.1	24.97±12.9	29.26±3.5	28.47±9.4	Alkene
7	Ocimene<(E)-beta->	0.08±0.0	0.10±0.0	0.11±0.0	0.13±0.0	Monoterpene
8	Sabinene hydrate<trans->	0.08±0.1	0.14±0.1	0.11±0.1	0.16±0.1	Monoterpene
9	Methyl benzoate	42.71±11.2	45.77±16.6	45.55±8.7	42.63±14.8	Aromatic ester
10	Terpineol<alpha->	3.35±2.9	1.93±3.3	6.45±2.8	7.80±0.5	Monoterpenes
11	Methyl salicylate	1.66±2.9	5.95±5.2	0.00±0.0	0.00±0.0	Aromatic ester
12	[1,1'-Bicyclopentyl]-2-one	1.43±0.7	0.00±0.0	0.00±0.0	1.96±0.1	Aromatic ketone
13	Indole	1.30±0.4	1.51±0.6	1.57±1.0	1.06±0.3	Aromatic amine
14	Anthranilate<methyl-	4.11±0.8	2.92±0.9	3.46±2.0	1.96±0.7	Aromatic ester
15	Eugenol	0.49±0.1	0.63±0.2	0.65±0.4	0.55±0.1	Aromatic alcohol
16	Methyl eugenol	0.00±0.0	0.00±0.0	0.67±0.5	0.00±0.0	Aromatic alcohol
17	Longicyclene	0.02±0.0	0.05±0.1	0.00±0.0	0.00±0.0	Monoterpene
18	Cyclopentanone, 2-cyclopentylidene	0.05±0.1	0.00±0.0	0.00±0.0	0.00±0.0	Aromatic ketone
19	Bourbonene<beta->	0.04±0.1	0.45±0.2	0.00±0.0	0.31±0.0	Alkene
20	Methyl eugenol	0.54±0.2	0.63±0.3	0.00±0.0	0.00±0.0	Aromatic alcohol
21	Longifolene	0.02±0.0	0.12±0.0	0.13±0.1	0.00±0.0	Sesquiterpene
22	Himachalene<gamma->	0.05±0.1	0.35±0.2	0.21±0.1	0.00±0.1	Sesquiterpene
23	Isoeugenol<Z->	0.93±0.1	0.85±0.2	0.62±0.2	0.00±0.1	Aromatic alcohol
24	Germacrene D	0.18±0.3	1.17±1.4	0.00±0.0	0.00±0.0	Sesquiterpene
25	Methyl isoeugenol<E->	1.16±2.0	0.54±0.9	1.77±2.1	0.22±0.0	Aromatic alcohol
26	Farnesol<2E,6E->	0.32±0.1	0.05±0.1	0.17±0.1	0.82±0.0	Aliphatic alcohol
27	Benzyl benzoate	2.21±0.4	2.76±1.2	1.86±1.2	0.00±0.6	Aromatic ester
28	Isopropyl tetradecanoate	0.13±0.0	0.00±0.0	0.11±0.2	0.47±0.0	Aliphatic ester

±SD refers to means of area under peak with the standard deviation (Chromatogram shown in appendix 1)

Table 4.9   Showing the number and type of functional groups present in the tuberose floral volatiles

Monoterpenes C_{10}	Sesquiterpenes C_{15}	Oxygenated Compounds	Aromatic compounds	Total
Alpha Thujene>	Longifolene	1,8-cineole	Indole	4
Alpha Pinene	Himachalene	Benzaldehyde	-	3
Sabinene	Gamma	Methylbenzoate	-	3
Myrcene	Gemacrene D	Methylsalicylate	-	2
Ocimene	-	1,1-bicyclopentyl-2-one	-	2
Sabinenehydrate	-	Methyl anthranilate	-	2
Terpineol	-	Eugenol	-	2
Longicyclene	-	Methyleugenol	-	2
Bourbonene	-	Cyclopentanone	-	2
-	-	Isoeugenol	-	1
-	-	Methyl Isoeugenol	-	1
-	-	2E,6E Farnesol	-	1
-	-	Benzyl benzoate	-	1
-	-	Isopropyl tetradecanoate	-	1
9	3	14	1	27

Table 4.10   Number of volatiles emitted at Sagana, Juja, Tigoni and Meru locations at the given ambient temperatures

Location	Altitude(m)	Emitted volatiles identified	Temperature at site (°C)
Sagana	1214	27	17.5 17.5–17.0
Juja	1520	21	21.0–19.0 19.0–18.0
Tigoni	1850	20	10.5 10.5–10.3
Meru	2068	26	9.0–8.0 8.0–7.0

The weather conditions under which the volatiles were collected were not similar in the different areas. Sagana and Tigoni were wet while Meru and Juja were dry. Temperatures during sampling ranged from 7–9°C in Meru, to 10.3–10.5°C in Tigoni, and 17–21°C in Sagana and Juja. More volatiles were identified in Sagana (27) and Meru(26) while Juja and Tigoni had 20 and 21compounds respectively (Table 4.10). The tuberose field in Juja was adjacent to a security light and beetles started foraging when the floral scent filled the air.

The two principal compounds 1,8-cineole and methyl benzoate contributed to 69–84% of the total floral fragrance data, based on 28 compounds. The total number of volatile compounds emitted was 28 for the three locations but individual locations had different numbers of volatile compounds identified. Meru, Tigoni and Sagana regions had one or two volatiles that were only found in these regions (Table 4.11).

Table 4.11   Volatiles compounds collected unique to Meru, Tigoni, Juja and Sagana

Location(s)	Chemicals	Temp. (ºC) at collection
Meru	• Muurola-4(14),5-diene<trans • 2-Pyrrolidinone, 1-methyl-	9–7
Tigoni	• Copaene<beta-> • Methyl geranate	10.5–10.3
Meru and Tigoni	• Bourbonene<beta->	10.5—7
Juja	• None	21–18
Sagana	• Cyclopentanone, 2-cyclopentylidene	17.5–17
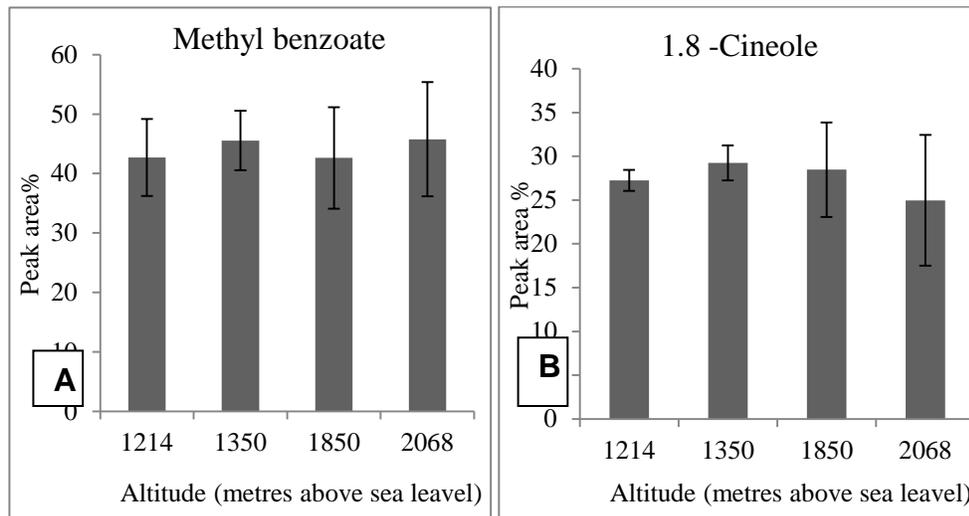
Figure 4.16   Emission of main volatile compounds at various altitudes A) Methyl benzoate and B) 1,8-cineole

There was no clear relationship between altitude and the peak area of the key volatiles methyl benzoate and 1,8-Cineole (Fig. 4.16). The peak area ranged between 40% and 50% for methyl benzoate and 20% and 30% for 1,8-cineole and the samples were not significantly different with very high standard errors. The methyl benzoate emission decreased during the second hour while 1,8-cineole increased (Fig. 4.17).
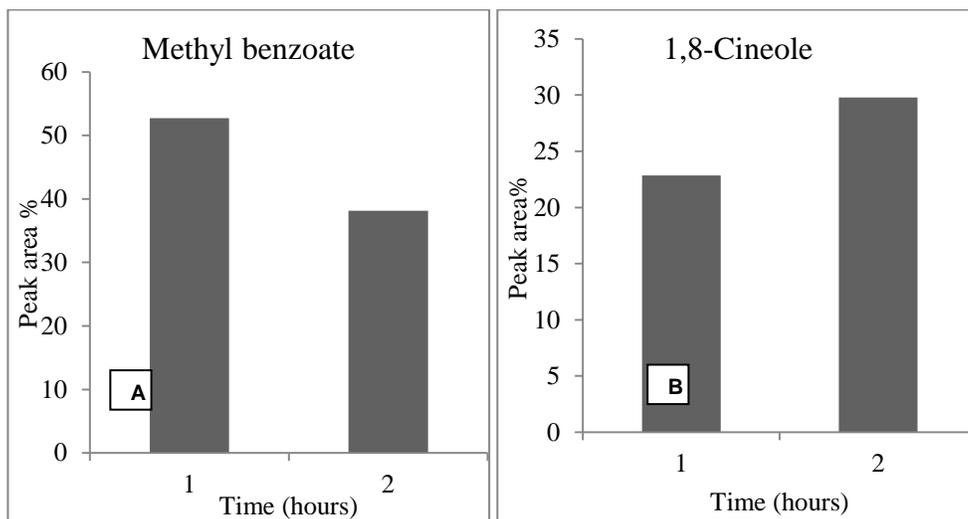
Figure 4.17   Total emissions of the two main compounds at all locations: A). Methyl benzoate; and B).1,8 - cineole emission over time

CHAPTER FIVE

5.0   DISCUSSION

5.1   Effect of nutritional factors on the colour development of *Polianthes tuberosa* Linn

During the study period, the mean temperatures at the KARI -Thika experimental field ranged from 18.2°C to 21.2°C,which was favorable for anthocyanin accumulation with anthocyanin values ranging from 0.88–2.64% w/w (Table 4.5). These are relatively high compared to anthocyanin values of 1.40% that were obtained for a temperature range of 15.9–25.6°C in the fruit of red-fleshed kiwifruit (Montefiori *et al*., 2005). This has also been shown for petals of petunia (Shvarts *et al*., 1997; rose (Oren-Shamir and Nissim-Levi, 1997) and *Lisianthus* (Oren-Shamir *et al*., 1999). Weather conditions were favorable for tuberose growth with the exception of low rainfall during the months of June, July andSeptember at KARI -Thika meaning that irrigation was necessary. Leaf growth in terms of leaf area and dry weight was similar between the different fertilizer applications. This can be attributed to the fact that the same amount of nitrogen was supplied in all the treatments. Despite the differences in soil pH, uptake of nutrients followed a similar pattern; hence growth parameters were not significantly different among the fertilizer applications. In the same way, SPAD (chlorophyll) values were generally similar irrespective of the fertilizer type.

Nitrogen is important in terms of influencing growth, morphology, and tissue composition. Optimal nitrogen supply is important to secure high-quality horticultural

products. Nitrogen is an integral part in the formation of chlorophyll pigment. Nitrogen availability affects chlorophyll content in the leaves and canopy (Schlemmer *et al.*, 2005). No differences in the greenness of the leaf could be detected. This can be attributed to plants having access to similar amounts of nitrogen though from the different fertilizer sources. The nutrient content of N, K, P, and Mg found in the plant tissue gives further evidence that their uptake was relatively similar among the fertilizer types.

The soil pH was expected to be reduced after the application of the fertilizers AS and $Mg(NO_3)_2$. Although there was a decline in the soil pH over time after the fertilizers were applied, only AS had lowered the soil pH as expected by the 18^{th} week. The $Mg(NO_3)_2$ treated soils had a higher pH than expected and this could be attributed to the NO_3 reaction with the soils (Taiz and Zeiger, 2010). Thus, plants treated with AS experienced a much lower soil pH, followed by plants in the CAN treatment, while those in $Mg(NO_3)_2$ had more or less a neutral pH.

Lightness values, which provide a measure of brightness, did not change significantly in response to the fertilizer types. However, the lightness values were higher in plants treated with AS at the 18^{th} week, when the soil pH was the lowest in that treatment. The general decline in lightness value from about 68–70 radians at 16^{th} week to 55–65 radians at the 18^{th} week could be attributed to the change in colour of the florets. Similar findings were reported in cherry, where the lightness values declined as colour changed during fruit ripening yet there were no pronounced declines in chroma and hue indices. In fact, a negative correlation between chroma and hue with

anthocyanin content in cherries and *Hippeastrum* (Goncalves *et al*., 2007; Byamukama *et al*., 2006) were demonstrated, but this was not the case in our study. In Eustoma flowers (*Eustoma grandiflorum* Gries), total anthocyanin content increased as lightness decreased, but increased as chroma increased (Uddin *et al*., 2004). In red *Hippeastrum* flowers, (Byamukama *et al*., 2006) lower lightness values ranging from 33–- 38radians with chroma and hue values ranging 40– - 63 radians, and 22– - 35radians, respectively were reported. In Eustoma, most purple-flowered cultivars had lightness values ranging from 21–42, chroma and hue values in the ranges of 21- 42, 45–76 radians, and hue values of319– 342; respectively, reddish- purple cultivars had value ranges of 55–58 radiansfor lightness, 40– 50radians for chroma, and 326–341 radiansfor hue;, and, pink-flowered cultivars had lightness values of 49–84, 3–49 radians, and 349–357 radians, respectively and white flowered cultivars 85—88 radians, 1–11 radians, and 17–343radians respectively (Uddin *et al*., 2004).Tuberose lightness values indicated a relatively light colour with lightness values between 54–67 radians. The lightness and chroma values for tuberose in this study are similar to reddish purple and purple flowered Eustoma, while the hue values are similar to that of white Eustoma.

Soil applied amendments were expected influence the soil pH and uptake of Mg by the plant leading to variation in tissue magnesium concentrations. This in turn was expected to influence floret colour through effects on lightness, chroma, hue, and anthocyanin content. The anthocyanin content of tuberose showed a weak link with floret tissue magnesium content (Table 4.4). The plants supplied with $Mg(NO_3)_2$ were expected to have higher amounts of magnesium in their tissues, however, this was not

the case, as magnesium content was not different among the treatments. Magnesium is commonly leached in soils, to ensure it is absorbed it should be sprayed as a solution. In aster flowers (Shaked-Sachray *et al.*, 2003), it was shown that application of magnesium salts to the flowers or whole plants increased the metal levels in the petals, and that anthocyanin production was increased at elevated temperatures. In this study, anthocyanin accumulation increased with low soil pH.

5.2 Effect of light quality on colour formation of *Polianthes tuberose* Linn. florets

The mean solar radiation in the open was highest while it was lofest in the brown bag. Red and black nets permitted lower solar radiation intensity than beige netting at the same shading level of 50%.Tuberose florets acquire a blush/tinge seen as reddish pink or purplish pink, according to the Royal Horticultural Society Colour Chart (RHSCC, 1984), Chart No. 4. L*, a* and b* values of the colour pink are shown in Table 4.6. The pink colour has more red as evidenced by the RHSCC reference colour 411, as the red value reduces and yellow reduces the pink colour becomes dull (412). When the yellow value increases beyond b* 35 radians, the yellowness becomes noticeable even though the redness is above 70 radians and therefore the colour is denoted as yellowish pink. The flowers from Meru and Thika differ in the yellowness and redness. Meru flowers have more redness and less yellow, while Thika flowers have more yellow and less redness.

The shading material varied in the percentage of light they allowed through as shown in Table 4.5. The brown bag had tuberose florets with high yellowness at b* 31.5 radians compared to 22.9 radians for the open. The open florets had significantly more red 54.6 radians compared to a* 38.1 radians for the bagged. Florets under 50% shading, with black, red and beige nets had more yellowness at b* range of 30.69–34.7 radians compared to florets in the open with 22.9 radians. The L*, a* and b* values measured for the standard colours are shown in Table 4.6. The calculated colours were similar to those of the RHSCC. Meru flowers had a strong pink colour while those in the open treatment had a yellowish pink colour similar to 10R 1004 of the RHSCC code. The colour parameters for florets under black and beige nets were not significantly different.

Lightness was not significantly different between treatments. However, the beige colour net gave the highest lightness while the red net gave the lowest (Fig.4.2). The average lightness values in the study ranged from 65.1 to 78 radians, there were no negative values for both redness and yellowness. The values for redness were greater than those of yellowness in all the light conditions. The values for Lightness in Meru flowers were different from those in Thika. Flowers under black net in Thika had highest value for Lightness at 78.0 radians, while those from red nets and open were statistically similar with a range of 72.9–77.0 radians. In Thika, there was no significant difference in Lightness value between the treatments, with the Lightness ranging between 76.0 and 77.9 radians.

5.3   The influence of magnesium on colour development of the florets of
**   *Polianthes tuberosa*Linn. under transient temperature**

The RHSCC colours were visually similar to calculated colours and thus gave
the basis for use of the colour calculator to derive the other colours from the measured
coordinates. Also the florets all gave positive values for both a* and b*, this indicated
that at the stage of the flower spike elongation, green or blue hues do not camouflage the
yellowness or redness of the flower. This suggests that regardless of light levels the
shades were yellowish and reddish in appearance. All the values of redness and
yellowness, in this study were low and therefore the hº lay between 22.7 and 46.4º for all
light conditions. Wanyama *et al*., (2010) found that yellow and orange shades developed
by *Morinda lucida, Curcuma. longa* L and *Mangifera indica* plant had lightness values
of 69-74. The shades for the majority of the florets were closer to yellow than red and
could be conclusively adduced from Table 4.7 that most of the colours are yellowish
pink.

Comparing these CIELAB values to work done by others show differences in
values for pink Uddin *et al.*, (2001), petal values of pink *Eustoma grandiflorum* cv
Asuka no Asa with the following values: lightness (64.8 radians), redness (17.6 radians),
-yellowness (-12.9 radians), chroma (21.9 radians) and hue angle (-36.3 radians). The
reddish pink from the Meru site in our study had the following values: lightness (62.1
radians), redness (55.9 radians), yellowness (23.4 radians), chroma (60.6 radians) and
h^{ab} (22.7 radians) the values for pink from the RHSCC code were: redness (69.5

radians), yellowness (22.5 radians), lightness (71.5), chroma (73) and h^{ab} (17.94radians). Schmitzer *et al*., (2012) used prohexadione-Ca to modify colour in roses and obtained for red flowers: redness (54.0 radians), yellowness (8.3 radians), lightness (28 radians), chroma (54.6 radians) and h^{ab}(8.8 radians) while the modified light pink flower values were: redness (48.9 radians), yellowness (1.4 radians), lightness (43.3 radians), chroma (49 radians) and hue (11.5 radians). This shows that there may be different values for the same colour under different conditions however, the changes in anthocyanin accumulation is evident. He *et al*. (2011) reported the purple *Lycoris longituba* petals to have lightness values 45.95-87.81radians for the red variant ranged while the orange variant ranged 64.6-86.39 radians and the yellow variant was above 86.3 radians. Lightness value above 70 radians are closer to bluish green, yellow green and orange yellow (Pascale, 2006).

5.4   The composition of the floral fragrance of *Polianthes tuberose* Linn.flower grown in Kenya

Colour formation at 19ºC once formed was not reversed at higher temperatures in the presence of Mg within the three days and was significantly different from those without magnesium in colour measurements. Tin, copper, and aluminium ions were capable of forming stable complexes with anthocyanin and magnesium formed stable ternary complexes (Shaked-Sachray *et al*., 2002; Yoshida *et al*.,2009;Abdullah *et al.*, 2011).In addition, Oren *et al.*, (2002) using detached flower buds described how

magnesium partially prevented colour fading at elevated temperatures in aster flowers. Thus the colour change and maintenance shown Fig. 4.13 (iv) and development of intense colour on petals as well as the bracts can be attributed to the magnesium treatment. Higher a* was an indication of more redness and this could be attributed to the presence of magnesium.

5.4.1 Volatile Composition and identification

Beetles present on the flowers in Juja (Fig. 4.16) were observed after fragrance emission was evident. The air was filled with the strong sweet fragrance of tuberose and beetles are known to be attracted these scent (Knudsen and Tollsten, 1993). Though the initial attraction of the beetles may have been the bright security lamp but they ended up on the flowers due to the floral scent. Such strong scents belong to specific classes of volatile compounds commonly used in perfumery (Knudsen and Tollsten, 1993). There is no documentation of tuberose pollinators.

The tuberose volatiles identified were 28 with varying amounts in abundance. The floral fragrance in tuberose was observed to vary over time within the collection period. Most of the compounds found in the tuberose volatiles (Table 4.7) are common floral volatile compounds that are present in flowers of many other plant species (Knudsen and Gershenzon, 2006). The oxygenated monoterpene 1,8-cineole was the dominant compound of the monoterpenes, comprising 29%. Methyl benzoate, an aromatic ester, comprised 47% of the total peak area of volatile compounds emitted in

all the sites. There was more methyl benzoate emitted within the first hour than the second hour of trapping. The emission of 1, 8-cineole increased with time (Fig 4.17). Methyl benzoate has been associated with nocturnal maxima rhythm (Kolosova *et al*., 2001). In *Mahonia japonica* it was reported that emission of monoterpenes and aromatic compounds was controlled by diurnal changes in light levels (Picone *et al*., 2002).

5.4.2   Abundance of methyl benzoate and 1,8-cineole

Though cultivation practices and inflorescence stage were similar, the temperatures at the various locations were different. Methyl benzoate and 1,8-cineole were the most abundant at all altitudes, other components were not all present at all locations. The abundance of the volatile ester methyl benzoate is similar to findings by Rakthaworn *et al*., (2009) who found methyl benzoate was the most abundant compound regardless of extraction method. Other authors relate the abundance to pollinator activity as shown in snapdragon, passion fruit, orchids and petunia (Dudareva *et al*., 2000; Kolosova et al.,2001; Negre *et al*., 2003; Salzmann *et al*., 2006). Variation in floral fragrance composition has been indicated in flowers of a similar species that have different pollinators at different altitudes (Knudsen, 2002). Commercially, methyl benzoate is known to possess flavor/aroma qualities giving a sweet floral scent with a fruity undertone, it is used to flavor berry and cherry condiments. 1, 8-cineole, also known as eucalyptol, found naturally in eucalyptus genus and is used as a food additive (baked goods, confectionery, meat products and beverages), medicinal additive (mouthwash and cough suppressant), pesticide (Nidiry and Babu, 2005; Sangita *et al*.,

2006), and a perfuming agent and tonic as well. The compound 1,8-cineole is typical of many essential oils from bay leaves, tea tree, rosemary and sage. It is used to give a minty, spicy or peppery flavor to condiments.

The emission patterns were not similar and there were unique volatiles emitted exclusively at some locations. Juja was the warmest location (18–21°C) and did not emit the following: methyl salicylate, (1,1'-bicyclopentyl)-2-one, longicyclene, cyclopentanone, 2-cyclopentylidene, bourbonene<beta-> and germacrene D (Table 4.4). Sagana second warmest location (17–17.5°C) had the highest number of volatile compounds identified 28, Tigoni was cool at 10.3°C to 10.5°C Tigoni had 20; and Meru which had the lowest temperature (7–9°C) had 26 compounds.Tuberose volatiles are known to be unstable with variable volatility and concentration. They are easily oxidised by contact with air or degraded by heat (Guenther, 1948).The advantage of using the push and pull is that though there is a possibility for oxidation of the volatiles during trapping, there is no exposure to high temperature; therefore the fragrance may be a close representation of the natural fragrance of the tuberose flower.

According to the Food and Chemical Toxicology registry (Anon, 2000), the tuberose concrete under this registration has the main compounds as farnesol, methyl anthranilate, eugenol, methyl benzoate, benzyl benzoate, methyl anthranilate, benzyl alcohol, geraniol, and nerol. Our study showed that the four altitudes were different in fragrance profile but there was no specific trend associated to the altitude/temperature or weather (wet or dry). The solvent extracted fragrance had more volatiles identified and some were in trace amounts. Methyl benzoate and anthocyanin biosynthesis originate

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from the same phenylpropanoid pathway and when methyl benzoate emission increased it is possible that the metabolic flow was diverted from anthocyanin biosynthesis to benzoic acid production (Dudareva and Pichersky 2008). The volatile profile of the solvent extracts was not similar the *in vivo* emitted profile.

A total of 28 compounds from intact flowers of *P. tuberosa* were separated and identified. alpha -pinene, sabinene, myrcene, *cis*-ocimene, benzaldehyde, terpinolene, were the characteristic compounds of the fragrance tuberose flowers. These results demonstrated that push pull technique using the portable volatile collection pump SPME–GC–MS is a simple, method suitable for the analysis of volatile compounds emitted from intact flowers of *P. tuberosa* in different localities. It is a useful method to distinguish the difference of volatile compounds emitted from flowers at different locations. As demonstrated in this study, 20 of the 28 were consistently identified with a purity of ≥90%. However, in this case we observed an overwhelming dominance of hydrocarbon derivatives, probably because of the low volatility of oxygenated compounds (Bouvier-Brown *et al*., 2009).

Manufacture of fragrance from tuberose flowers by extraction of volatiles would add value to the crop and make it more profitable as recommended for Rwanda in their agribusiness development.However, more studies are necessary to establish extraction protocols and economical volumes of tuberose flowers for commercial volatile extraction and the prerequisite climatic conditions associated with good quality fragrance compounds.

CHAPTER SIX

6.0   CONCLUSIONSAND RECOMMENDATONS

6.1   CONCLUSIONS

The main objective of this study was to determine factors that influence tuberose flower attributes for improved incomes and livelihoods leading to food security. To determine the effect of nutritional factors on the colour development of *Polianthes tuberosa* Linn.The nutritional conditions that enhance colour development were examined.

6.1.1. Effect of nutritional factors on the colour development of *Polianthes tuberosa* Linn.

Nutritional factors that affect colour formation in tuberose florets were not so apparent in soil applied amendments. Magnesium supplied through fertilizer application did not increase accumulation of magnesium in floret tissues, low soil pH led to the accumulation of anthocyanins in the florets. The amendments lowered the soil pH, especially for the AS and CAN. Floret tissue elements and chlorophyll contents were similar between the amendments, implying that magnesium applied as $Mg(NO_3)_2$ did not lead to accumulation of magnesium in floret tissues. The colour parameters and concentration of anthocyanins in the florets were not significantly correlated to the soil pH following the amendments. The results of the nutritional studies will help farmers to upgrade cut flower quality.

6.1.2 Effect of light quality on colour formation of *Polianthes tuberosa* Linn.florets

The effect of light on colour formation in tuberose issimilar to results reported in earlier studies (Albert *et al*., 2009; Huang *et al*., 2009),which show that planting tuberose in the open gives a more intense colour. This study specifically showed that under modified light using colour nets, thered nets gave a more intense colour than beige and black nets. Growing tuberose under red coloured net would be an economically attractive enterprise to the farmer as well as being climate-smart farming.**T**he effect of temperature on colour formation in tuberose showed that at lower temperature the colour formed was more intense (Ubi *et al*. 2006; Steyn *et al*. 2009; Wang *et al*., 2011).

6.1.3 The influence of magnesium on colour development of the florets of *Polianthes tuberose* Linn.under transient temperature

Under transient temperature, the effect of foliar-applied magnesium, applied as $Mg(NO_3)_2$, led to colour stability three days after colour formation at a low temperature of19 ºC, since magnesium plays an important role at pro chlorophyll level. It is significant to note that irrespective of the period at which flower spikes were under treatment, results indicated that colour formation needed 6 days.

Effects of global warming include increased rising temperatures and extreme events of major flooding or heat waves. Mitigating global warming means having innovative strategies such as using a magnesium foliar spray to stabilize colour under fluctuating temperatures to make tuberose a crop resilient to climate change since it is recommended for growing in the lower and medium highlands ranging inaltitudefrom1200 to 1800 m.

6.1.4 Influence of location on composition of biochemical compounds of tuberose floral volatiles

The influence of location on the composition of the biochemical compound of tuberose floral volatiles shows that at least 28 compounds were detected at all altitudes though there were differences between samples and not all the compounds were detected in all locations. The presence of methyl benzoate and 1,8-cineole in large amounts indicates the potential for industrial exploitation. This is the first reported study of floral fragrances emitted *in vivo* for intact tuberose flowers. The study chemically characterized the floral fragrance in tuberose and also analyzed the floral fragrance between altitudes. The study shows that the mobile volatile collection pump can be used to collect and identify volatiles. The study on floral volatiles of tuberose grown in Meru, Juja, Sagana and Tigoni show the potential for value addition product diversification.

This is the first reported study of floral fragrances emitted in vivo for intact tuberose flowers. The study chemically characterized the floral fragrance in tuberose and

also analyzed the floral fragrance between altitudes. The study shows that the mobile volatile collection pump can be used successfully to collect floral volatiles. The study on floral volatiles of tuberose grown in Meru, Juja, Sagan and Tigoni show the potential for value addition well suited for the vibrant Kenyan cottage industry(Jua Kali).It could be one avenue of wealth creation and distribution in the various rural areas where tuberose is grown. Interventions include building capacity of farmers in processing, packaging, and branding and risk mitigation.

The overall conclusion is that tuberose production can be profitable by introducing coloured tuberose germplasm, adopting technologies to enhance colour formation and the alternative use of flowers for extraction of industrial raw materials. This information is important to stakeholders in the floriculture industry, including policy makers to put in place strategies that promote new products.

6.2 RECOMMENDATIONS

These recommendations follow up those of the tuberose survey on production status that recommended a system of certification and regulation for tuberose planting material to increase quality of cut flowers and reduce distribution of pests. Further to that, new coloured tuberose germplasm be introduced in the country for it to remain competitive in tuberose cut flower production. Below are recommendations with an application approach:

- The nutritional regime for tuberose production should have magnesium applied to increase anthocyanin accumulation in the florets.

- Use the red net for production of tuberose in areas with high light intensities to modify light quality.

- A floral spray of magnesium solution should be used when temperatures are high to increase accumulation of anthocyanins and stabilize the colour by forming the magnesium-anthocyanin complex under transient temperatures conditions

- Floral volatiles can be extracted from tuberose grown at any location in Kenya as large quantities methyl benzoate and 1,8-cineole, are present regardless of location.

- Floral volatiles from tuberose should be exploited for industrial use and product diversification for the smallholder farmer's improved livelihood.

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LIST OF APPENDICES

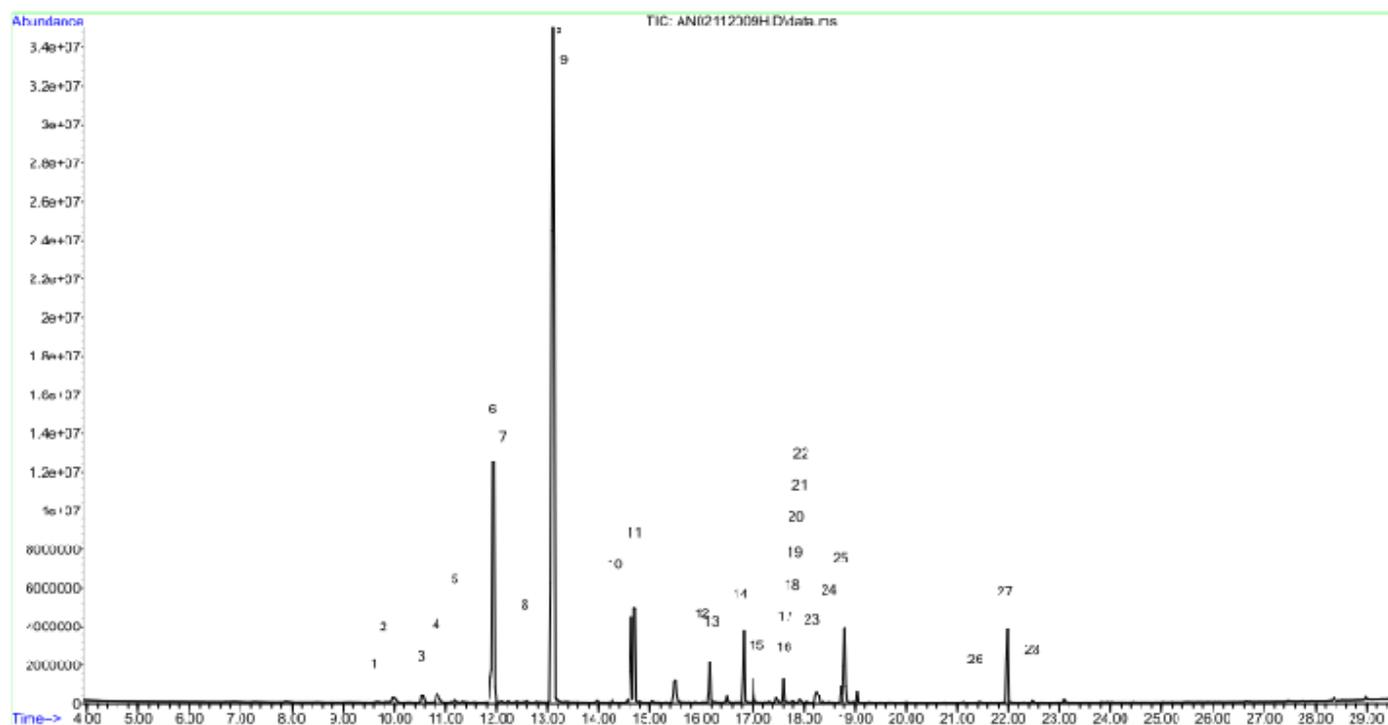## APPENDIX I: SAMPLE CHROMATOGRAM

Figure A. 1   GC-MS chromatogram with retention times showing the abundance of floral volatiles identified whose details are shown Table 4.8.