BIOCHEMICAL CHANGES AND MICROBIAL PROFILES AFFECTING QUALITY AND VASE LIFE OF CUT GLADIOLI (*Gladiolus grandiflorus* L. 'FADO') FOLLOWING PULSING AND COLD WET STORAGE

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

EGERTON UNIVERSITY

APRIL, 2021

DECLARATION AND RECOMMENDATION

Declaration

I hereby declare that this thesis is my original work and has not been submitted for award in any institution of learning to the best of my knowledge

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Recommendation

This thesis has been submitted with our approval as University supervisors.

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DEDICATION

This work is dedicated to my entire family members, my husband Henry Chore; and my dear children Allan Amiani, Kelly Everia, Tony Kahi and Mercy Cheredi. Your moral support, endurance and belief in me carried the day for me.

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ABSTRACT

Floriculture in Kenya remains among the top three foreign exchange earners. *Gladiolus* is one of the famous cut flowers in the world. Annual losses amounting to 30,000 metric tonnes valued at USD; 166.6 is incurred by farmers due to postharvest challenges. The aimof this study was to determine the vase life and quality parameters of cut *Gladiolus grandiflorus* L. cv.'Fado' as affected by pulsing with 600 ppm 8-hydroxyquinoline sulphate plus 5% sucrose prior to wet cold storage duration(0 – 5 days). The *Gladiolus* were grown in the open field from corms at the Horticulture Research and Teaching Field, in the department of Crops, Horticulture and Soils, Egerton University, Kenya, during the season of September to December 2014. A two by six factorial experiment embedded in a Completely Randomized Design with four replicates was adopted using Proc GLM model in two way ANOVA. Differences in means were determined using Tukey's test at 5% level of significance. Pulsing treatments and interactive wet cold storage durations significantly affected cut *Gladiolus* quality parameters including fresh weight (P< 0.0031; 11.67% increase).,dry weight (P< 0.0272 ;17.62% increase)., mean water uptake (P < 0.0001; 38.14% increase)., opened florets (P< 0.00095; 59.8% increase) and number of unopened buds (P <0.0256 ; 61-28.88% reduction). The pulsing and cold storage treatments and their interaction significantly (P< 0.0001., P<0.0003.,P<0.0001 respectively) affected the vase life of the cut *Gladiolus* (11.5 ± 0.0.148 days versus 4.75 ± 0.148 days; 142% increase).The pulsing treatment coupled with wet cold storage and their interactive effects significantly (P < 0.0001) affected the concentrations of the senescence biomarkers: Total Free Amino Acids, (P<.0001; 21.97% reduction)), Total Soluble Sugars (P < 0.0001; 101.16% increase) and Total Starch(P <0.0001; 309.39 % increased) on the third day in the vase compared with the control. The levels of Total Phenols, anthocyanins and microbial proliferation were also significantly affected (P< 0.0003 (104.14% increase)., P < 0.0012 (26.98% increase)., P < 0.0001 (6.032% reduction) respectively) on the third day in the vase in pulsed spikes wet cold stored for 3 days.*Gladiolus* cut flowers can be wet stored at 3 ± 1°C up to a maximum of 4 days without any adverse effect on their subsequent vase life and quality. Small scale farmers can adopt pulsing cut*G. grandiflorus cv.*Fadowith subsequent storage of upto 4 days at 3 ±1 ^{O}C to manage bulking and transportation of their produce to reduce postharvest losses. This may increase domestic livelihood withenhanced foreign exchange and consequently food and job security in Kenya.Knowledge from this study will augment existing technologies in improving quality and market value of *Gladiolu*s cut flower.

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LIST OF ABBREVIATIONS AND ACRONYMS

ABA	Abciscic acid
AIPH	International association of horticultural producers
ANOVA	Analysis of variance
APVC	Agricultural Product Value Chain
ATP	Adenine 5’-triphosphate
BA	Blood agar
BAP	6- Benzyl amino purine
BCDMH	1-Bromo–3–chloro–5-5– dimethylhydantoin
BHT	Butylated hydroxytoluene (2,6-di-tert-4-methylphenol
BSM	*Botrytis* selective medium
BSTM	*Botryti*s spore trapping medium
CA	Controlled atmosphere
CFC	Cut flower center
CFU	Colony forming units
CHI	Cycloheximide
CLED	Cystine lactose electrolyte deficient agar
CRD	Completely randomized design
CW	Chen and Wu
Dev. Co. Ltd	Development company limited
DHL	Deoxycholate hydrogen sulphide lactose agar
DICA	Dichloroisocyanuric acid
DIPP	Promoting Danish import programme
Dw	Dry weight
EMB	Eosin methylene blue
EPK	Eastern produce Kenya Limited
FAO	Food and agricultural organization of the United Nations
G.	*Gladiolus*
GA_3	Gibberellic acid
GLM	Generalized linear mode
HCD	Horticultural Crop Directorate
HCOOH	Methanoic acid/formic acid/carbonic acid
HVAPs	High value agricultural products
HPLC	High performance liquid chromatography

8-HQC	8-Hydroxyquinoline citrate
8-HQS	8-Hydroxyquinoline sulphate
IVP	The internatiomal vision project
IMVIC	Indole, methyl red, Voges Pros Kaur and citrate tests.
JMP	Java memory profiler
KALRO	Kenya agricultural & livestock research organization
KARI	Kenya agricultural research institute
KBS	Kenya bereau of statistics
KFC	Kenya Flower Council
KHCP	Kenya horticultural competitiveness project
KSLP	Kenya seeds for life project
MA	Modified atmosphere
MMA	Match maker associates limited
MoALF&I	Ministry of Agriculture, Livestock, Fisheries and Irrigation
NUV	Near
ultraviolet	
NPK	Nitrogen phosphorus potassium
1-MCP	1-Methylcyclopropane
PhHD-Zip	Protein encoded by a homoebox-Homeodomain-leucine zipper
protein	
PDA	Potato dextrose agar
SDA	Sabouraud dextrose agar
SOD	Superoxide dismutase
SS	*Salomonella-Shigella* agar
SSA	5’-Sulfosalicylic acd
TCA	Trichloroacetic acid
TFAA	Total free amino acids
USAID-KHCP	United States Agency for Development- Kenya Horticultural Competitiveness Project
TP	Total phenols
TS	Total starch
TSI	Triple sugar iron
TSS	Total soluble sugars

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CHAPTER ONE
INTRODUCTION

1.1 Background information

Floriculture industry is a branch of horticulture that deals with the cultivation, marketing and production of field-grown or green-house grown plants for their flowers or showy leaves (Debajit &Sudip, 2016; DIPP, 2009;Wani *et al*., 2018).The global floriculture market involves a variety of products such as cut flowers, potted flowering plants, foliage plants, seeds, tubers and bedding plants (Alberto, 2007; Donkor *et al*., 2017;Gebreeyesus, 2015;KFC, 2017; Xia *et al* 2006). Floriculture is a fast growing profit making agri-business with an estimated current Global market of over US$ 109 billion annually (Dastagiri, 2017). This is a great stride in upward dynamism from 1950s when the Global flower industry trade was only valued at US$ 3 billion (Kargbo *et al.,* 2010).The annual Global market of floriculture in 2009 was only US$ 40 billion (Getu, 2009). The Netherlands ranks top in the world and commands 52% of the global floriculture trade, followed by other exporting countries such as; Columbia (15%), Ecuador (9%), Kenya (7%), Belgium 3%, Israel, Ethiopia and others (14%) (Melese, 2018; van Rijswick, 2015). The export value of cut flowers from Kenya in 2019 was US$ 971 million, which was a drop fromUS$ 1.06 billion for the previous year (KBS, 2020). Kenya has to remain relevant in the international floriculture agri-business amongst aggressively competitive countries such as Columbia, Ecuador and Israelin view of a stagnant market demand against increased production (Krishnan, 2018). Among the contries in the East African Community, Kenya has signed a 25-yearEconomic Partnership Agreement with the European Union region that would make it benefit from duty free and quota free trade in goods to European market, including the United Kingdom market (Adeola *et al*., 2018; NMG, 2021).

Important floricultural crops in the international cut flower trade are roses (*Rosa hybrida*), carnations *(Dianthus caryophyllus),* chrysanthemum (*Dendranthema grandiflora*), *Gerbera spp.*, *Gladiolus sp.* (**Plate 2.1**), *Gypsophila sp.Liatris sp.*, orchids *(Orchidaceae)*, *Archilea millefolium*, *Anthurium sp.,* Tulip *(Tulipa) and* lilies(*Lillium sp*) (KFC, 2017). Floriculture sub-sector directly employs 100,000 people and 2 million people in related industries (Babu *et al*., 2016; KFC, 2017; Leopold & Morgante, 2013). While floriculture crops such as roses and carnations are grown in green houses, others such as *Chrysanthemum and Gladiolus* among others are grown in the open field (Castillo *et al*., 2018; Kargbo *et al.,* 2010; Vahoniya *et al.,* 2018;).

Gladiolus is a summer flower which gained popularity in Kenya after the introduction of nine new varieties; six of which were approved to growers for multiplication (KARI, 2003; Saleem *et al*., 2013). These cultivars were ranked based on the time taken for inseption of inflorescence and also their susceptibility/ tolerance to *Fusarium* corm rot and rust. Trials were also done on other *Gladiolus* varietis (Her Majesty, Friendship, Green star, Amsterdam and Chinon) in North Western Kenya (Wambani *et al*., 2009).Modern day gladioli are tetraploid hybrids of different wild species or hybrids of subsequently obtained hydrids that belong to *Gladiolus hybridus* (Alam *et al*., 2013; Rakosy-Tican, 2012). Hybrids designated as *Gladiolus grandiflorus* belong to a complex of atleast 11 species grown from the corms that are propagated from cormels (Wambani *et al*., 2009). 'Fado' is one of the exotic cultivars belonging to *Gladiolus grandiflorus L.*evaluated for growth, yield and cormel production (Saleem *et al*., 2013). *Gladiolus* cut flower has been ranked among high value agricultural products and also holds a strong position in the summer flowers value chain in Kenya and Sub-Saharan Africa (Temu & Temu, 2006; KALRO, 2016). *Gladiolus* farming is a venture that can be undertaken by small scale holders since it is easier to manage in comparison to green-house grown crops (Mwangi, 2019; Zylberberg, 2013).This spike-like flowerholds a strong position in the domestic markets in many countries including USA (Florida and Michigan), Italy, Spain, India, Australia, Japan and Taiwan (FloraCulture, 2019; KFC, 2018); USAID, 2017). *Gladiolus*, Statice, leather fern, Strelitza and Tuberose are among flowers supplied by smallholders in Kenya (Muriithi, 2014).

*Gladiolus grandiflorus*is among the summer flowers with potential for production by small scale farmers in Kenya (Kazimierrezuk *et al.*, 2018).Elite *Gladiolus* varieties were introduced into the country after successful field trials were carried out inThika; Nairobi, in Kenya (KALRO, 2016). *Gladiolus* is popular to consumers because it has many spike forms, colors and color combinations: attributes that are an advantage in every floral arrangement (Ahmad*et al.,*2016). These qualities make *Gladiolus* a favorite in cut flower industry (Bhat & Sheikh, 2015). *Gladiolus* can withstand desiccation since it can be grown without irrigation except in very dry spells hence it can be subjected to diversified climatic changes (Manzoor *et al*., 2018; Wambani *et al*., 2009).The Global floriculture industry is faced by many challenges such as impact of climate change, high dependence on imported planted materials, high cost of investment, pests and diseases (Adeola *et al*., 2018). However favorable climatic conditions, local expertise and the on-going government initiative in improving infrastructure provide opportunity for future growth and expansion of floriculture in Kenya (KuIper, 2019). Qualitative and quantitative post-harvest losses of *Gladiolus* can be reduced by adopting

improved technologies (Aghdam *et al*., 2015). These include harvesting at the proper stage(Kumar *et al*., 2018; Sales *et al.,* 2018), use of floral preservatives(Mukherjee & Mukherjee, 2017; Sharma&Srivastava, 2014) and bud opening solutions(da Costa & Finger, 2016), pulsing(Jafarpour *et al.,* 2015),precooling, improved storage techniques such as low temperature and proper packaging among others. Pulsing and cool storage have been proven to have variable effects on the vase life of cut flowers (Jowkar *et al*., 2017; Viera *et al*., 2013). The longevity of cut flowers is one of the main challenges of florists (*Dung et al.,* 2017). Apart from external quality, the vase life of cut flowers remains a major concern for farmers.

It has been noted that during the course of petal ageing there is a drop in the level of macromolecular components such as sugars and cell wall polysaccharides (Ahmad & Tahir, 2016; Yoshinda *et al*., 2008.,). The postharvest behavior of flowers is an outcome of the physiological processes occurring in leaves, stem, the flower bud and the leafless penduncle (Jitendra, 2012). The display life of cut flowers is very short, owing to their perishable nature.The caroteinoids and anthocyanins, the two major classes of pigments responsible for different flower coloration change significantly during development and maturation of plant organs (Cavaiuolo *et al*., 2013). Preservation of *Gladiolus* cut flowers by use of a biocide such as 8-hydroxyquinoline sulphate in combination with a carbohydrate such as sucrose components in pulsing treatment could minimize the physiological effects due to microbial proliferation and macromolecular breakdown (Sudaria, 2017). The endogenous release of ethylene by the cut flower could be slowed down leading to the decline in the overall symptoms associated with senescence in the cut flower. Cold storage of cut flowers slows down the effects due to microbial proliferation, macromolecular breakdown and the ethylene factor (Rani & Singh, 2016). The cold storage of cut flowers facilitates the adjustment of flowers for distant shipment and makes it possible to prolong the sale period for cut flowers and also alleviate probable risks incurred by farmers in the advent of natural disasters (Senapati *et al*., 2016). Pulsing of cut flowers has been found to alleviate sensitivity of cut flowers to ethylene during prolonged cold storage (Makwana *et al*., 2015).

Monitoring of anthocyanin content, microbial genera and biochemical changes in pulsed *Gladiolus grandiflorus* in Kenya could equip stake-holders with technical information required to promote the export and import trade involving this cut flower. Vase life of cut flowers represents the commercial value of cut flowers from the time of harvesting to the time of delivery to the consumer (Khattab *et al*., 2017). In this study the effect of pulsing and duration of cold wet storage of cut *Gladiolus grandiflorus*was evaluated. The impact in quality parameters: vase life, ability to retain fresh / dry weight, water uptake, carbohydrate, protein

and anthocyanin changes were determined using chemical pulsing treatment of 8-hydroxyquinoline sulphate supplemented with exogenous sucrose prior to wet cold storage. Results from this study established that the vase life, water uptake and floral attributes of wet cold stored cut *Gladiolus* improved when subjected to 24 hour pulsing treatment with 8-HQS plus 5% sucrose. Information derived from this study has been disseminated to stakeholders through peer reviewed journal publications and conferences in view of maximizing the commercial value of this cut flower in the flower market chain. This study has contributed towards bridging the gap in the literature review on the biochemical changes and the microbial profiles affecting the post-harvest life of cut cold-wet stored *Gladiolus* in Kenya.Cut *Gladiolus* pulsed with 600 ppm 8-HQS maintained superior vase life and other floral attributes when the cold storage duration was upto 4 days compared with the control. The trend in the quality biomarkers starch, total soluble sugars, total phenols and anthocyanins correlated with improved vase life and oher floral attributes in pulsed wet cold stored *Gladiolus*. The levels of total free amino acids were lower in the pulsed wet cold stored *Gladiolus* compared with the control. The microbial proliferation in the vase water of the pulsed wet cold stored *Gladiolus* was also lower compared with the non- pulsed spikes

1.2 Statement of the problem

Cut flowers are by nature perishable, as such farmers incur 20% loss of 30,000 metric tons yearly most of which is attributed to poor quality and vase life of the produce There is shortened shelf life of cut flowers due to effect of endogenous and exogenous ethylene that continues even during cold storage. There are also biochemical and metabolic changes thatcontinue even under low temperatures. Floriculture in Kenya remains one of the top three foreign exchange earners.Kenya exported 159, 961 metric tonnes of cut flowers valued at Ksh. 82249 million were exported in 2017, which represented an increment of 20% in volume and value. *Gladiolus* is one of the summer flowers grown by small scale farmers in Kenya, The short vase life of gladioli necessitates optimum storage and preservation to lower the adverse physiological and biochemical changes coupled with microbial proliferation associated with senescence.This study demonstrated that the postharvest quality of *Gladiolus grandiflorus* cv. Fado could remain economically viable after pulsing with 600 ppm 8-HQS plus 5% sucrose followed by wet cold storage of upto 4 days. This was portrayed by the ability of the cut spikes to maintain vase life, quality parameters and floral attributes were higher compared with the control. The flower integrity of starch and protein macromolecules was also preserved from the trend shown by the concentration of the former and total free amion acid profiles from the

latter. The elevated mean total phenols and anthocyanins in pulsed wet cold stored gladioli could be indicative of improved keeping quality of the cut flower. It is therefore economically ffeasible for farmers to bulk and transport their produce within this stipulated time without compromising the vase life and quality of the spikes.

1.3 Objectives

1.3.1 General objective

To contribute to the knowledge in the enhancement of post harvest quality and vase life of cut gladioli through pulsing and wet storage

1.3.2 Specific objectives

(i). To investigate the effect of pulsing and wet cold storage duration on vase life and post harvest quality of cut gladioli.

(ii). To determine changes in total free amino acids, total starch, total phenols and total soluble sugars as biochemical markers during senescence of pulsed and non-pulsed wet cold stored cut gladioli flowers.

(iii). To determine the anthocyanin content in pulsed and non-pulsed gladioli flowers at harvest, during storage and at senescence.

(iv). To identify and quantify the bacterial and fungal flora in vase water of pulsed and non-pulsed cut gladioli during the wet cold storage duration.

1.4 Hypotheses (Ho)

(i). There is no significant difference in the vase life and post harvest qualities between the pulsed and non-pulsed cut gladioli after pulsing and wet cold storage.

(ii). There is no significant difference in the changes of the biochemical biomarkers total free amino acids, total starch, total soluble sugars and total phenols between the pulsed and non-pulsed wet cold stored cut gladioli flowers during senescence.

(iii). There is no significant difference between the anthocyanin content of pulsed and non-pulsed cut gladioli flowers at harvest day, during storage or at senescence.

(iv). There is no significant difference between the diversity and quantities of microorganisms in vase water of pulsed and non-pulsed wet cold stored cut gladioli.

1.5 Justification

Gladiolus cut flower is ideal for the local growing as it is heat tolerant, being able to withstand dessication and it can be grown without irrigation except in very dry spells. *Gladiolus* can therefore be subjected to diversified climatic conditions. This cut flower is also cheaper to grow compared to green house grown crops tha are more financially demanding. Cut flowers occupy an important position in the local and foreign markets because of their importance as a source of national income. According to the Horticultural and Crop directorate this value had increased to 159, 961 metric tons in 2017, with a market value of USD 833 million. Cut flower growing in Kenya has created employment for the low income earners and has a work force of over 100,000 workers directly, with another 2 million people indirectly in gainful employment. The greatest percentage (95%) of cut flowers in Kenya are for export. There is therefore a need to extend the vase life of cut flowers to meet international destination of cut flowers. There is a need to pulse cut flowers in combination with cold storage to minimize microbial growth, biochemical changes and the effects due to endogenous and exogenous ethylene that continue even under low temperatures. There is a need to evaluate the effect of pulsing *Gladiolus* (*Gladiolus grandiflorus* cv. Fado) with 600 ppm 8-HQS plus 5% sucrose in combination coupled with cold storage as it is documented that there is differential responses between species and even amongst cultivars or strains to flower prervatives/pulsing/holding solutions. The stringent rules attached to market requirements on sanitary and phyto-sanitary matters calls for need to look for formulations that would help adhere to the international safety requirements. This study sought to unravel the keeping quality, vase life, microbial profiles and biochemical biomarkers in cut *Gladiolusgrandiflorus* L. cv. 'Fado' as affected by the pulsing treatment of 8-HQS plus 5% sucrose and wet cold storage treatments. According to available literature this treatment had not been administered on this cultivar of *Gladiolus*. Pulsing *Gladiolus grandiflorus* cv 'Fado' cut flower with 8-hydroxyquinoline sulphate and wet storage might give a post-harvest trend that could be utilized by stakeholders in improving the commercial value of this floricultural product in both local and export markets.

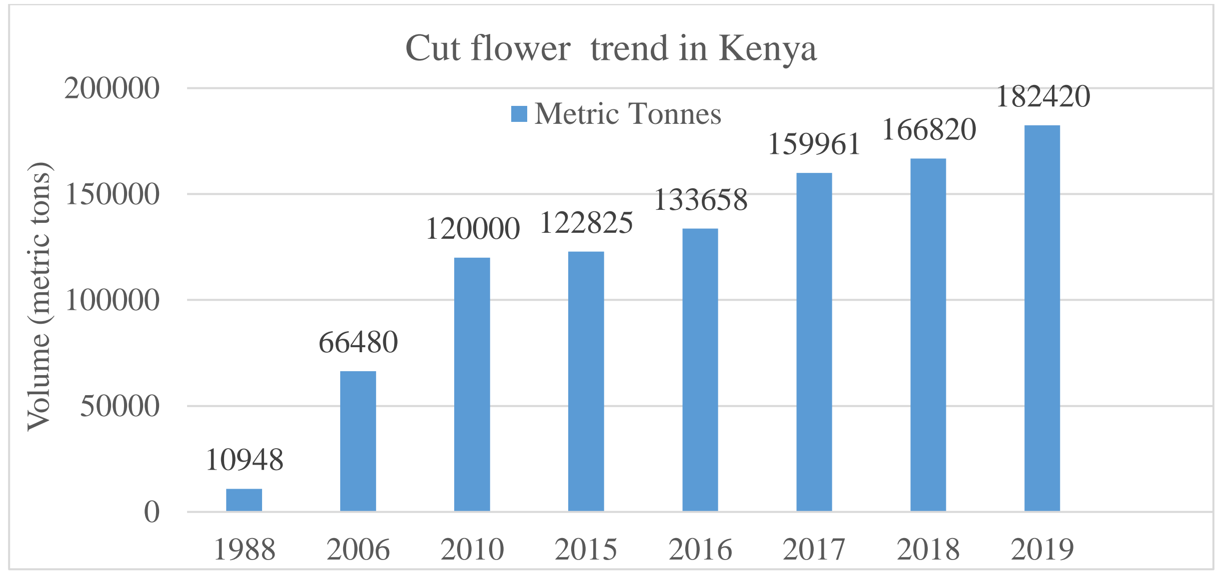
CHAPTER TWO
LITERATURE REVIEW

2.1 The cut flower industry in Kenya

The demand for Kenyan cut flowers has increased steadly with a forecast annual growth rate of 5%, which is extrapolated from sales of Ksh.40 billion, Ksh. 92 billion and projected Ksh.145 billion in 2008, 2019 and 2030 respectively (AIPH, 2019). This has seen Kenya retain a competitive standin both the domestic and international markets over the last two decades (Adeola, 2018; Recha, 2018). This is due to a change in lifestyle among Kenyans where flowers are sought for during occasions such as weddings, birthday parties, religious festivals and office decorations (Mwangi, 2019). Improved infrastructure and favorable climate in the country are some of the factors that have fostered the growth of floriculture (KFC; 2018 Rikken, 2012). The adoption of shipment over airfreight of cut flowers has contributed towards favourable pricing of Kenyan products over the competitors (AIPH, 2019). Floriculture crops not only have higher profit margins in terms of sales but they take a relatively shorter time to mature compared to conventional crops such as sugar cane and tea.Cut flowers come second to tea among the top six crops grown in Kenya. Which together constitute 90 % of the country's crop market value (MoALF & I, 2019). In Kenya floriculture farming is practiced in the following areas: Nakuru, Naivasha, Kericho, Limuru, Kiambu, Athi-River, Thika and in the Mount Kenya region (Kuiper, 2019). The leading counties in cut flower production are Kiambu (47%) and Nakuru (5%) (KFC, 2017).

Over 80 different varieties of cut flowers are grown in Kenya for their commercial market value (KALRO, 2016). The major flowers grown in Kenya ranked in decreasing order in terms of areas under cultivation are Roses, Easter lilies, Arabicum and Carnations (KFC, 2017). Roses constitute 90% of the total value of domestic horticulture and they comprise of 75% of total exports of the cut flowers from Kenya. Other flowers cultivated in Kenya include *Hypericum*, *Alstromeria,Agapanthus*, mobby dick, ornamental *Amaranthus*, *Eryngium*, Statice, *Gypsophilla*, Phlox, *Gladiolus*, *Anthurium*, *Scobiosa*, *Solidago*, leather leaf fern, lilies and tuberose (Kazimierazuk *et al*., 2018). Summer flowers comprise 2.9% of the total cut flowers produced in Kenya and are grown by smallholder growers in the following counties;Nyandarua, Nakuru, Kiambu, Nyeri, Meru, Machakos, Trans Nzoia, Siaya, Migori, Bomet, Nyamira, Mombasa, Kwale, and Muranga (Rikken, 2012). Small scale farmers have experienced problems in the auction market as they have had to rely on middle men for sale of their produce (Hall *et al*., 2017).

Elaborate distribution channels of floricultural products from the producers to the final destinations gives the Kenyan large scale producers an edge over their competitors (Kuiper, 2019). Over 90% of cut flowers produced in Kenya are exported through the international market which is accessed by large companies such as Finlay Flowers Ltd, Oserian Dev. Co. Ltd, Sian Roses Ltd and Mt. Elgon Flower Ltd among others (KFC, 2018). The USAID-KHCP with Equity bank in Kiambu and Nyeri counties are working on a package that promotes Kenyan smallholder competitiveness in the Dutch auction flower market (Rikken, 2012).Regional dominance of the global floriculture market in receding ordrer is Europe, North America, Asian Pacific, Latin America and the Middle East/ Africa (European Commission, 2017; Lawrence, 2019).Kenya dominates the floriculture market in the listed Middle East and Africa region, followed by Ethiopia (Ahmed *et al*., 2018). The floriculture industry in Kenya has expanded in volumes very fast over the years, as shown in **Figure 2.1** below (Flora Daily, 2019; HCD, 2017;KFC, 2018;USAID, 2013)



Year of cut flower production

Figure 2.1: The yearly cut flower production in metric tons from 1988-2019.

Other countries whose floriculture industry has been growing at a fast rate in terms of hectares under cultivation and quality of fresh cut flower produce that are fetching favorable prices in the Global value market chain are Belgium, Ethiopia, Malaysia, Italy, Germany and Israel (AIPH, 2019). The Kenyan flower growers are faced with a number of challenges such as double taxation especially now that the country has a devolved system of government (Keror *et al*., 2018). The other challenge is as a result of poor postharvest processing which results in losses to the tune of 20% of the fresh farm produce (KALRO (formally KARI), 2003; Darras, 2020). Infestations by pests is a major concern as Global phyto-sanitary requirements are set

to maintain stringent quality for cut flowers' export market chain (Cai *et al*., 2018). Several crises emerging in the local, political and international spheres contribute negatively to the market value of cut flowers. In retrospect is the ash cloud of 2010 which adversely affected the production and quality of cut flowers (KCF, 2012). On the local scene calamities such as drought and floods have impacted negatively on floriculture (Kazimierczuk *et al*., 2018). Prohibitive investments and the reliance of local farmers on imported planting materials reduce the market profit margin of Kenyan cut flowers (Kuiper, 2019). Inspite of these challenges, the Kenyan Horticulture sector continues to grow (KFC, 2018).

2.2. Overview of *Gladiolus* cut flower

Gladiolus is an annual bulbous ornamental plant with spike-like leaves grouped among summer flowers and is planted from seeds, corms or cormels (Alam *et al*., 2013). The genus *Gladiolus* is found in the familyIridaceaewhich contains 300 species (Hembrom *et al*., 2019; Rakosy-Tikan *et al.,* 2012). The wild *Gladiolus* flower originated from South Africa and is called Corn Lily or Sword Lily due to its sword shaped leaves (Bhat & Sheikh, 2015). This genus is found throughout Africa, Eurasia and the Mediterranean area, the greatest concentration being in South Africa (da Costa & Finger, 2016).*Gladiolus* is a popular cut flower known asqueen of the bulbousplants in terms of its market value (Shaukat *et al*., 2013). The fact that *Gladiolus* has many spike forms, colors (**Plate 2.1**) and color combination attributes makes it a favorite by both florists and consumers (Hossain *et al*., 2012; Safeena *et al,* 2014). All the modern cultivars of gladioli are of complex hybrid origin raised from the sexual wild South African species and their morphology differs from one genotype to the other (Kumar, 2015). *Gladiolus* is an ethylene insensitive flower whose exogenous ethylene and ethylene inhibitors have no effect on the petal senescence process (Arora *et al.,*2006). But ethylene affects the rate and quality of unopened florets on the spikes (Khattab *et al*., 2017).

Consequently Abciscic Acid (ABA) could be the initiating agent in petal senescence in the *Gladioli* flowers (Reid &Jiang, 2012). A number of gladioli species such as *G. newii*, *G. watermeyeri* and *G. watsonioides* have been identified by environmental conservationists as being native to Kenya (Feilen, 2011; KSLP, 2006; Martins, 2011). Among the species,*G. watsonioides* grows on Mt Kenya and Mt Kilimanjaro and on other Kenyan mountain ranges (Goldblatt, 1996). It is found growing in the forests and other mountainous places to an altitude of 3900m, being the highest growing *Gladiolus* (Feilen, 2011). *G. imbricatus* is a rare species that spreads from the South Scadinavian hills to the coast of the Mediterranean Sea,whose

mode of propagation (seeds) take eight months to break dormancy making it commercially unviable (Rakosy-Tican *et al*., 2012).

Gladiolus is among the flowers selected by stakeholders for analysis in field trials as High Value Agricultural Products (HVAPs) for their economic potential and market value (KALRO (formally KARI), 2003; KALRO; 2012; Temu & Temu, 2006). *Gladiolus* is the 13th most important cut flower at the flower auctions at the Netherlands, holding a strong position in the domestic markets worldwide in countries such as USA (Florida and Michigan), Italy, Spain, India, Pakistan, Australia, Japan and Taiwan (Ahmad*et al*., 2008; Alam *et al*., 2013; Shaukat *et al*., 2013).

Based on evaluation trials conducted at KALRO (formally KARI), (2003; 2016) research center and on farm trials the following cultivars had been recommended: friendship (pink and white), prosperity (white), spic and span (pink), Carmen (red), Fidelio (purple) and hunting song (orange). Recent trials done on gladioli genotypes in India established that they could be rated assusceptible, moderately susceptible, highly susceptible, resistant, moderately resistantor or highly resistant depending on disease progression by*Fusarium oxysporum* f. sp. *gladioli* (Hembrom *et al.,* 2019). Varieties of *Gladiolus* grown under similar environment conditions display significant difference in postharvest characteristics including vase life (Kumar *et al.,* 2018). It has also been established that origin of the planting material and the cultivar adopted significantly affected gladioli resistance to *Fusarium* (Hembrom *et al.,* 2019). The postharvest quality of *Gladiolus* vary between varieties and the planting dates (Akpina & Bulut, 2011; Nagar *et al*., 2018). Soil-borne *Fusarium oxysporum* is among the most destructive microorganisms affecting the production of ornamental plants (Lecomte *et al*., 2016). The demand for exotic ornamentals such as gladioli and orchids and the associated International production and movement between countries has led to disease introduction into new areas (Srivastava *et al*., 2018).

The use of organic substrates such as vermicompost and microorganisms such as *Tricoderma* sp (as biocontrol agent) are among strategies adopted in the improvement of quality of *Gladiolus* stems (da Cruz *et al*., 2018). The use of large volume of chemicals, sometimes beyond the allowable levels is a challenge faced by producers to control flower diseases (AIPH, 2019). Research has been intensified to find means to curtail transfer of diseases between countries through undertaking stringent phytosanitary regulations (KALRO, 2016; MMA, 2017; MoALF & I, 2019). Soiless substrates have been adopted in the growing of *Gladiolus* in an attempt to seclude the natural reservour of *Fusarium* (El-Hanafy *et al*.,

2018). Integrated management of *Fusarium* have also been adopted which involve corm treatment before planting and drenching of the soil (Joshi *et al.,* 2020; Yasmin *et al*., 2018).

The pixiola types of *Gladiolus*flowers have been developed in USA and are good for export purpose due to the lighter weight of their spikes (Abhishekr *et al*., 2019; Safeena *et al*., 2014). These include the following types (Anna Bel (red), Flaura Belle (yellow and purple), Astro (white), Little fox (yellow and purple) Jay vee (yellow) and Spring (greenish) and Chinon (El-Hanafy *et al*., 2018). In the USA, *Gladiolus* cultivation is popular for local consumption though its market share has reduced due to diversification of floral products available (USAID, 2017). According to the National Cut Flower Center, UK, the field grown *Gladiolus* as well as daffodil were economically viable upto 2012 (CFC, 2016). *Gladiolus* as well as tulips are harvested and sold for export to the Czech Republic in Germany (Flora Culture, 2019). *Gladiolus* is one of the summer flowers supplied in Kenya by small scale farmers (Muriithi, 2014). This cut flower still fetches market both locally and internationally in some countries such as Pakistan, Egypt,and USA(Abd-El-Kafie *et al*., 2016; Ahmad *et al.,* 2013; El-Hanafy *et al.,*2018).

Plate 2.1: The pink, white , red and purple colored *Gladiolus* hybrid

In North Eastern Kenya *Gladiolus* was among the flowers grown successfully together with *Arabicum,Molucella,Tuberose*, *Asters*, *Mobbydick*, *Lilies*, *Eryngium* and *Ammi,* by small and medium scale farmers (HCD, 2005). The hybrid species *G. grandiflorus* had the most potential for production and marketing by small scale farmers in Turkana County (Wambani *et al.,* 2009). The most adapted *Gladiolus* cultivars for cut flower production in terms of yield in this County are Friendship, White goddess, Amsterdam, Chinon and Her Majesty (USAID,

2017). Among the gladioli cultivars studied in Turkana Countyearly maturity at 68 days was noted in the cultivars Her Majesty, Friendship and Green star(Wambani *et al*., 2009).Genotype differences within cultivars of *Gladiolus grandiflorus* L. affected quality cultivars includingdays taken for complete sprouting, the average spike yield per hectare and the weight of corms and cormels among others (Abhishek *et al.,* 2019). *Gladiolus* is valued in the flower trade owing to its attractive shapes, brilliant colours, varying sizes and excellent keeping quality (Joshi *et al*., 2020; Safeena *et al.,* 2014: Yasmin *et al*., 2018).The ability of *Gladiolus* to withstand drought as long as air, humidity and moisture are optimum makes *Gladiolus* a favorite in cut flower industry (Milandri *et al.,* 2008; Riaz *et al*., 2009). An introduction of elite *Gladiolus* varieties improved flower quality and yield in Thika and Nairobi, Kenya (USAID, 2017).

2.3 Biochemical changes associated with senescence

Senescence in plants is a complex highly regulated process that involves structural, biochemical and molecular changes that in many cases bear the hallmarks of programmed cell death (Ahmad & Tahir, 2016; Buchannan-Wollaston & Morris, 2000; Shahri *et al*., 2011 Yamada *et al.,* 2009). It is an integral part of the normal developmental cycle of plants and it can be viewed on a cell, tissue, organ or organization level (Shahri & Tahir, 2010). It involves a decline in photosynthesis, dismantling of chloroplasts, and degradation of macromolecules such as proteins, nucleic acids and lipids (Elgimabi & Sliai, 2013). This is accompanied by loss of chlorophyll and mobilization of nutrients to developing parts of the plant (Tania *et al.,* 2001: Van Doorn &Woltering, 2008). Protein turnover has been found to be mediated through proteosomes and various classes of proteome- independent proteases whose transcripts have been found to accumulate in senescent floral tissues (Jones *et al.,* 2005). This has been confirmed by the use of cycloheximide (a protein synthesis inhibitor at the translational stage) which delays senescence in the flowers *Dianthus sp, Gladiolus sp*, *Hemerocallis sp, Ipomoea sp, Iris sp and Narcissus sp* (Shahri & Tahir, 2010). Flower senescence is the terminal phase of a developmental process that leads to the death of a flower (Ahmad & Tahir, 2016). This phase is accompanied by wilting, shedding of flower parts and fading of blossoms (Shahri & Tahir, 2010). Flower senescence is a rapid process compared to other parts of a plant hence providing a perfect model to study cell death. During flower senescence, developmental and environmental stimuli enhance the up regulation of catabolic processes causing breakdown and remobilization of cellular constituents (Siddhartha & Narendra, 2007).

Endogenous and exogenous ethylene sources have been documented to enhance the up regulation of catabolic processes causing breakdown and remobilization of cellular constituents in ethylene sensitive flowers (Chang *et al*., 2014). Abscisic acid (ABA) is thought to be the primary regulator of senescence in ethylene-insensitive flowers (Siddhartha & Narendra, 2007). In *Hemerocallis* (day lily) flowers oxidative stress and antioxidant activity have been established as the cause of senescence (Claudia & Christophe, 2010; Van Doorn & Woltering 2008). It has been reported that ethylene sensitivity, lipid peroxidation and membrane damage are involved in petal deterioration of both ethylene sensitive and ethylene insensitive flowers (Arora *et al*., 2006). Petals are the floral organs which primarily determinethe commercial longevity of flowers. Thus, the functional life of a flower is terminated by senescence (Shahri & Tahir, 2010).

2.4 Anthocyanins and phenolic changes in plants

Anthocyanin synthesis is an integral part of flower development and generally occurs at later stages of petal development (Yoshinda, 2008). Flower pigments of red, pink, blue and purple come mainly from the pigments called anthocyanins. Naturally occurring pigments, carotenoides are responsible for colouration in fruits and flowers (Wang *et al*., 2018). Anthocyanins are water soluble polyphenol pigments in the class of chemicals called flavonoids, which are phenolics (Kaššák *et al,* 2013).Phenylalanine is the precursor molecule for anthocyanins (Tanaka *et al*., 2008). They are secondary plant metabolites with a wide range of biological and physiological activities such as antoxidant and/or anti-inflammatory functions (Miguel *et al*., 2011; Ponmozhi *et al*., 2011).

Polyphenolic compounds also have antifungal and antibacterial activities (Kaššak, 2012). The biochemical pathways of the Shikimate, pentose phosphate and phenylpropernoid are involved in the biosynthesis of phenolic compounds (He, 2010; Lin *et al.,* 2016). Anthocyanins are found in cell vacuoles and are glycosides whose color variation is pH dependent (Schmitzer *et al*., 2010).Protocatechuic acid, chlorogenic acid, caffeic acid, p-coumaric acid, L-DOPAand garlic acid areamong phenolic compounds commonly found in plants whose structures range from simple phenolic molecules to polymerized compounds (Lin *et al*., 2016). A study on the concentration of major anthocyanins; quercetins, catechin and phenolic acids during flower development of *Rosahybrida* L. ‘KORcrisett’ flowers showed tenfold increase in concentration of garlic acid in buds than in senescent flowers (Schmitzer*et al*., 2010). This is in contrast to anthocyanins in senescent *Digitalispurpurea* flowers whose

levels have been reported to remain stable (Stead & Moore, 1977). Conversely, some flowers such as *Hibiscus mutabilis*and *Nicotiana mutabilis* have shown an increase in the level of anthocyanins at senescence as compared to mature stage of development (Macnish *et al*., 2010; Meng & Wang, 2004).

Other studies have indicated that ornamental flowers contain high levels of antioxidant compounds including phenolics, flavonoids and anthocyanins whose concentration is developmental stage dependent, constitutive and varies from species to species (Brunetti *et al.,* 2013; Cavaiuolo *et al*., 2013). A comparative study on African ginger (*Siphonochilus aethiopicus* indicated that inducing stress by varying irrigation and nitrogen regimens during growth caused production of secondary metabolites such as phenols (Mokgehle *et al*., 2017). The activity levels of antioxidant enzymes in both ethylene- dependent and independent flowers shows a pattern that is species dependent (Prata *et al*., 2017). Studies done on cut flowers of the genus *Iris* showed that different species showed differing trends of phenol concentration during the stages of flower development (Ahmad & Tahir, 2016). But a study on the cut flower, *Tagetes erecta* showed that the total phenols decreased as the flower development progressed towards senescence (Desai *et al*., 2012). The levels of superoxide dismutase (an antioxidant enzyme) were found to decrease with the progression of senescence in petals from rose, daylily,carnation, chrysanthemum, *Freesia* and *Gladiolus* cut flowers (Cavaiuolo *et al*., 2013). A research done on the concentration of antioxidative compounds such as anthocyanins, polyphenols and caroteinoids, proved a correlation between induction of antioxidant enzymes and the process of senescence in the above cut flowers (Kamal & Suman, 2008; Kazemi & Shokri, 2011).

The longevity of cut flowers is one of the main challenges of florists (Jalili *et al*., 2011; Thomas, 2013). One of the most important factors for consumers, apart from the quality of cut flowers is their vase life. For *Gladiolus*, the vase life is just 4 to 6 days. Appearance of senescent florets, at the bottom of the spikes marks the end of quality life of the florets in spite of opening of the upper florets (Khattab *et al*., 2017). Increased ethylene concentration in ambient atmosphere is one of the important factors that reduce longevity of cut flowers (Sakine *et al*., 2011). It is also well documented that one of the main causes for inferior cut flowers quality is the blockage of xylem vessels by micro-organisms that accumulate in the vase solution or in the vessels themselves (Auyeung *et al.,* 2017). With stem vessels blocked, continuing the water uptake and transpiration by the leaves of cut flowers results in net loss of water of flower and stem (Schouten *et al.,* 2018).

Several biochemical and metabolic changes take place in the flower during development and senescence including activities such as breakdown of starch into sugars, proteins into amino acids and changes in the phenol content (Ahmad & Tahir, 2016; Mwangi *et al.,* 2003; Singh *et al*., 2007). Plant senescence is regulated by a coordinated genetic program mediated by changes in ethylene, abciscic acid and cytokinin contents (Ferrante *et al*., 2012; Manzoor *et al*., 2018). A number of treatments have therefore been undertaken to delay senescence of cut flowers (Jowkar *et al*., 2017). It is recommended that fresh cut flowers be placed in clean water free of fluorides and chlorine to minimize loss of water through transpiration and respiration. Postharvest treatments on cut flowers have included pre-chilling of cut flowers in ice cold water, pulsing treatments, maintaining the cold chain storage and use of holding solutions among others (Bergamann & Dole, 2020; Manzoor *et al*., 2018; Senapati *et al*., 2016).

2.5.1 Pulsing of cut flowers

Pulsing is a short term treatment given to cut flowers whose effect lasts the entire shelf life of the flower, even when they are put in water (Jitendra *et al*., 2012). Different pulsing solutions have been used including dimethylsulphoxide (2%), 8-hydroxyquinolene sulphate, silver thiosulphate, quaternary ammonium salts, slow release chlorine, aluminium sulfate, citric acid, nitric oxide and 8-hydroxyquinoline citrate among others (Jowkar *et al*., 2017; Lazar *et al.,* 2010; Li-Jen *et al*., 2001; Seyyedeh *et al*., 2013; Singh *et al.,* 2007). The term 'pulsing' refers to placing freshly harvested flowers for duration of time (ranging from a few seconds to several hours) in a solution formulated to extend their vase life.

The pretreatment solutions contain sucrose which allows the flower to continue its normal metabolism, a biocide, a weak acid and an anti- ethylene agent and some may also have plant growth regulators (Sharma & Srivastava, 2014). It has been proved that some pulsing solutions significantly increased vase life and floret opening percentage (Sudaria *et al*., 2017), decreased contamination in vase solution and improved water balance for cut flower spikes and also maintained flower quality i.e. anthocyanin content in petals (Tilahum *et al*., 2015). Post harvest characteristic of cut *Gladiolus* flowers with exogenous calcium delayed the onset of senescence, (Bai *et al.,* 2009). Plant growth regulators such as benzylpurine and gibberellic acid have been found to impact positively on the postharvest life of cut Gerbera flowers (Abd-El-Kefie *et al*., 2016; Watershed *et al*., 2009). A study done on cut stems of *Sedum aizoon* gave

results contrary to the norm when a pulsing solution of 200 mg/l 8- hydroxyquinoline sulphate failed to increase the vase life of this flower (Krzyminska *et al.,* 2014).

Combining of pulsing of cut *Gladiolus* with cold storage improved flower quality due to control of bacteria growth (Bhat & Sheikh, 2015; Serek *et al*., 1994). Pulsing of *Gladiolus* cut spikes with treatment of 20% sucrose plus 200ppm 8-HQS for 20 hrs at 23±2°C increased the flower vase life compared to non-pulsed flowers that were stored at 5±1°C (Kushal *et al.,* 2000). There has been a search for more effective and safer treatments fot increasing the vase life of cut flowers (Senapati *et al*., 2016). Cut flowers had generally been treated with anionic complex of silver thiosulphate, an inhibitor of ethylene for increased vase life. The silver thiosulphate is reported to have some antimicrobial activity inside the plant tissues hence most effective in improving flower vase life (Mohsen & Ameri, 2012).

The use of silver thiosulphate has been banned in many countries since it is a potential environmental pollutant (Shahri & Tahir, 2010). The use of salicylic acid and essential oils as safe preservatives for maintenance of food, cut flower and agricultural products has been documented (Abdolahi *et al*., 2010; Kazemi & Shokri, 2011). Salicylic acid is a phenolic compound involved in regulation of many processes in plant growth and development including stomatal movement, seed germination, ion absorption, sex polarization and induction of disease resistance (Khattab *et al*., 2017; Zhang *et al.,* 2003). Essential oils that have been used as preservatives include extracts from mint (*Mentha* spps), *Thymuskotschyanus* and *Carum copticum* among others, (Bagamboula *et al*., 2004; Jalili *et al.,* 2010; Jalili *et al*., 2011; Kotzekidoe *et al*., 2011). Essential oils have been known to prolong vase life of *Rosa* (*Rosa hybrida* L cv. 'grand') (Nermeen, 2012). In *vitro* efficacy of plant essential oils against *Botrytis cinerea*, and *Mucor piriformis* has been documented (Abdolahi *et al.,* 2010).

The phenolic constituents in essential oils contribute to their anti microbial activities (Bagamboula *et al.,* 2004; Šilha *et al*., 2020). Flowers are strongly dependent on the carbohydrate status and the acceptable amounts of metabolic sugars are factors that affect the rate of senescence (Mwangi *et al.,* 2003; Senapati *et al*., 2016). The vase life of cut flowers has been enhanced when germicides are used together with a carbohydrate source (Ahmad *et al.,* 2016; Manzoor *et al*., 2018). The experimental study on different concentrations of the carbohydrate sucrose in pulsing solutions showed 12% to be ideal for spike preservation (Regmi *et al.,* 2000). Indeed different cut flowers species requirements for the amount of carbohydrates necessary to keep vitality of post harvest flowers vary (Senapati *et al*., 2016; Yamada *et al*., 2003).

2.5.2 Storage of cut flowers

It has been observed that total starch, total soluble sugars, total free amino acids and total phenols in the petals of cut flowers (´Golden Gate' cut roses) are significantly affected by pre-cooling with ice cold water spray, pulsing, cold storage and also packaging materials (Bayleyegn *et al*., 2013; Mwangi *et al.,* 2003). Modification of pre-storage temperatures coupled with subsequent pulsing and post storage vase solutions contributed in improving *Gladiolus* vase life up to 17 days (Bhat & Sheikh, 2015; Singh *et al.,* 2007). Cut flowers are either transported in holding solutions (wet storage) or they are pretreated with pulsing or hydrating agents and packed dry for storage (Ahmad *et al*., 2013; Flora Culture, 2019; Senapati, 2016; Shahri *et al*., 2011; Macnish *et al*., 2009). The proper understanding of the physiology of material to be stored is paramount in choice of cold storage temperature in order to avoid chilling injury or acceleration of senescence in cut flower (Ahmad *et al*., 2013). Different flowers have different minimal temperatures below which there is risk of chilling injury. Consequently gladioli are stored between 2-4 ^{o}C; Bird of Paradise and *Anthurium sp.* are stored at 13°C such that each cut flower has its own optimal cold storage temperature. Flowers from the tropics are stored separately as low temperatures below 10°C lead to chilling injury (Senapati, 2016; Reid & Jiang, 2012).

There are different cold storage methods including Controlled Atmosphere (CA) or Modified Atmosphere (MA), in which increased CO_2 and reduced O_2 concentrations in association with cold storage increase storage life of cut flowers and plants (FAO, 2004; Krishi, 2010). Some plants such as *Aster* and *Delphinium* have been refrigerated for storage at -2.8^{o}C and -2.2^{o}C at very high relative humidity to avoid chilling injury and desiccation risks. Because ethylene production continues at low levels even during cold storage, different treatments such as silver nano particles, controlled atmospheres or use of nutritive solutions that prevent depletion of carbohydrates are utilized to help prevent cut flower deterioration (Ahmad *et al*., 2013; Jitendra *et al*., 2012; Manzoor 2018; Reid & Jiang, 2012).

It is recommended that for proper storage management, maintenance of the cold chain at every step from harvest until consumer receipt of cut flower be adhered to (Senapati *et al*, 2016). Hence before storing cut flowers for a period of time or transporting over a long distance it is recommended that they are pre-cooled (Ahmad *et al*., 2013). Precooling methods available include hydro cooling, air cooling and also vacuum cooling. In the absence of these systems precooling can be done by placing cut flowers in open boxes in a cold room for a period of at least 8 hours to decrease the temperature (Senapati *et al.,*2016). The cut flowers or plants can then be shipped or air freighted either in dry cold storage or wet cold storage (FloraCulture,

2019; Skutnik *et al*., 2020). Wet storage of cut flowers at cool temperatures is recommended for flowers that are for short storage duration (Pranuthi *et al*., 2018). Conversely, dry cool handling of cut flowers is applicable when long storage is needed especially when long distance destinations are involved for cut flower transportation (Dastagiri *et al*., 2017; Macnish *et al*., 2009; Shahri *et al*., 2011; Srivastava *et al*., 2015).

Chemical treatments administered during cold storage help to alleviate flower deterioration due to chilling injury and nutrient depletion (Senapati *et al*., 2016; Skutnik *et al*., 2020). It has been documented that *Gladiolus* have been cold stored dry at 1-4°C for 3-4 weeks while *carnation* cut flowers were wet cold stored at 4°C and dry cold stored at 0-1°C for durations of 4 weeks and 4-12 weeks respectively (Ahmad *et al*., 2013; Bhat & Sheikh, 2015) Cut flowers/plants experience physiological changes and ethylene accumulation on removal from storage and hence chemical treatments are employed to preserve quality (Senapati *et al*., 2016; Skutnik *et al*., 2020). Inclusion of growth regulators such as cytokinin, gibberellins, auxin and others has been found to improve post harvest quality of cut flowers (Aziz *et al*., 2020; Bhagheri *et al*., 2013; Sakine *et al.,* 2011). An innovationthatutilized an electrochemically treated solution containing potassium chloride, hypochloric acid and dissolved oxygen into which cut flower stems were dipped improved their quality and vase life (Svetlana & Sampson, 2010). Fresh cut flowers and foliage have enhanced quality when dyed in preservative solutions containing glycerol and surfactants (Kazuo *et al.,* 2005). The positive attributes of nanotechnology have been employed to enhance postharvest quality of cut flowers and other horticultural products (Manzoor *et al*., 2020)

2.6 Microbial genera associated with senescence

Occlusion by microbes and the extracellular polysaccharides that they produce is by far the most common cause of poor water relations in cut flowers (Reid & Jiang 2012). This is more so in cut flower as a wounded stem placed in water quickly depletes the oxygen in vase solution, providing perfect growing conditions for microbes (yeasts and bacteria) (Rafi & Ramezanian, 2015; Reid & Jiang, 2012). The bacteria isolated in vase solution of cut flowers include: *Pseudomonas* (80%), Enterobacteriaceae (5-10%), and others such as *Aeromonas*, *Alcaligenes*, *Citrobacter*, *Flavobacterium*, *Staphylococcus xylosus*, *Streptomyces griseus, Streptomyces albus* and *Erwinia* (Evans *et al*., 2010; Mohammed *et al*., 2012; Mwangi *et al.*, 2003; van Doorn & Witte, 1997).

The following fungi have been isolated and identified from cut flower vase solutions; *Aspergillus species, Alternaria altanata, Alternaria brassicola, Aureobasidium pullulescens, Acremonium sp., Dreschlera specifera, Cladosporium cladosporoides, Cladosporium oxysporum and Fusarium pallidoroseum* (Mwangi *et al.,* 2003; Narayanasamy, 2011). *Fusarium* corm rot is a common disease affecting *Gladiolus* flowers while *Botrytis* infection has been associated with decreased vase life of cut flowers (Lopez – Moya *et al*., 2019; Mwangi *et al*., 2003). Germicides such as 1-bromo-3-chloro- 5, 5-dimethylhydantoin (BCDMH) and dichloroisocyanuric acid (DICA), 8-hydroquiquinoline compounds, aluminium sulphate and silver nano particles are among compounds that have been reported topossess antiproliferative effect on microbial flora (Kazemi *et al*., 2010; Manzoor *et al*., 2018; Rodney & Megan, 1993). Malic acid is another bacteriocide which was used either alone or in combination with sucrose and found to reduce bacteria populations in cut flowers of carnations' vase solutions (Kazemi *et al*., 2010). That microorganism indeed reduced vase life of cut flowers has been confirmed by the observed that different microbes differentially have adverse effects in postharvest lives of roses, *Chrysanthemums grandiflorum* hybrids (syn. *Dendranthemum*) and carnations (Zagory & Reid, 1986).

2.7 The dynamics and technological developments in floriculture industry

A number of technologies have been adopted to prolong the market value of cut flowers including establishing optimal regimes for 1-methylcyclopropene (1-MCP) and natural antimicrobials on cut flowers and potted plants (Ali *et al*., 2012; Song & Fan, 2012). Functional analysis of regulatory genes associated with senescence and abscission has been done with a view of breeding long lasting flowers (Chang *et al*., 2014). The use of plant extracts such as tea and the antibiotic rifampicin to prolong vase life of cut *Denderanthema* (*Chrysanthemum grandiflorum* L. cv. Purple) has been employed as part of a search for alternative treatments to improve market value of the produce (Hashemabadi & Bagheri, 2014). The pathogenicity to plants by *A. raphani* and *A. brassicicola* has been brought under control by use of biological agents such as actinomycetes, fungi, some plants and plant products (Yen-Ting & Wein-Hsiang, 2014). *Chetomium globosum*, *Trichoderma harzianum*, *T. koningii* and *Fusarium* sp. have effectively been used as antagonists in bio-control of seed-borne *Altenaria* pathogenicity against cut flowers (Mangain *et al*., 2013). According to FloraCulture (2019), the use of biological controls in ornamentals seems to be inevitable. These could involve the use of predatory mites, parasitic wasps and other preadatores. Biopesticides have also been

recommended in floriculture for being nvironmental friendly and being less harmful to non-targets (Kazimierczuk *et al.,* 2018).

National Cut Flower Trial Programs administered by Carolina state University, USA, and Association of Specialty Cut Flower Growers has revealed varied responses by new flower cultivars to commercially available holding preservatives and hydrating solutions (Clark *et al*., 2010). This variability has also been confirmed in the use of 8-hydroxyquinole citrate as a preservative on cut Rose flowers Golden Gate and Cherry Brandy cultivars in which their vase lives were enhanced and adversely affected respectively (Mohammad *et al*., 2012; Mwangi *et al*., 2003;). 8-Hydroxyquinoline sulphate has been reported to have beneficial effects on postharvest life of other cut flowers such as *Gladiolus* and *Gerbera* (Lazar *et al*., 2010; Sujatha *et al.*, 2003). Hence the use of preservatives in improvement of postharvest life of floricultural products should be examined and recommended based on the flower species and even the cultivar under consideration (Gent &McAvoy, 2000;Ritu *et al*., 2015). The use of 8-hydrxyquinoline as a preservative did not increase the vase life of cut *Sedum aizoon* L. stems (Krzyminska*et al*., 2014). But 8-hydroxyquinoline sulphate in combination with sucrose increased the vase life of cut rose, waxflower and *Gladiolu*s flowers (Bhat & Sheikh, 2015; Dung *et al*., 2017; Ichimura *et al*., 1999).

This study focused on the effects of the biochemical changes and the diversity of microorganisms on the vase life of cut *G. grandiflorus* cv, 'Fado' during pulsing and wet storage. Dissemination of these research findings has been done through publications and conferences for consumption by stakeholders to improve the market value of this cultivated variety of *G. gradiflorus*. The target group involving small scale flowesrs can utilize the formulation of 600 ppm 8-HQS plus 5% sucrose with cold storage at 3 ±1 ^{o}C for postharvest handling of of cut flowers within 4 days without adverse effect to cut *G. grandiflorus* cv. Fado. The bulking and transportation of this cultivar of *Gladiolu*s has to be accomplished with 4 days of harvest to minimize loss in vase life and other quality parameters. Results generated from this study showed that this cultivar improved in vase life and other quality parameters after pulsing with 600 ppm plus 5% sucrose with subsequent wet cold storage duration of upto 4 days. This data pertaining to vase study of this cultivar could be utilized to improve the quality and market value of this *Gladiolus*cultivar contributing to income and foreign exchange generating crop.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Experimental site description

The *Gladiolus* flowers were grown in open field from corms at the Horticulture Research and Teaching Field, in the Department of Horticulture, Egerton University, Kenyaduring the seasonofSeptember to December 2014. The field lies at a latitude of 0°23’ S and longitude 35°35’ E in the Lower Highland III Agro-Ecological Zone (LH3) at an altitude of 2,238 m above sea level. Average maximum and minimum temperatures range from 19°C to 22°C day temperature and 5^0C to 8°C night temperature respectively, with a total annual rainfall of 1200 to 1400 mm. Soils are predominantly mollic / andosols with a pH of 6.0 to 6.5 (as described by Kinyanjui, 1979).

3.2 *Gladiolus* cut flower production

3.2.1 Planting material and crop establishment

Gladiolus grandiflorus corms cv. ‘Fado’ of uniform grade (2.0 – 2.5 cm diameter) were sourced from a grower in Nakuru County, Kenya and they were kept evenly spread out at room temperature for one month to break dormancy. The corms were then treated with a copper based fungicide, Funguran® - Progress (also referred to as Funguran ® –OH or Funguran ® – OH 50WPwhich was administered at 3 grams / liter (K¨uhne *et al.,*2018). The corms were then evenly spread out to air dry for at least one week. Commercial corms with a diameter of more than 2.5 cm were selected for planting.The experimental field was prepared manually by digging and harrowing to a fine tilth using a plough.

The corms were planted in two open field experimental plots on beds measuring 1m x 1m at spacing of 25 cm by 20 cm and each experimental unit consisted of four rows with ten plants per row. Uniform sprouting of corm sheaths was observed 16 days after corm planting. Two weeks after the corms emerged from the soil surface a fertilizer dose of 50 grams NPK (17:17:17) was applied to the vegetative plants Watering was done using drip irrigation and the corms were allowed to grow to maturity within 90 days. Flower inflorescence was observed after 85 days while spike emergence occurred on the 86th day. Standard good agricultural management practices such as weeding, irrigation and crop protection was applied as necessary (**Plate 3.1**).

3.3 Experimental design and treatment application

Cut flowers of uniform grade of *G.grandiflorus* cv. 'Fado' were harvested after 90 days from the open field of Horticulture Teaching and Research Field 3. The flower spikes were harvested at the color break stage when the basal two florets in the spike had shown color. The flower spikes were harvested in the morning hours (when the rate of flower metabolism is not high) and brought to the biotechnology laboratory, Egerton University, Njoro (in Nakuru County, Kenya) in a clean plastic bucket containing tap distilled water. The stem ends were then recut at 2 cm from the base with sharp and clean secateurs after harvesting: The experiment was laid in a Completely randomized design (CRD) with four replications as shown in the experimental layout below (**Table 3.1**):

Plate 3.1: *Gladiolus* (*Gladiolus grandiflorus* L. cv 'Fado') under cultivation using standard agricultural practices

Treatments employed to fresh cut flower were (i) pulsing with sucrose (5%) + 8-HQS (600 ppm) and (ii) pulsing (in distilled water) at room temperature for 24 hours. These flowers were transferred immediately to a bucket containing distilled water and kept in a refrigerated cold storage chamber maintained at 3 ± 1°C, in two different sets in separate buckets. After 0, 1, 2, 3, 4 and 5 days of storage, each of the two sets of flower treatments were brought to room temperature and compared with freshly harvested flowers for their keeping quality in tap water in the ambient temperature.

Table 3.1: The experimental layout for the completely randomized design

P0	**NP1**	**P 5**	**P 1**	**NP 0**	**NP 3**	**P 2**	**NP 2**	**P 3**	**NP 4**	**NP 5**	**P 4**
NP 3	P 4	NP 1	P 5	P 0	NP 0	P 3	NP 5	NP 4	NP 2	P 2	P 1
P 2	P 0	P 3	NP 3	NP 0	NP 5	NP 4	NP 1	NP 2	P 1	P 4	P 5
NP 4	NP 0	NP 5	NP 1	P 3	NP 3	P 0	NP 2	P 1	P 5	P 2	P 4

Legend: *P = Pulsing with sucrose (5%) + 8-HQS (600 ppm) at room temperature for 24 hours; NP = Pulsing (in distilled water) at room temperature for 24 hours; Numbers 0, 1, 2, 3, 4, and 5 = Cold storage duration (days) at 3 ± 1°C.*

3.3.1 Study of vase life and quality of flowers

Vase life studies and quality of non – pulsed and pulsed gladioli flowers described in section 3.3 above was done after cold storage duration of 0,1,2,3,4 and 5 days.Each treatment and respective cold storage interval consisted of quadruplets of five flowers. The total number of flowers in the pulsed and non – pulsed cut gladioli was 240. These flowers were then slightly recut at the base and transferred to 1000 ml plastic cylindrical vases containing tap water to study the vase life and quality of flowers at ambient temperature (as shown in **Table 3.2**).

3.4 Collection of flower samples for biochemical analysis

Petal samples for biochemical analysis were collected at different stages from both non-pulsed and pulsed flowers:

(i) On the day of harvest (day zero)

(ii) Following storage (0, 1, 2, 3, 4, 5, days)

(iii) At senescence (at day 4.5 and 11.5 for non-pulsed and pulsed *Gladiolus* respectively)

The collected samples were dried in an oven at 70 °C to a constant weight for the analysis of Total Soluble Sugars, (TSS), Total Starch (TS), Total Free Amino Acids (TFAA), Total Phenols and Anthocyanins (**Figure 3.1**).

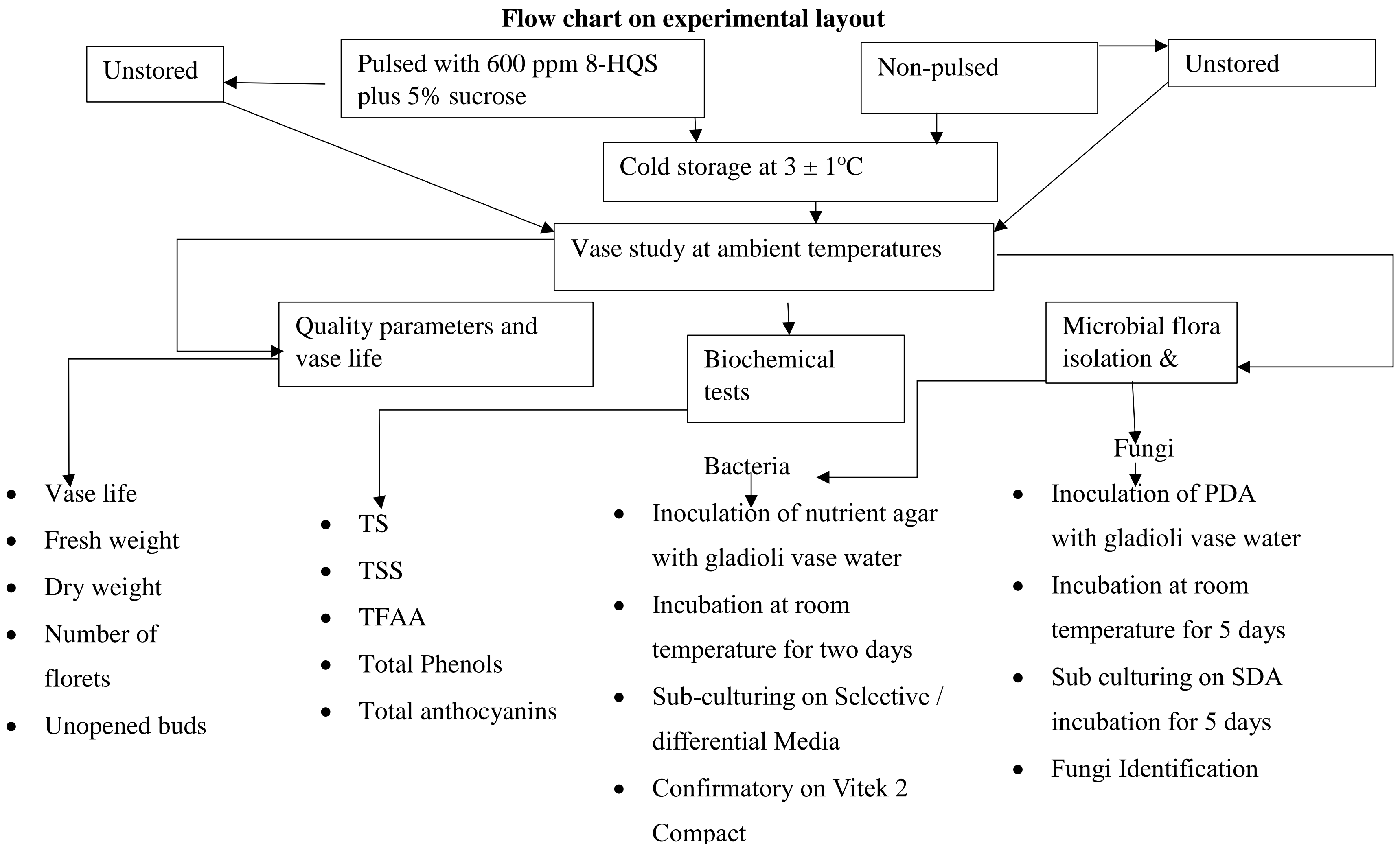


Figure 3.1: Flow Chart for the experimental layout

Table 3.2: Study of vase life and quality of pulsed and non-pulsed wet stored *Gladiolusgrandiflorus* L. cv. 'Fado' cut flowers

Treatment (24 hours)	Changes in fresh weight (grams)			Changes in dry weight (grams)			Vase life (days)	Water uptake (mls)
	After storage	On 3rd day	At senescence	After storage	On 3rd day	At senescence		
600 ppm + 5 % sucrose	A	B	C	D	E	F	β ±	α ±
Distilledwater	A1	B1	C1	D1	E1	F1	$β_2$ ±	$α_2$ ±

Legend:*Letters A, B, C, D, E and F represented the changes in fresh weight and dry weight thatoccurred in gladioli flowers pulsed with 600 ppm 8-hydroxyquinoline solution containing 5% sucrose. This was compared to non-pulsed (kept in distilled water) wet stored flowers and the accrued weight changes were denoted by A1, B1, C2, D1, E1, and F1.*

The symbols β and $β_2$ represented the respective vase life in days for the wet stored pulsed and non-pulsed gladioli flowers.

The symbolsα and $α_2$ represented the water uptake for the pulsed and non-pulsed gladioli on the 3rd day and at senescence depending on the 0, 1, 2, 3, 4, or 5 days of wet storage.

3.4.1 Extraction and determination of Total Phenolics (TP)and anthocyanins by high performance liquid chromatography

The extraction of phenolic compounds from the gladioli petals was done using a gradient system of solvents coupled with isocratic elution (Schmitzer *et al*., 2010). Petals from pulsed and non-pulsed *Galdiolus* cut flower stored under wet storage over a period of time (0,1, 2, 3, 4 and 5 days) were ground to a fine powder with liquid nitrogen. Extraction of phenolics was done by adding 3 ml methanol containing 3% (v/v) HCOOH and 1% (w/v) 2, 6-di-tert-butyl-methylphenol in an altrasonic water bath for 1 hour. Treated samples were centrifuged for 7 minutes at 12000 rpm. The supernatant was filtered through a polyamide filter (Chromafil

AO-45/25; Machery- Nagel, Düren Germany) and transferred to a vial before injection in a high performance liquid chromatography (HPLC) system. Samples were analysed using Thermo Finnigan Surveyor HPLC system (Thermo Scientific, San Jose, CA) with a diode array detector at 280 nm (gallic acid, protocatechuic acid, catechin, chlorogenic acid, caffeic acid, p-coumaric acid), 350nm (querrtins) and 530 nm (anthocyanins). A HPLC column (18,150 x 4.6 mm, Gemini 3µl; Phenomenex Torrance CA) protected with a Phenomenex security guard column operated at 25^0C will be used. The injection volume was 20µl and the flow rate was maintained at 1ml min $^{-1.}$ The elution solvents was aqueous 1% formic acid (A) and 99 % acetonitrile (B). Samples were entered according to the linear gradient described by Marks *et al*., (2007): 0 to 5 minutes, 3% to 9% B ; 5 to 15 minutes, 9% to 16% B; 15 to 45 minutes, 16 5% to 50% B; 45 to 50 minutes , 50% isocratic and finally washing and reconditioning the column.

All compounds were expressed as µg mg^{-1} dry weight. The quantitative and qualitative determination of phenolics from pulsed and non-pulsed cut gladioli were done by running sample extracts against standards of gallic acid, catechin, quercetin-3-oprutinoside, cyanin chloride, kuromanin chloride and chlorogenic acid from Sigma-Aldrich (Steinheim, Germany), catechin, protocatechuic acid, caffeic acid, p-coumaric acid, quercetin-3-glucoside, quercetin-3-o-rhamnoside and peonidin-3-o-glucoside from Fluka (Buck, Switzerland).The chemicals for sample preparation and mobile phase were methanol, di-tert-butyl-4-methylphenol (BHT) and acetonitrile from Sigma- Aldrich and formic acid from Fluka. The water used was double-distilled and purified with a Millipore-Q – water purification system by Millipore (Bedford, MA) (Schmitzer *et al*., 2010).

3.4.2 Extraction of anthocyanins for spectrophotometric analysis

The amount of 2g sample of dried petals were weighed and placed in a 25 ml Erlenmeyer flask. Twenty five milliliters of a 1% HCL in methanol solution was added and the flask was capped with a marble. The mixture was boiled for ten minutes and cooled to room temperature for 1 hour. Absorbance was taken at 530nm using a spectrophotometer. Distilled water was used as a blank (Hillebrand *et al*., 2009; Shi *et al*, 1992).

3.4.3 Extraction and determination of Total Starch(TS)

Extraction of starch from cut powderedgladioli petals was done byhomogenizing in ethanol followed solubulising the residual in perchloric acid and eventually assaying

quantifying the equivalent reduced sugars by the anthrone reagent at 630 nm (Kaur & Mukherjee, 2012). Two grams of finely powdered dried petal samples of the pulsed and non-pulsed *Gladiolus* were homogenized with 10 ml of 80% ethanol by stirring thoroughly, allowing standing for 5 minutes and then centrifuging at 5000 rpm for 15 minutes. The residue was retained after removing the alcoholic solution (to remove the sugars). To the residue 10 ml of fresh hot 80% ethanol was added while stirring and the contents were centrifuged again, retaining the residue as before by discarding the alcoholic solution. The washing treatment was repeated twice to get rid of the sugars and the residue was dried over a water bath. To the dried residue 5 ml of water was added and the contents were cooled in ice before adding 6.5 ml of 52% perchloric with constant stirring for at least 30 minutes. The contents in tubes were then centrifuged at 5000 rpm for 15 minutes and the aqueous starch solution was poured into a 100 ml volumetric flask. The extraction was repeated twice and the starch solution was pooled together. The volume was made up to 100 ml with distilled water and was then filtered through Whatman No.1 filter paper.

Starch was extracted by pipetting out 0.2 ml of the filtrate from above into a test tube and bringing the volume to 1 ml with distilled water. Then into the tube contents 4 ml of freshly prepared anthrone reagent was added under ice. Each tube contents was thoroughly mixed and then heated in a water bath for 8 minutes at 100°C. The solutions in the tubes were then rapidly cooled to room temperature and the intensity of green to dark green color was read at 630 nm in a digital UV-visible spectrophotometer. A glucose standard calibration curve was prepared by making a stock glucose solution of 100 mg in 100 ml of water. A 1:10 dilution of the stock glucose solution in distilled water was prepared. Aliquots ranging from 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 ml of the working glucose standard solution were made to 1 ml with distilled water to obtain standard glucose concentrations of 0, 10, 20, 30, 40, 50, 60, and 70 µg/ ml. To each 1 ml of glucose at different concentrations, 4 ml of freshly prepared anthrone reagent was added under ice and the contents stirred thoroughly. The test tubes were heated for 8 minutes in a water bath at 100 °C and then cooled rapidly to room temperature. The intensity of green to dark green color was read in a digital spectrophotometer for the different glucose standards. A standard curve of glucose was prepared of absorbance against the different glucose concentrations. The amount of glucose in the samples of pulsed and non-pulsed petals and was extrapolated from the glucose calibration curve. Quantity of starch was calculated in terms of glucose equivalents and a factor of 0.9 was used to convert the value of glucose to starch. Quantity of starch was expressed in terms of mg/g of dry weight of leaf and petal tissue.

3.4.4 Extraction and determination of Total Soluble Sugars(TSS)

The Extraction and determination of total soluble sugars from cut gladioli was done using phenol reagent in concentrated sulphuric acid (Mozzam & Zeynab, 2012). The amount of 0.2 g of finely powdered dried petal samples was homogenized in 10 ml of 80% ethanol with constant stirring and kept overnight. Centrifugation of the contents was then done at 2000 rpm for 20 minutes. Aliquots of 0.04 ml were then pipette to test tubes and the volume of 1 ml 5% phenol was added followed by 5 ml of concentrated sulphuric acid with concurrent agitation of the tubes vigorously. The solution in the tubes was left to stand at room temperature for 15 minutes. The development of golden yellow color formed in each sample tube was followed by reading the absorbance at 490 nm after proper setting and calibration of the instrument with a blank. The total sugar content in the flower samples was worked out by referring to a standard curve of sugar (glucose) and expressed in mg/ml of dry weight.

A 0.2 g per cent stock glucose solution was prepared by dissolving 100 mg of glucose in 50 ml of 80% ethanol. A set of standard glucose solutions of strengths 0, 20, 40, 60 and 80 µg/ ml were arranged by taking 0, 0.01, 0.02, 0.03 and 0.04 ml of stock solution and respectively adding into each tube 0.04, 0.03, 0.02, 0.01 and 0 ml of 80% ethanol so that the volume in each case was made up to 0.04 ml equivalent to the volume of the alcoholic aliquot taken from the alcoholic extract of the powdered flower sample. To each of the test tube contents 1 ml of 5% phenol was added, followed by the addition of 5 ml of concentrated H_2SO_4 with concurrent stirring to mix. The solution in each test tube remained at room temperature for 15 minutes for color development. The optical density (OD, absorbance) of individual contents was read in a spectrophotometer at the wavelength of 490 nm. A standard curve of glucose was prepared by plotting a graph of absorbance against the glucose concentration. Glucose concentration from the flower samples from the pulsed and non-pulsed gladioli was read off from the graph (Mozzam &Zeynab, 2012).

3.4.5 Extraction and determination of Total Free Amino acids (TFAA)

Extraction of amino acids from petals of the *Gladiolus* cut flower was done by use of a phosphate buffer followed by by removal of organic pigments by use of a solvent (Soudry *et al.,* 2005). Approximately 2 g of slurry from wet stored pulsed and non-pulsed flowers were suspended in 20 ml of phosphate buffer of pH 7.0 in 250 ml beakers. The suspension wascentrifuged in a refrigerated centrifuge at 300 rpm for ten minutes and the supernatant poured into a separating funnel and shaken with 10 ml petroleum ether to remove the organic pigments.

The top phase was discarded and the aqueous phase which contained protein and amino acids was retained. The proteins were precipitated from aqueous phase by adding 5 ml of 10% (w/v) trichloroacetic acid (TCA) to 5 ml of the extract. The mixture was shaken and kept in the freezer for 10 mins. The precipitate formed was used for total amino acids profile determination using reverse Thin Layer Chromatography (TLC). The quantitative estimation of amino acids was done using the colorimetric method (Soudry *et al*., 2005). The supernatant of each leaf and petal sample was prepared and used to determine the concentration of each amino acid present. Each supernatant was made up to 2 ml with distilled water and 1 ml ninhydrin solution was added. The absorbance was taken at 570 nm using a spectrometer. A control test tube containing 2 ml distilled water and 1 ml ninhydrin solution was set up. The concentration of each amino acid wasinterpolated from a luecine standard curve.

A standard curve was prepared for determination of total amino acids using a leucine standard solution which was prepared by dissolving 10 mg of leucine in 10 ml of phosphate buffer of pH 5.34. From this solution 1 ml was made up to 100 ml with the phosphate buffer to give a working solution with the concentration of 50 µg / ml. The amounts of 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 ml of the working solution were made up to 1 ml with the phosphate buffer to obtain standard leucine concentrations of 0, 5, 10, 15, 20, 25 and 30 µg / ml. To each 1 ml of leucine of different concentrations 0.5 ml of ninhydrin solution was added. After thorough shaking to mix the tubes were immediately immersed in a hot water bath at 100ºC for 15 minutes. During heating the solution gradually turned purple. Immediately after removing the tubes from the hot water bath 5 ml of 50% isopropyl alcohol wiere added to each tube. This was followed by vortexing and then cooling the tubes to room temperature. The absorbance of the standards was read at 570 nm and a standard curve of leucine was prepared by plotting a curve of concentration against absorbance.

3.4.6 Chromatographic separation of amino acids

The amino acid contents of extracts were separated by reverse phase thin layer chromatography (Avalaskar *et al*., 2011). The reverse thin layer chromatography plates were dried at 110°C before use as TLC plates tend to pick up moisture from air and this could considerably reduce their ability to absorb sample. Aliquots of 50µl of each filtrate was spotted on thin layer glass plates along with 20 µl reference standard amino acids (0.1% w/v). The standard amino acids used were isoleucine, lysine, tryptophan and methionine. One dimensional ascending chromatography was used. The solvent system employed for the separation was n-butanol: glacial acetic acid: water at a ratio of 4:1:4 (v/v/v) and separation

was carried out for 3 hours. The chromatogram was air dried and amino acids located by spraying with 0.2% (w/v) ninhydrin in ethanol. These were allowed to air dry and then oven dried at 100°C for 5 mins for the spots to develop. Identification of the separated amino acids was done using the reference standards.

3.5.1 Isolation and identification of fungi in vase water

Fungal pathogens from vase water of pulsed and non-pulsed *Gladiolus* cut flowers were isolated, quantified and identified on zero and third days in vase and at senescence by the use of general and selective media as well as microscopic and colony morphological characteristics (Narayanasamy, 2011). The vase water was transferred to sterile Petri dishes containing standard media such as Potato Dextrose Agar (PDA) supplemented with streptomycin sulfate at the rate of three to four pieces of tissue per Petri dish and incubated at room temperature (25-27°C) that could favor pathogen development. A portion of mycelium developing on the nutrient medium was transferred to agar slants for purification and storage for further examination. Purification of cultures of fungal pathogens in agar slants was by either the hyphal tip method or the single spore isolation method. In the single hyphal method a segment of fungal growth in the agar medium was transferred to the center of Petri dishes containing nutrient medium using a flame sterilized inoculating needle and incubated at room temperature or optimum temperature for a few days. As the mycelium grew in the medium the advancing edge of mycelium had hyphal tips that were well separated from each other. They were marked by a glass marking pencil by observing under the microscope. The marked hyphal tips along with bits of agar were carefully transferred individually to separate agar slants in tubes using sterile inoculating needles. The hyphal tips in tubes develop into a pure colony of the fungal species under investigation. The fungus were sub-cultured at regular intervals to maintain vigor.

The fungal cultures were alternatively purified by the single spore isolation method in which a spore suspension was prepared by transferring the fungal growth in the agar slant to sterile water kept in sterilized test tubes followed by vigorous shaking in order to disperse the spores from spore-bearing structures. A serial dilution of spores was prepared by transferring serially 1 ml aliquots to a series of test tubes containing 9 ml of sterile water. Aliquots (1 ml) of spore suspension at optimal dilutions mixed with melted nutrient agar (at about 45°C) was transferred to sterile Petri dishes and the mixture were spread by tilting the dishes gently in different directions for uniformly covering the entire surface of the bottom plate. The Petri dishes were then kept at temperatures that favor spore germination. The dishes were examined

under the microscope at regular times and the locations of germinating spores were marked by using a glass marking pencil. Bacterial and mites contaminants were eliminated by use of a technique based on fact that they inhabit the upper surface of the agar medium. The contaminated cultures were placed upside down. A thin layer of agar with the inverted agar culture was gently removed and transferred to plates containing sterile medium. Colonies developing on this medium were free of contaminants present in the original cultures.

Identification and morphological characterization of the fungal pathogens was based on the sexual and asexual stage structures, hyphae, conidia, septation, concentric zone, pigmentation, fruiting bodies, various spore forms and any other visible structures that could be observed under a compound microscope at the magnifications of 100X, 400X and 1000X. Microscopic characteristics were used to identify fungi up to the genus level (Meer *et al*., 2013). For some fungal pathogens, specific conditions were provided for sporulation (production of spores / conidia) and morphological criteria were determined for appropriate placement in different species, genera, family and any other organization according to standard keys of identification of fungi. Some fungal pathogens were given certain treatments so as to induce spore formation. Exposing fungal cultures to wavelengths of near ultraviolet (NUV) region of light was effective method of inducing sporution in *Alternaria solani* and *Septoria lycopersiei* species. Sporulation in *Fusarium oxysporum f. sp. niveum* was induced by incubating the plates at 25°C for 10 days in darkness. Alternation of light and darkness was required for some fungal pathogens such as *Altarnaria altarnata* to sporulate. *Bortrytis sp.* and a host of other plant pathogens grown on PDA and isolates were incubated at 20-22°C for 7 days under light to induce production of conidia. Production of sclerotia was induced in these species by incubation at 8 ± 1°C in darkness.

The characteristics of both the conidia and sclerotia was used in the identification of species of *Botrytis*. Czapek selective medium containing 20% sacharose was used to distinguish cultural and micro morphological characteristics of the different species of *Fusarium* based on variations in colony color and texture. Bringing the pH of the medium to 4.4 down from 7.7 was used to intensify the morphological difference within the species of *Fusarium* without altering their characteristics. Inhibition of the commonly found soil species *Rhizoctonia* sp. was accomplished by incorporation of a combination of rifampicin and toiclofosmethyl. CW medium (a semi-selective medium) which suppressesed the growth of saprophytic fungi was used for the detection of the pathogen *Alternaria brassicola*. *Botrytis* selective medium (BSM),was used to isolate and enumerate the polyphagous fungal pathogen *Bortrytis cinerea* because it favored development of higher numbers of colony forming units

(CFU) in individual Petri dishes due to restricted radial growth of colonies. *B. cinerea* colonies on BSM were surrounded by a dark brown halo clearly visible against the purple color of the medium. *Botrytis* spore trapping medium (BSTM) containing fenarimol in place of Rose Bengal in BSM was used for trapping the conidia of *Botrytis* in air and also for accurate enumeration of spores.

3.5.2 Isolation and identification of bacteria in vase water

The isolation and identification of bacteria in vase water was done by use of general purpose, differential and selective media, and eventually and also Vitek 2 –compact Biomereux (Model No. VK2C9938, bioMerieux) system (Al-shallah, 2017; Bora *et al.,* 2016). Isolation of bacteria from vase water of gladioli was carried out using nutrient agar medium. Aliquots of vase water taken on the third day of vase life and at senescence from the pulsed and non-pulsed cut flowers were diluted 100 times. Then 25 µl aliquots of the diluted solutions were spread out on sterile nutrient agar in sterile Petri dishes. The plates were allowed to incubate at room temperature for 48 hours and individual colonies of microorganisms representing the most common morphological types were picked from the agar medium with sterile inoculating needles and streaked on eosin methylene blue (EMB) agar and tellurite agar media for purification. Purified microbe populations were maintained on EMB agar and tellurite agar for Gram negative and Gram positive bacteria respectively and were transferred daily on fresh media (Kazemi & Shokri, 2011). The colonies from purified cultures were subjected to the Gram's reaction and for the study of morphological distinguishing features of Enterobacteriaceae (*Klebsiella*, *Escherichia*, *Entorobacte*r, *Citrobacter*, *Yersini*a, *Shigella*, *Proteus* and *Serratia*) and *Pseudomonas* bacteria. Cultural characteristics from colonies on EMB medium were used to differentiate the bacteria into lactose fermenters and non-lactose fermenters. Further tests used to identify the Gram negative bacteria were as directed by biochemical tests including pigment formation (*Pseudomonas* species), motility, oxidase test, catalase test, H_2S production, indole test, methyl red test, Vogas- Proskauer test, citrate utilization, starch hydrolysis and gelatin liquefaction.

The species of *Erwini*a were distinguished from one another on the basis of ability to metabolize the substrates inositol, sorbitol, esculin, melibiose, D-raffinose, β-gentibiose and gelatin hydrolysis. The *Pseudomonas* were further identified on the ability to metabolize mannitol, glucose, sorbitol, sucrose and growth at 5% NaCl. Silver staining method was used on the cytological study of *Pseudomonas sp*. Features such as flagella, cross- walls or septa, capsules and slime were demonstrated. Bacteria belonging to the *Aeromonas* species were

identified based on the IMViC reaction. *Alcaligenes sp*. were identified from their ability to utilize dextrose, sucrose, lactose and glucose sugars and also their reaction with phenylalanine deaminase.Further differentiation of colony forming units from cell cultures of Gram positive bacteria from the tellurite agar was based on chemical and physiological characteristics such as cell wall composition, enzyme production and substrate utilization among others. Positive coagulase and catalase tests were indicative of *Staphylococcus aureus.* The microscopic appearance was singly or in clusters in contrast with catalase negative *Streptococcus* sp. that appearedin chains. Blood agar plates were used to differentiate hemolysis in members of the genera *Stathylococcus*, *Streptococcus* and *Enterococcus* that appeared as alpha, beta or gamma hemolysins. Enterobacteriaceae were identified by te use of Tripple Sugar Iron Agar (TSI); Deoxycholate hydrogen sulphide lactose Agar (DHL) was employed as a selective medium to Gram negative bacteria while *Salmonella – Shigella* medium (SS) was a selective medium in isolation of isolates of *Salmonella*. Mannitol agar was the selective medium in isolation of Gram positive bacteria such as *Staphylococcus*. Bacteria were isolated and identified based on morphological, growth on differential and selective media and biochemical tests.

3.6 Data Analysis

A two by six factorial experiment embedded in a completely randomized design with four replicates was adopted. Pro GLM model in two way ANOVA was used to determine differences in pulsing and cold storage treatments on the flower quality and vase life. Differences in means were determined using Tukey's test at 5% level of significance. All the analyses were done usingJava Memory Profiler (JMP) software. Changes produced by the treatments represented deviations around the general mean and variability was the deviation of all parameters around the grand mean. Four Null hypotheses were tested regarding the vase quality, concentrations of biochemical markers, anthocyanin changes and microbial profiles in the pulsed and non- pulsed cut gladioli.

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1.0 Effect of pulsing and wet cold storage duration on the vase life and post harvest quality of cut *Gladiolus*

In this study, the effect of pulsing and wet cold storage durations significantly affected the cut *Gladiolus* vase life and quality parameters: floral attributes, water uptake, fresh and dry weight. Pulsing with 600 ppm 8-HQS + 5% sucrose for 24 hours and wet storing the cut

Gladiolus at 3 ±1°C showed a gain in fresh weight just after storage of both pulsed and non-pulsed flowers (**Table 4.1, Apendices A1-A6**). After the pulsing treatment of 600 ppm 8-HQS plus 5% sucrose solution for 24 hours, there was significant difference (P = 0. 0031) in the spike mean fresh weight (82.77 ± 0.7934 g) as compared to the control whose mean was 79.214 ± 0.7934 g (**Table 4.1**). The two way interactive effects were significant (0.0376; 0.0004 and 0.0016) on the *Gadiolus* weight after pulsing, afterstorage and the dry weight at senescence, respectively. The flower quality parameters of vase life, the number of open florets, open buds and water uptake were significantly superior in spikes pulsed with 600 ppm + 5% sucrose for 24 h followed by wet cold torage at 3 ±1 °C, compared with the control. In this study, the biomarkers of quality (total soluble sugars, starch, phenols and anthocyanins) significantly varied in the pulsed and the non pulsed *Gladiolus*. There was also significant difference in the concentrations of these biomakers in the pulsed and non-pulsed cut spikes as a result of wet cold storage duartions of 0, 1, 2, 3, 4 and 5 days which may have had an influence on vase life and other quality parameters in the cut *Gladiolus* flowers.

4.1.1 Influence of pulsing and wet cold storage duration on subsequent weight of cut *Gladiolus* flowers

In this study, *Gladiolus* wet cold stored for 0, 1, 2, 3 and 4 days invariably gained in weight on the third day in the vase in regard to their respective weight after pulsing for 24 hours with 600 ppm 8-HQS plus 5% sucrose (**Table 4.1).** For unstored (0 day storage) spikes, there was insignificant difference in weight between the pulsed and the non-pulsed *Gladiolus* (**Table 4.1**). However, *Gladiolus* pulsed after wet cold storage durationsof 2, 3 and 4 days exhibited higher mean fresh weights (85.99 ±.0.29g, 93.57±0.29g, 97.37±0.29g)versus (78.05±0.29 g, 83.54 ±0.29g., 91.69 ±.0.29 g) for the non pulsed spikes respectivelyon the third day in the vase (**Table 4.1**). The highest fresh weight gained (97.37 ±0.29) on the third day in the vase was recorded in cut*Gladiolus* pulsed with 600 ppm HQS plus 5% Sucrose and wet stored for 4 days which also displayed the second best vase life (11.25 ±.0.48 days) (**Table 4.1; Appendix**A**9**). The same trend was exhibited in the dry weight on the third day in cut *Gladiolus* which was pulsed and wet cold stored for 4 days prior to vase study had the highest dry weight (8.75±.2.7 grams) which was significantly different from that of non pulsed control (**Table 4.1**). The lowest dry weight (5.15 ±.2.7 g., 5. 75 ±.2.7g) on the third day was attained in cut *Gladiolus* wet cold stored for 5 days prior to vase study in both the pulsed and non pulsed spikes respectively.The same trend was displayed on the fresh and dry weight values in both the pulsed and non pulsed *Gladiolus* at senescence (**Table 4.1**).For the overall model, pulsing

treatment of 600 ppm 8-HQS plus 5% Sucrose for 24 hours, had significant difference (P = 0. 0031) on the spike mean fresh weight (82.77 ± 0.7934 g) as compared to the control whose mean was 79.214 ± 0.7934 grams (**Table 4.1**). Pulsing with 600 ppm 8-HQS + 5% Sucrose for 24 hoursand wet storing the cut *Gladiolus* at 3±1°C showed a gain in fresh weight just after storage of both pulsed and non-pulsed flowers (**Table 4.1, Appendix A**). The two way interactive effects were also significant (0.0376; 0.0004 and 0.0016) on the *Gladiolus* weight after pulsing, after storage and the dry weight at senescence respectively.

It is evident from **Table 4.1**, that the highest increase in fresh weight of the *Gladiolus* spikes just after storage duration was recorded in the treatment of flowers pulsed with 600 ppm HQS + 5% sucrose and wet cold - stored for 3 days followed by flowers pulsed with 600 ppm HQS + 5% Sucrose and wet cold - storedfor 2 days. However, they were not significantly different from each other. In the case of non-pulsed flowers, the highest increase in fresh weight was recorded with the flowers wet stored for 4 days, which was significantly different from all the other treatments of storage duration (**Table 4.1**). Incidentally, the least mean weight after cold storage was 83.43 ± 0.645 grams thatwas observed in spikes that had been subjected to five days cold storage duration and pulsed with 8-HQS + 5% sucrose. Prolonged storage of cut flowers has been found to have adverse effect on cut flowers, probably due to microbial proliferation. However, the significant interactive effect (P = 0.0004) of pulsing and cold storage on weight after cold storage period is in agreement with work done on cut roses where similar observations were made(Makwana *et al.*, 2015). In their study, cold storage without pulsing treatment led to decrease in vase life and floret opening of cut tuberose inflorescence. Pulsing with 600-ppm 8-HQS + 5% Sucrose solution and cold storage durations also significantly affected the rate of water uptake ($P < 0.0001$). Similar results were observed in cut carnations stored at 5°C and packaged in prolypropylne which significantly enhanced water uptake (Pranuthi *et al.*, 2018). Treating cut flowers with chemical preservatives before storage is generally the acceptable procedure adopted to extend the vase life and quality in floriculture (Bhat & Sheikh, 2015). However, there are exceptions to this rule according to studies done on Yarrow “Cassis” in which the longest vase life was attained by placing the cut stems in water (Clark *et al.*, 2010).

Table 4.1: Effect of pulsing with 600 ppm 8-HQS + 5% sucrose solution (P)versus distilled water (NP) and wet cold storage duration on weight (grams) changes during post-harvest life of cut *Gladiolus grandiflorus* cv. 'Fado'

Days of storage	Flower weight (grams)												Vase life (days)	
	Just after 24 hours Pulsing		Just after storage		On third day in vase				On senescence					
					Fresh weight (grams)		Dry weight (grams)		Fresh weight (grams)		Dry weight (grams)			
	Pulsed	Non pulsed	Pulsed	Non pulsed	Pulsed	Non pulsed	Pulsed	Non pulsed	Pulsed	Non pulsed	Pulsed	Non pulsed	Pulsed	Non pulsed
0	82.96^{b}	77.36^{b}	-	-	85.93^{abc}	85.89^{abc}	7.70^{ab}	6.69^{abc}	57.49^{abc}	58.38^{abc}	6.89^{bc}	6.78^{bc}	8.75^{b}	4.75^{c}
1	76.58^{b}	79.08^{b}	84.43^{b}	85.95^{b}	82.69^{abc}	87.62^{abc}	7.13^{abc}	7.19^{abc}	62.53^{ab}	63.90^{a}	9.00a	6.80^{bc}	10.25^{ab}	9.5^{ab}
2	83.39^{ab}	80.52^{b}	92.37^{ab}	88.86^{b}	85.99^{abc}	78.05^{cd}	7.17^{abc}	7.05^{abc}	63.00^{ab}	64.48^{a}	6.56bc	6.69^{bc}	10.00^{ab}	9.75^{ab}
3	90.34^{a}	79.44^{b}	100.20^{a}	86.63^{b}	93.57^{ab}	83.54^{abcd}	7.65^{abc}	7.38^{abc}	64.36^{a}	58.13^{abc}	6.87^{bc}	6.67^{bc}	11.50^{a}	11.00^{ab}
4	83.19^{ab}	81.95^{ab}	89.00^{b}	99.76^{a}	97.37^{ab}	91.69^{abc}	8.75^{a}	7.12^{abc}	55.70^{abc}	59.81^{ab}	8.12ab	6.18^{bc}	11.25^{ab}	10.2^{ab}
5	80.23^{b}	76.94^{b}	83.43^{b}	86.35^{b}	72.93^{a}	80.23^{bcd}	5.15^{d}	5.75^{c}	49.95^{bc}	53.45^{bc}	5.97c	6.90^{bc}	10.33^{a}	9.3^{b}
Mean	82.77^{a}	79.21^{b}	89.86^{a}	89.91^{a}	82.72^{a}	84.46^{a}	7.4^{a}	6.8^{b}	59.4^{a}	59.10^{a}	7.20^{a}	6.66^{b}	10.10^{a}	9.00^{b}
C.D at 5%	0.79	0.79	0.99	0.99	2.7	2.7	0.16	0.16	0.81	0.81	0.163	0.163	0.148	0.148
Pulsing (P)	**0.0031**		0.9690		0.2994		0.0188		0.7879		0.0272		0.0003	
Period of storage	**0.0082**		< 0.0001		0.1035		0.0006		< 0.0001		0.0118		< 0.0001	

Continuation of Table 4.1

Treatment * Storage	**0.0376**	0.0004	0.6440	0.2754	0.1311	0.0016	0.0041

Means followed by the same letter within evaluation period are not significantly different according to Tukey's test at 5% level of confidence

Lowering storage temperature is the easiest option adopted to increase the relative humidity to within 90 to 95 percent to prevent water loss in cut flowers (Reid & Jiang, 2012). Cold storage of cut flowers facilitates the adjustment of flowers and other planting material supplies against market demand also enables accumulation of large quantities (Senapati *et al.*, 2016). It has been established that long periods of storage ultimately have negative effects on the ultimate vase life of the product (Bayleyegn *et al.*, 2013). Pulsing treatment has been proved to control xylem vessel blockage that occurs due to air embolism, tissue injury or microbial proliferation (Kwon &Kim, 2000). 8-HQS is an antimicrobial agent that improves water uptake in cut flowers. According to this study, there was no significant difference in the fresh weight of cut *Gladiolus* flowers on the third day in vase after the cold storage duration

4.1.2 Effect of pulsing and wet cold storage treatment on subsequent percentage weight changes in cut *Gladiolus*

In this study 0,1,2,3 and 4 days' cold stored *Gladiolus grandiflorus* spikes pulsed with 600 ppm 8-HQS + 5% sucrose solution significantly (P < 0001) increased in fresh weight on the third day in the vase compared with the control. (**Table4.2**) The same trend was observed in *Gladiolus* spikes pulsed with distilled water for 24 hours and subjected to wet cold storage durations of 0,1,3,4 and 5 days respectively (**Table 4.2**). The highest percentage gain (17.04±3.00% of initial pulse weight) on the third day in of the vase study was in *Gladiolus* spikes pulsed for 24 hours with 600 ppm 8-HQS + 5% sucrose solution and then wet cold stored for 4 days. Comparatively, *Gladiolus* spikes subjected to a similar storage duration but pulsed for 24 hours in distilled water had 11.88 ±3.00% gain in fresh weight on the third day in the vase (**Table 4.2**). There was a decrease in fresh weight on the flowers pulsed with 600 ppm 8-HQS + 5% sucrose solution (-9.09% ±3.00loss of initial pulse weight) on cut *Gladiolus* spikes cold stored for 5 days prior to vase study (**Table 4.2**).

This was comparable to the *Gladiolus* spikes pulsed with water and wet cold stored for two days which registered loss in weight (-3.06 ± 3.00% of initial pulse weight (**Table 4.2**). There was insignificant difference in fresh weight of unstored cut *Gladiolus* irrespective of whether pulsing treatment used was 600 ppm 8-HQS + 5% sucrose solution or distilled water as shown in homogeneity in superscripts (^{abcd}) in mean separations (**Table 4.2**). However, the percentage gain in weight for the zero day stored spikes was 3.60 ±3.00% and 11.0 3 ± 3.00% for the pulsed and non –pulsed cut flowers respectively (**Table 4.2**). Hydroxyquinoline and its salts (8-hydrqxyquinoline citrate and 8-hydroxyquinoline sulphate) have been used as

preservatives to prolong the vase life of cut flowers due to their antimicrobial activity that retards bacterial and fungal growth that lead to xylem vessel occlusion (Sudaria *et al*.,2017). Inclusion of sugars in a pre –treatment solution is essential for crops such as gypsophilla, gladioli and other flowers which are harvested in a very immature state (Walton *et al*., 2010). Availability of sugars ensures the high respiratory activity of the petals does not deplete stored carbohydrates that would rapidly create a problem in hydration (Dung *et al*., 2017).

The two way interactive effects of cold storage and pulsing had non-significant effect (P = 0.6259, **Table 4.2**) on the *Gladiolus* fresh weight on third day. The pulsing treatment alone also had insignificant effect (P = 0.3068) on the percentage change in weight of the cut *Gladiolu*s in vase. However, the period of cold storage duration at 3 ± 1°C significantly (0.0348) affected the percentage change in weight of the cut *Gladiolus*. These results are relative to observations made on rehydration capacity of *Gladiolus grandiflora* Hor*t.*cultivars (Blue Frost, Gold Field, Green Star and Jester). Hence the fresh weight of *Gladiolus* decreased as the storage duration progressed. Though storage of *Gladiolus* stems in water even at low temperatures during storage or transport is not recommended (da Costa *et al*., 2017; Veilling, 2009). But on the study on *G. grandiflorus* cv. Fado, the maximum fresh weight (97.76 ± 4.27 grams) on the third day was observed in *Gladiolu*s pulsed and cold stored for 4 days before vase study.

A similar was in which cut flowers gained weight for the first 1 hour, which remained constant for next 24 hours (da Costa *et al*., 2017). However it is contradictory to the observation he made that the control treatments increased in weight in the first 6 hours, with little variation after 12 hours, maintaining fresh weight below that obtained in the pre-storage treatments for 12, 24 and 36 hours. In another study done on the keeping quality of cut *Gladiolus grandiflorus* L. cv. White prosperity, pulsing with 20% sucrose + 300 ppm $Al_2 (SO)_4$ + GA_3 ppm gave superior results in terms of days to open basal florets, vase life, number of florets opened, floret size, longevity of open florets and fresh weight/gain/loss (Bhat & Sheikh, 2015). The use of cold dry/wet storage of cut flowers can be used only when there is proper knowledge on the ideal time to be used. Also the concentrations of proper chemical preservatives and sugar solutions must be adjusted for species and even cultivars being evaluated. For instance HQS at 200mgL^{-1} can become toxic but a quarter of this amount could extend the vase life and the number of days when the stem fresh weight of wax flower was above the initial stem weight (Dung *et al*., 2017)

Table 4.2: Effect of pulsing with 600 ppm 8-HQS + 5% sucrose solution and wet cold storage duration on percentage change in weight (grams) during post-harvest life of cut *Gladiolus grandiflorus* cv. 'Fado'

Days of storage (days)	% Change in Weight after Pulsing										Vase Life (days)	
	Weight after Pulsing (days)		Third day in the Vase				Senescence					
			Fresh Weight (grams)		Dry Weight (grams)		Fresh Weight (grams)		Dry Weight (grams)			
	Pulsed	Non-pulsed	Pulsed	Non-pulsed	Pulsed	Non-pulsed	Pulsed	Non-pulsed	Pulsed	Non-pulsed	Pulsed	Non-pulsed
0	82.96 ^{bc}	71.36 ^{h}	3.58 ^{a}	11.95 ^{a}	9.32 ^{b}	8.22 ^{def}	30.72 ^{c}	24.53 ^{f}	8.2 cd	8.76 cd	8.75^{b}	4.75^{c}
1	76.88 ^{fg}	79.08 ^{de}	8.02 ^{a}	10.44 ^{a}	9.38 ^{b}	9.17 ^{bc}	18.25 ^{g}	19.19 ^{g}	11.75 a	8.59 cd	10.25^{ab}	9.50^{ab}
2	83.39 ^{cde}	80.52 ^{de}	3.11 ^{a}	-3.06 ^{a}	8.59 ^{cde}	8.19^{ef}	24.45 ^{f}	19.92 ^{g}	7.86 defg	8.31 cde	10.00^{ab}	9.75^{ab}
3	90.34 ^{a}	79.44 ^{ef}	3.57 ^{a}	5.16 ^{a}	8.80 ^{cd}	9.32 ^{b}	28.75 ^{d}	26.82 ^{e}	7.6 efg	8.31 def	11.50^{a}	11.00^{ab}
4	83.19 ^{b}	81.95 ^{ab}	17.28 ^{a}	11.88 ^{a}	10.38 ^{a}	8.52 ^{de}	33.04 ^{b}	27.01 ^{e}	9.76 b	7.54 g	11.25^{ab}	11.13 ^{ab}
5	80.23 bcd	76.94 ^{g}	-9.04 ^{a}	4.27 ^{a}	6.3^{g}	7.70^{f}	37.74 ^{a}	30.53 ^{c}	7.44 fg	8.90 bc	10.33 ^{a}	10.2^{ab}
Mean	82.82 ^{a}	78.33 ^{b}	7.66 ^{a}	12.25 ^{a}	8.82 ^{a}	8.52 ^{b}	29.05 ^{a}	24.80 ^{b}	8.53 ^{a}	8.21^{b}	10.10 ^{a}	9.13 ^{b}
CD at 5%	2.028	2.028	3.00	3.00	3.49	**3**.49	3.00	3.00	2.02	2.02	0.148	0.148
Pulsing (P)	<.0001		0.3068		0.0002		<.0001		0.0013		0.0003	
Period of storage	<.0001		0.0345		<.0001		<.0001		<.0001		<.0001	

Treatment * Storage	<.0001	0.6259	<.0001	<.0001	<.0001	<.00041

Means followed by the same letter within evaluation period are not significantly different according to Tukey’s test at 5% level of confidence

4.1.3 The fresh weight of *Gladiolus grandiflorus L.* cv. 'Fado' as affected by cold storage duration

There was significant difference in the fresh weight of cut *Gladiolus* (P < 0.0001) due to the wet cold storage durations of 1,2,3,4 and 5 days (**Table 4.3; Appendix A3**). The highest fresh weight after storage (100.2 ± 0.99 g) was observed in cut *Gladiolus* pulsed with 600 ppm plus 5% sucrosed after cold storage of three days. Incidentally, these gladioli registered the highest mean vase life (11.50 ± 0.148 days) which was significantly different (P < 0.0001) from that of the control. The wet cold storage durations of 1, 2, 3, 4, and 5 days also significantly (P < 0.0001) affected the quality weight parameter of the pulsed and non- pulsed cut *Gladiolus*. The interactive effect of wet cold storage and the pulsing treatment also significantly (0.0004) affected the weight of the cut spikes. Cut spikes cold stored for four days and pulsed with distilled water registered the next highest weight (99.76 ± 0.99 g) just after storage. These spikes also recorded the next highest vase life (11.25 ± 0.148 days). In this study, the wet cold storage durations of 1, 2, 3, 4, and 5 days were significant determinants on fresh weight compared with the pulsing treatment whose effect was insignificant (P < 0.9690). (**Table 4.3**). The lowest fresh weight (83.430 ± 0.99g) was in cut *Gladiolus* spikes that had undergone five days wet cold storage duration after pulsing with 600 ppm 8-HQS + 5% sucrose solution. In comparison, a similar storage duration had enhanced weight (86.350 ± 0.99g) in non-pulsed.spikes **(Table 4.3**).

This result is comparable to observations done on cut Tuberose (*Polianthes tuberose* L). in which pulsing with double distilled water plus 2 % sucrose significantly improved the vase life in comparison with preservatives including 8-HQS, citric acid and silver nitrate (Motaghayer & Esna-Ashari, 2009). This observation concurs with work done on cut *Gladiolus grandiflorus* cv. Prosperity in which increased storage duration in days negatively affected the vase life and floral attributes of the spikes (Bhat & Sheikh, 2015). It is evident from **Table 4.3**, that the highest increase in fresh weight of the *Gladiolus* spikes just after storage duration was recorded in the treatment of flowers pulsed with 600 ppm HQS + 5% Sucrose and wet stored for 3 days followed by those similarly treated and wet stored for 2 days. Germicides such as 8-HQS inhibit microbial proliferation while the sugar in preservatives supplies food for respiration (Manzoor *et al*., 2018). This combination in the pulsing solution of 600 ppm HQS plus 5% sucrose could have maintained the fresh weight during storage compared with the control. In case of non-pulsed flowers the highest increase in fresh weight was recorded with non-pulsed flowers and wet stored for 4 days which was significantly different from all the other treatments of storage duration (different superscript in mean separations, (**Table 4.3**).

The low storage temperature of 3 ±1°C plays the role of decreasing loss in water through respiration and transpiration (which are minimized) in both the pulsed and non-pulsed gladioli spikes (Senapati *et al.,* 2016). The low storage temperaturein the chamber coupled with increased relative humidity also plays the roles of inhibiting bacterial growth and also slows down the metabolic rate in the cut flower (Reid &Jiang, 2012). The duration of cold storage (whether dry or wet) compromises the ultimate vase life and quality of cut flowers (da Costa *et al*., 2017). A study done on rose flowers showed storage time significantly ($P < 0.001$) affected the solution uptake, relative fresh weight and TSS contents of petals (Bayleyegn *et al*., 2013). Cold storage of cut flowers facilitates the adjustment of flowers and other planting material supplies against market demand and enable accumulation of quantities of flowers (Senapati *et al*., 2016). However, the choice of the ideal storage time and temperature will vary in terms of the species in question and even the cultivars (Bhat & Sheikh, 2015; Clark *et al*., 2010). It has also been observed that the storage temperature is specific for each type of flower and also the climatic conditions in which they happen to have been grown (Mapeli *et al*., 2011). Storing freshly harvested flowers in a chilled room and treating it with chemical before transporting is the standard international practice to kill undesired microorganisms, reduce damage and prolong vase life (World Bank Group, 2016).

Table 4.3: The fresh weight of *Gladiolus grandiflorus* L. cv. 'Fado' as affected by cold storage duration

Days of storage duration	**Weight (grams) just after storage**		**Vase life (days)**	
	Pulsed	Non pulsed	Pulsed	Non-pulsed
0	-	-	8.75 b	4.75 c
1	84.43 [b]	85.95 [b]	10.25 ab	9.5 ab
2	92.37[ab]	88.86 [b]	10.00 ab	9.75 ab
3	100.20 [a]	86.63 [b]	11.50 a	11.00 ab
4	89.00 [b]	99.76 [a]	11.25 ab	10.2 ab
5	83.43 [b]	86.35 [b]	10.33 a	9.3 b
Mean	89.86 [a]	89.91[a]	10.10 a	9.00 b
CD at 5%	0.99	0.99	0.148	0.148
Pulsing (P)	0.9690		0.0003	
Period od storage	< 0.0001		<0.0001	
Treatment* storage	0.0004		0.0041	

Means followed by the same letter within evaluation period are not significantly different according to Tukey's test at 5% level of confidence

4.1.4 Dry weight of cut *Gladiolus* as affected by pulsing with 600 ppm HQS plus 5% Sucrose and cold storage on the third day in the vase

According to this study, cut *Gladiolus* maintained the dry matter upto 4 days in the vase as shown by homogeneity in the mean separations (**Table 4.4, Appendix A4**). For the *Gladiolus* that were pulsed with 600 ppm HQS plus 5% sucrose the mean dry weight for spikes wet cold stored for 1, 2, 3, and 4 days were significantly elevated and different from the control (8.22 ±0.035 g; **Table 4.4**). The effect of pulsing with distilled water and wet cold storage also increased the dry weight of the spikes for storage durations of 1,2, 3 and 4 days prior to vase study compared with the control (**Table 4.4**). This trend correlated with the increase in the vase life of the pulsed and wet cold stored *Gladiolus* compared with that of the control (4.75 ±0.148 days; **Table 4.4**). However, the percentage change in the dry weight of the *Gladiolus* was significantly affected by the pulsing treatment compared with the control (P= 0.0002). The effects of pulsing, storage durations of 1 – 5 days and the interactive effects of these treatments also significantly (<.0001) affected the percentage change of the dry weight of the cut *Gladiolus* (**Table 4.4**) on the third day in the vase.. These results are comparable to work done on three species of cut *Iris* scapes where storage for 72 hours at 5°C followed by transferring to distilled water and sucrose solutions improved the flower floral diameter, membrane integrity and maintained higher fresh and dry mass of the flowers (Ahmad *et al*., 2013).

Findings in other studies have indicated that 8-HQS inhibits microbial proliferation and also averts physiological blockage in cut flowers (Elgimabi & Yagi, 2016). The effectiveness of 8 – hydroxyquinoline derivatives in inhibiting pseudohyphae formation by *Candidaalbicans* and other dermatophytes has been documented (Pippi *et al*., 2017; Pippi *et al*., 2019). In another study metal scaffolding potential by 8-HQS inhibited mycobacterium tuberculosis proliferation (Shah *et al*., 2016). Aluminium sulphate, silver nano particles, cycloheximide, HQC, quaternary ammonium compounds and sodium bemzoate are among the compounds used in floriculture to improve the postharvest physiology of cut flowers (Damunupola *et al*., 2008; Shahri & Tahir, 2010). The pulsing of cut *Gladiolus* flowers with edible dyes such as Brilliant Blue FCF and Ponceau 4R in combination with growth regulaters also improved their post-harvest quality (Ritu *etal*., 2015). However, a study done on cut inflorescence stems of *Sedum aizoon* revealed that the use of the biocide 8-HQS as a pulsing agent did not improve the quality of these flowers (Krzyminska *et al*., 2014). All flower preservatives used have advantages and disadvantages on the postharvest quality of cut flowers and/or the environment (Damunopola & Joyce, 2008).

Table 4.4: Effect of pulsing with 600 ppm HQS plus 5% Sucrose and cold storage on the dry weight (grams) changes during post harvest life of cut *Gladiolus grandiflorus* cv. Fado on the third day in the vase

Days of storage	**Weight (grams)**						**Vase life (days)**	
	Just after storage		Third day in vase					
			Fresh weight		Dry weight			
	Pulsed	Non pulsed	Pulsed	Non pulsed	Pulsed	Non pulsed	Pulsed	Non pulsed
0	-	-	85.93 ^{abcd}	85.89 ^{abcd}	7.70^{ab}	6.69 ^{abc}	8.75 ^{b}	4.75 ^{c}
1	84.43 ^{b}	85.95 ^{b}	82.69 ^{abcd}	87.62 ^{abcd}	7.13^{abc}	7.19^{abc}	10.25 ^{ab}	9.5^{ab}
2	92.37^{ab}	88.86 ^{b}	85.99 ^{abcd}	78.05 ^{cd}	7.17^{abc}	7.05^{abc}	10.00 ^{ab}	9.75^{ab}
3	100.20 ^{a}	86.63 ^{b}	93.57 ^{ab}	83.54 ^{abcd}	7.65^{abc}	7.38^{abc}	11.50 ^{a}	11.00 ^{ab}
4	89.00 ^{b}	99.76 ^{a}	97.37 ^{ab}	91.69 ^{abc}	8.75^{a}	7.12^{abc}	11.25^{ab}	10.2^{ab}
5	83.43 ^{b}	86.35 ^{b}	72.93 ^{a}	80.23 ^{bcd}	5.15^{d}	5.75^{c}	10.33 ^{a}	9.3 ^{b}
Mean	89.86 ^{a}	89.91^{a}	82.72 ^{a}	84.46 ^{a}	7.4 ^{a}	6.8 ^{b}	10.10 ^{a}	9.00 ^{b}
CD at 5%	0.99	0.99	2.7	2.7	0.16	0.16	0.148	0.148
Pulsing	0.9690		0.2994		0.0188		0.0003	
Storage duration	< 0.0001		0.1035		0.0006		< 0.0001	
Treatment * **Storage duration**	0.0004		0.6440		0.2754		0.0041	

Means followed by the same letter within evaluation period are not significantly different according to Tukey's test at 5% level of confidence

4.1.5 Fresh weight and dry weight of cut *Gladiolus grandiflorus* L, cv. 'Fado' at senescence as affected by cold storage and pulsing treatment of 600 ppm HQS plus 5% Sucrose for 24 hours

At senescence, the fresh weight was less than the initial weight after pulsing. The maximum fresh weight (64.36 ± 0.81 g) at senescence was recorded in pulsed flowers wet stored for 3 days; which was not significantly different from the pulsed flowers and wet stored for 4 days which also gave the maximum vase life (11.50 ± 0.895 days). The decrease in fresh weight was relative to the increase in storage duration from 4 to 5 days. With the non-pulsed flowers, maximum fresh weight was recorded with flowers wet stored for 2 days (64.48 ±0.128 g) which was not significantly different from flowers wet stored for 3 and 4 days. The maximum dry weight at senescence was recorded with pulsed flowers and wet stored for 4 days which also gave a maximum vase life of (11.25 ± 0.332 days) and was statistically similar to pulsed flowers wet stored for 3 days.

There was significant difference between the dry weight offlowerspikes pulsed with8-HQS + 5% Sucrose compared with the ones that had been placed in distilled water (7.202 ± 0.164; 6.669 ± 0.164, P = 0.0272 respectively) with a strong interactive index between the treatments and the storage durations (P = 0.0016; (**Table 4.5; Appendices A5; A6**). There was also significant difference (P = 0.0118) in the dry weight of the gladiolus spikes depending on the storage duration. This study has also indicated a two way interactive index (P = 0.0016) between the pulsing treatment and the period of cold storage and their effect on the dry weight of the cut flowers. However, there was no significant difference (P = 0.7879) in the fresh weight at senescence between the pulsed and control cut flowers (**Table 4.5).**

The use of saccharides such as sucrose and trehalose has been reported to have a suppressive effect on apoptotic cell death of cut *Gladiolus* petals (Yamada *et al*., 2003). These findings can be compared with observations done on inflorescence of cut tuberose *Polianthestuberosa* L. in which pre-storage treatment with hydroxyquinoline citrate plus 20% sucrose solution and cold storage significantly improved its quality parameters(Waithaka *et al*., 2001) In another study, the use of vase solution treatment of GA_3 (50 mg l-1), followed by BA (50 mg l-1) with sucrose (50 g l-1) significantly increased solution uptake, fresh weight and dry weight of cut spikes of *Gladiolu*s (*Singh et al*., 2008). Pretreatment of flowers with CHI at 0.01 and 0.05 mM concentrations was found to delay visible signs of senescence, maintain high fresh and dry mass of flowers and lower electrical conductivity of ion leachates. It has been observed that the use of CHI at higher concentrations had negative effects on desirable flower display physiology characteristics (Shahri &Tahir, 2010).

Table 4.5: Influence of pulsing and wet cold storage on the subsequent fresh and dry weight of cut *Gladiolus grandiflorus* L, cv. 'Fado' at senescence as affected by cold storage and pulsing treatment of 600 ppm HQS plus 5% Sucrose for 24 hours

Days of storage	**Just after 24 hours Pulsing**		**On senescence**				**Vase life (days)**	
			Fresh weight (grams)		Dry weight (grams)			
	Pulsed	Non pulsed	Pulsed	Non pulsed	Pulsed	Non pulsed	Pulsed	Non pulsed
0	82.96 ^{b}	77.36 ^{b}	57.49^{abc}	58.38 ^{abc}	6.89 ^{bc}	6.78 ^{bc}	8.75 ^{b}	4.75 ^{c}
1	76.58 ^{b}	79.08 ^{b}	62.53 ^{ab}	63.90 ^{a}	9.00 a	6.80 ^{bcbc}	10.25 ^{ab}	9.5^{ab}
2	83.39 ^{ab}	80.52 ^{b}	63.00 ^{ab}	64.48 ^{a}	6.56 bc	6.69 ^{bc}	10.00 ^{ab}	9.75^{ab}
3	90.34 ^{a}	79.44 ^{b}	64.36 ^{a}	58.13^{abc}	6.87^{bc}	6.67 ^{bc}	11.50 ^{a}	11.00 ^{ab}
4	83.19 ^{ab}	81.95^{ab}	55.70 ^{abc}	59.81^{ab}	8.12 ab	6.18^{bc}	11.25^{ab}	10.2^{ab}
5	80.23 ^{b}	76.94 ^{b}	49.95 ^{bc}	53.45 ^{bc}	5.97 c	6.90^{bc}	10.33 ^{a}	9.3 ^{b}
Mean	82.77^{a}	79.21 ^{b}	59.41^{a}	59.10 ^{a}	7.20^{a}	6.66^{b}	10.10 ^{a}	9.00 ^{b}
C.D at 5%	0.79	0.79	0.81	0.81	0.163	0.163	0.148	0.148
Pulsing (P)	**0.0031**		0.7879		0.0272		0.0003	
Period of Storage 0.0082			< 0.0001		0.0118		< 0.0001	
Treatment * Storage	**0.0376**		0.1311		0.0016		0.0041	

Means followed by the same letter within evaluation period are not significantly different according to Tukey's test at 5% level of confidence

4.1.6 Influence of pulsing and wet cold storage on the vase life and subsequent floret opening in cut *Gladiolus*(*Gladiolus grandiflorus* L. cv. 'Fado'

The pulsing treatment had a significant effect (P =0. 003) on the vase life and the number of opened florets as can be seen in **Tables 4.6; Appendix A7.** The maximum vase life of 11.5 days ± 0.287 was recorded in *Gladiolu*s pulsed with 600 ppm HQS plus 5% Sucrose and wet stored for 3 days at 3 ± 1°C prior to vase study while the minimum vase life (4.75 ±0.287 days) was observed in spikes pulsed in distilled water without any cold storage treatment.A study done on two cut chrysanthemum varieties showed that refrigerated storage at 3 – 4°C plus 6 hours of simulated transit resulted in enhanced quality and vase life (Sharma and Srivastava, 2014). The supply of carbohydrates has been proved to increase the vase life of cut rose (*Rosa hybrida* L. cv. 'Audio' and 'Black Magic' flowers that had been pre-treated with 200mg L^{-1} 8-HQC + 20 g/L sucrose (Nabigol *et al*., 2014). The highest number of opened florets (11.0) were found on the spikes pulsed with 8-HQS + 5% sucrose solution and wet stored for 4 days prior to vase study.

These results are synonymous with the observations made on cut *Gladiolus* spikes cv. Peter Pears (Alka *et al.*, 2006). According to this study, pre-storage treatment of the cut *Gladiolus* spikes with 300 ppm 8-HQ for one hour followed by 20% sucrose for 12 hours prior to post storage treatment with 300 ppm 8-HQ plus in combination with 5% sucrose enhanced flower vase life (17 days). These results are comparable with the studies done on cut tuberose (*Polianthestuberosa* L. in which the vase life and floret opening of the inflorescences were significantly improved by pre-storage treatment of 20% sucrose containing HQC as compared with the control (Waithaka *et al*., 2001). It has been documented that cut *Gladiolus grandiflorus* L. cv. White prosperity inflorescences subjected to cold storage before chemical pulsing treatment display lowest values in terms vase life, number of open florets and gain in fresh weight (Bhat & Sheikh, 2015). A similar trend has been observed in cut tuberose where the vase life and floret opening was significantly decreased by cold storage (Waithaka *et al*., 2011). A study on *Gladiolus* cv. Her Majesty revealed the superior effect of pulsing with 20% sucrose plus 200 ppm HQS prior to cold storage at 4°C for 48 hours which attained vase life of 11.3 days (Beura & Singh, 2001). This was in contrast with spikes subjected to deionized water whose vase life was 7.3 days. The concentrations of the biocide HQS used in combination with sucrose affects the vase life and physiology of the cut flowers under study (Dung *et al.,* 2017). The added advantage of polypropylene packaging of cut *Rose* var, Sun King enhanced the vase life and quality of the flowers (Makwana *et al*., 2015).

Table 4.6: Influence of pulsing and wet cold storage on the vase life and subsequent floret opening in cut *Gladiolus* (*Gladiolus grandiflorus* L. cv. 'Fado'

Days of storage	**Number of florets opened**		**Vase life (days)**	
	Pulsed	Non pulsed	Pulsed	Non pulsed
0	8.60 ^{bc}	6.88 ^{c}	8.75 ^{b}	4.75 ^{c}
1	10.00 ^{ab}	10.00 ^{ab}	10.25 ^{ab}	9.5^{ab}
2	9.75 ^{ab}	9.50 ^{ab}	10.00 ^{ab}	9.75^{ab}
3	10.50 ^{ab}	9.25 ^{ab}	11.50 ^{a}	11.00 ^{ab}
4	11.00 ^{a}	10.25a^{b}	11.25^{ab}	10.2^{ab}
5	8.75 ^{bc}	10.00 ^{ab}	10.33 ^{a}	9.3 ^{b}
Mean	9.76 ^{a}	9.31^{a}	10.10 ^{a}	9.00 ^{b}
CD at 5 %	0.159	0.159	0.148	0.148
Pulsing	0.0512		0.0003	
Period of storage	< 0.0001		< 0.0001	
Treatment*Storage	0.0095		0.0041	

Means followed by the same letter within evaluation period are not significantly different according to Tukey's test at 5% level of confidence

4.1.7 The number of unopened buds in cut *Gladiolus grandiflorus* L. cv. 'Fado' as affected by pulsing and cold storage duration

In this study, there was significant difference in themean number of unopened buds between the pulsing treatments, storage durations and their interactive effects (P=0.0256, 0.0012, 0.0209 respectively). The highest mean number of unopened buds (8 ±0.2159 buds, different superscript) was found in the spikes pulsed with distilled water and not cold stored (**Table 4.7; Appendix A8**). Spikes that were pulsed with 600 ppm 8- HQS plus 5% sucrose solution and subjected to zero day storage prior to the study of vase life registered significantly reduced mean unopened buds (5.00 ± 0.2159, **Table 4.7**). This trend was observed in spikes wet cold stored for 1, 2, 3, 4 and 5 days after pulsing with either600 ppm 8- HQS plus 5% sucrose solution or distilled water.These observations are similar to those shown by cut scapes of three *Iris* species which registered better floral attribues when storage at 5 ^{o}C for 72 hours followed by transfer to either water or sucrose solution was applied (Ahmad *et al*., 2013).

A related study oncut gladioli (*Gladiolus grandiflorus* L.cv. White Prosperity showed improvement in their postharvest quality over a limited period of storage duration (Bhat &Sheikh, 2015). Studies done elsewhere on the postharvest life of snapdragon (*Antirrhinum majus* L. cv. Yellow butterfly) cut flowers highlighted beneficial effects of8-HQS plus sucose solution on theirfloral attributes (Asrar, 2012). Bud opening of cut *Dendrobium* "Burana Jade"was enhanced by the use of vase solution containing distilled water plus 2% sucrose (Chanjirakul *et al*., 2015). In the same study, 100 mg $Al_2(SO_4)_3$ plus 2% sucrose showed the highest vase life for *Dendrobium* "Ela Sakur". The use of sulfosalicylic cid at 100-200 ppm also improved the floral attributes in cut *Gladiolus* (*Gladiolus grandiflorus* L.) including the number/rate of floret opening and reduced unopened buds (Khattab *et al*., 2017). In another study a report indicated a two way significant interaction (P < 0.01) between pulsing solution and storage period on *Botrytis* incidence, maximum flower bud opening and vase life (Bayleyegn *et al*., 2013).

Table 4.7: The effect of pulsing with 600 ppm HQS plus 5% sucrose on the open florets and unopened buds in *Gladiolus grandiflorus* L. cv. 'Fado'

Days of Storage	Unopened buds (Number)		**Vase life**	
	Pulsed	Non-pulsed	Pulsed	Non-pulsed
0	5.00^{b}	8.00^{a}	8.75^{b}	4.75^{c}
1	4.25^{b}	4.50^{b}	10.25^{ab}	9.5^{ab}
2	5.13^{b}	4.50^{b}	10.00^{ab}	9.75^{ab}
3	3.75^{b}	5.08^{b}	11.50^{a}	11.00^{ab}
4	4.50^{b}	4.50^{b}	11.25^{ab}	10.2^{ab}
5	4.25^{b}	4.50^{b}	10.33^{a}	9.3^{b}
Mean	4.47^{b}	5.18^{a}	10.10^{a}	9.00^{b}
C.D at 5%	0.2129	0.2129	0.148	0.148
Pulsing (P)	0.0256		0.0003	
Period of storage (S)	0.0012		< 0.0001	
P * S	0.0259		0.0041	

Means followed by the same letter within evaluation period are not significantly different according to Tukey's test at 5% level of confidence

4.1.8 Effect of pulsing and wet cold storage on water uptake of cut*Gladiolus grandiflorus* L cv.'Fado'

There was significant effect on water uptake by cut *Gladiolus* spikes due to the pulsing and wet cold storage treatments and their interactive effects (P = < 0.0001)**.** The mean water uptake for spikes pulsed with 600 ppm plus 5% sucrose was 21.20 ± 0.16 ml in comparison with those pulsed with distilled water (12.41 ± 0.16) as shown in **Table 4.8; Appendices A10-A12**). *Gladiolu*s spikes wet cold stored for 1, 2, 3, 4 and 5 days gained more weight with prior pulsing using 600 ppm 8- HQS rather than water (36.18 ±0.25 ml; 32.72 ±0.25 ml respectively; **Table 4.8**). Cut spikes wet cold stored for one day and pulsed with 600 ppm 8-HQS had enhanced mean water uptake (36.18 ±0.25 ml**)** compared with those treated with distilled water (30.36 ± 0.25 ml) after storage duration. The same trend was observed for the mean water uptake after storage durations of 2,3, 4 and 5 days (**Table 4.8**). The same trend was observed after pulsing, on the third day in vase and at senescence (**Table 4.8)**. On the third day in the vase, unstored *Gladiolu*s previously pulsed with 600 ppm8-HQS plus 5% sucrose solution registered elevated mean water uptake (44.56 ± 0.19 mls compared with the control (29.32 ± 0.19 ml). This trend was replayed for *Gladious* spikes cold stored for 1,2,3,4,and 5 days after prior pulsing for 24 hours with 600 ppm8-HQS plus 5% sucrose versus distilled water prior to vase study.This pattern is manifested in thehigher mean water uptake (31.98 ± 0.193 ml) observed on the third day and 23.87 ± 0.26 ml at senescence respectively for *Gladiolus* pulsed with 600 ppm 8-HQS plus 5% sucrose solution).In contrast,the mean water uptake for 0, 1,2,3,4and 5 days'stored *Gladiolus* on the third day in the vase and at senescence was 23.14 ± 0.93 ml and 17.05 ± 0.26 ml respectively for the water pulsed flowers (**Table 4.8**).

Zero day stored *Gladiolus* pulsed with 600 ppm 8 HQS plus 5% sucrose had significantly (different superscripts) elevated mean water uptake (31.66 ±0.18 ml) compared with the control (27.27 ± 0.18 ml) at senescence. This trend was displayed for *Gladiolus*pulsed with 600 ppm 8 HQS plus 5% ssucrose and wet cold stored for 1, 2, 3, 4 and 5 days in which the mean water uptake was significantly higher compared with their counterparts placed in distilled water prior to storage and eventually vase study. Imbalance in water relations is the main cause of wilting and termination of vase life in cut flowers (Halevy,1976; Senapati *et al*., 2016). Lack of water conductance in the cut flowers is one of the reasons responsible shortened vase life in cut flowers (Carlson & Dole, 2013). The use of preservatives such as tea extracts, 8-hydroxyquinoline sulphate at designated concentrations, have been found to improve the vase life, petal protein, total chlorophyll, and petal caroteinoid and water absorption in cut

Chrysanthemum (*Denderanthema grandiflorus* L. cv, Purple) (Hashembadi &Bhagheri, 2014). A study averred that cold and dry storage of snapdragon (*Antirrhinum majus*) retarded their senescence and prolonged marketable period provided a post-storage solution consisting of 8-HQS in combination with sucrose and citric acid was used (Viera *et al*., 2011). Germicides such as HQS, HQC, aluminium sulphate, silver nano prticles are effective in inhibiting bacterial proliferation in cut flowers (Manzoor *et al*., 2018). This helps to avert microbial occlusion of the xylem vessels (Halevy, 1976). The use of saccharides such as sucrose and trehalose suppress nuclear fragmentation thereby suppressing apoptotic cell in senescing cut *Gladiolus* petals (Yamada *et al*., 2003).

The use of sucrose, tetracycline and 500 ppm 8-HQS by was found to increase quality of cut *Gladiolusgrandiflorus* through improvement in cut weight and water relations (Cong *et al*., 2010). A combination of 100 ppm HQS with 3% Sucrose was found to increase the vase life of cut rose (*Rosa hybrida*) flowers (Elgimabi & Ahmed, 2009). The treatment of snapdragon (*Antirrhinummajus* L cv Yellow Butterfly) cut flowers with a solution containing 2% sucrose plus 200 ppm for 12 hours gave best results in terms of water uptake, water balance, increase in fresh weight and vase life (Asrar, 2012). In another study, it was proved that the interactive effects of sugars and antimicrobial agents increased the vase life and improved the quality of two *Gladiolus* cultivars (Al-Humaid, 2004). In a study on cut spikes of sweet pea (*Lathyrus odoratus* L.), pulsing treatment of 200 ppm HQS in combination with 2% sucrose for 12 hours gave the best results among other preservatives in terms of water uptake, water balance , percentage increase in fresh weight of the cut stems and vase life (Elhindi, 2012).

For maintaining of cut flower freshness, there has been a need to design holding solutions containing ingredients that may include: hormones, bactericides, mineral ions, metabolic inhibitors or their combinations (Rani &Singh, 2014). Still in a study on a variety of cut flowers,the use of 300 ppm 8-HQS plus 40 g/l sucrose prolonged the vase life and caused maximum increase in fresh weight, lowest percentage of weight loss at initial day, increased water uptake of cut *Solidago* flowers(Elshereef, 2015).

Table Table 4.8: Effect of pulsing with 600 ppm 8-HQS + 5% sucrose solution and wet cold storage duration on water uptake (milliliters) during post-harvest life of cut *Gladiolus grandiflorus* cv. 'Fado'

Storage duration (days)	Water uptake (mls)									
	After Pulsing		After cold Storage		On third day in vase		At senescence		Vase Life	
	Pulsing	No pulsing	Pulsing	No pulsing	Pulsing	No pulsing	Pulsing	No Pulsing	Pulsing	No pulsing
0	18.51^{cd}	10.99^{ef}	-	-	44.56^{a}	29.32^{d}	31.66a	27.27b	8.75 ^{b}	4.75 ^{c}
1	23.93^{a}	11.70^{ef}	36.09^{c}	30.36^{d}	34.69^{c}	23.24^{e}	31.38a	21.98c	10.25 ^{ab}	9.50 ^{ab}
2	18.89^{cd}	11.57^{ef}	30.90^{d}	30.11^{d}	28.93^{d}	23.62^{e}	21.52c	20.23cd	10.00 ^{ab}	9.50 ^{ab}
3	20.34^{bc}	10.04^{f}	31.65^{d}	26.44^{e}	23.34^{e}	18.35^{g}	21.79c	14.45e	11.50 ^{a}	9.75 ^{ab}
4	24.31^{a}	12.55^{e}	51.66^{a}	47.25^{b}	39.63^{b}	23.77^{e}	17.27de	8.98f	11.25 ^{ab}	11.00^{ab}
5	21.28^{b}	17.62^{d}	30.61^{d}	29.53^{d}	20.75^{f}	20.56^{fg}	19.69cd	8.45f	10.25ab	10.25 ^{hi}
Mean	21.20^{a}	12.41^{b}	36.18 ^{a}	32.74 ^{b}	31.98 ^{a}	23.15 ^{b}	23.387a	17.05b	10.10^{a}	9.13^{b}
C.D at 5%	0.16	0.16	0.25	0.25	0.19	0.19	0.18	0.18	0.148	0.148
Pulsing (P)	< 0.0001		< 0.0001		< 0.0001		< .0001		0.003	
Period of Storage	< 0.0001		< 0.0001		< 0.0001		< .0001		< 0.001	
P*S	< 0.0001		0.0001		< 0.0001		< .0001		< 0.0041	

Means followed by the same letter within evaluation period are not significantly different according to Tukey's test at 5% level of significance

4.2.1 The effect of pulsing and wet cold storage duration on subsequent starch levels in cut *Gladiolus* (*Gladiolus grandiflorus* cv. 'Fado')

This study established pulsing cut *Gladiolus grandiflorus* L. cv. 'Fado' spikes with 600 ppm 8- HQS in combination with 5% sucrose solution had significant effect ($P < 0.0001$) on the total starch on the third day of the shelf life (**Table 9; Appendices A18; A19**). The maximum total starch (TS) (42.29 ± 0.30 µg/mg dw) in the pulsed spikes was observed in three days' wet cold stored *Gladiolus* flowers which incidentally registered the highest vase life (11.50 ±0.21 days). This was followed by spikes pulsed and wet cold stored for four days whose mean total starch was 41.96 ± 0.136 µg/mg dw) and registered the next highest vase life (11.25 ± 0.21 days). Similar results were obtained in postharvest study of Chincherinchee (*Ornithogalum thyrsoides* Jacq) cut flowers which improved in vase life when cellophane paper was wrapped and flowersstored for three days at 4°C before vase study (Dastagiri *et al*., 2017). Enhanced vase life, total starch and total soluble sugars parameters were registered in cut rose flowers pulsed with 3% 8-hydroxyquinoline citrate with subsequent wet storage at 3°C before vase study (Mwangi *et al*., 2003).

The effect of pulsing cut *Gladiolus* with 600 ppm 8- HQS in combination with 5% sucrose followed by wet cold storage elicited higher mean total starch (37.00 ± 0.136 µg/mg dw) which was significantly different (34.54 ± 0.136 µg/mg dw) from that of the non pulsed cut spikes (**Table 4.9**). In this study, the pulsing treatment enhanced levels of total starch when followed by wet cold storage durations of 1, 2, 3 and 4 days which translated into improved vase life compared with the zero day stored spikes. These results are comparable to accumulation of starch in *Gladiolus*, which was associated with stem maturation that allowed larger storage reserves, an attribute exhibited during opening stage (da Costa & Finger, 2016). However, in both the pulsed and non pulsed spikes, levels of total starch dropped in *Gladiolus* subjected to five days' wet cold storage period prior to vase study, which may have prompted a downward trend in the cut flower vase life (10.25 ± 0.21 days).Reduction in starch content as the storage duration progressed has also been reported in studies on peony flowers (Walton *et al*., 2010). The results from this study are comparable to results from lily flowers harvested before full opening which exhibited an increase in starch production (Vander Menten-Mulser *et al*., 2001). A small reduction of total starch and tepal soluble sugarsas days after harvesting progressed was also reported in calla lily flowers (Sales *et al*., 2018). The inclusion of sucrose in combination with kinetin and salicylic acid delayed petal senescence in cut flowers of

Matricaria parthenium L. and also minimized reduction in starch contents (Mukherjee & Mukherjee, 2017).

At senescence, there was significant difference (P< 0.0001) in the Total Starch between the pulsed and non-pulsed *Gladiolus.* There was progressive increase in the levels of TS as the wet cold storage increased from the one day to the fourth days' wet stored *Gladiolus*spikes. The meanconcentrations (21.60 ± 0.209 µg/mg dw; 19.90± 0.209 µg/mg dw. respectively) of total starch for both the pulsed and non pulsed spikes were lower at senescence compared with the levels on the third day in the vase (**Table 4.9**). The pulsed four days' cold stored *Gladiolus* registered the maximum TS (27.98± 0.209µg/mg dw) which was significantly different from the zero day's stored *Gladiolus*. While the trends in concentrations of TS were the same at senescence as well as during the third day in the vase, these levels were significantly (P < 0.0001) affected by the wet cold storage duration in days (**Table 4.9**). There was also significant variance (P < 0.0001) in concentrations of TS due to the interactive effect of the pulsing and wet cold storage treatments (**Table 4.9**). Postharvest qualities of cut Chincherinchee (*Ornithogalum thyrsoides*) Jacq cut flowers improved after three days,storage at 4°C and wrapping with cellophane (Dastagiri *et al.*, 2017).

The inclusion of exogenous carbohydrates such as glucose and sucrose in pulsing and holding solutions promoted quality parameters in cut *Dendrobium* inflorescence including delay of tepal senescence (Patatravayo *et el.*, 2013). Starch and sugar stored in the stem, leaves and petals provide much of the needed respiratory reserves for maintainance of cut flowers (Reid, 2009).Senescence comprises a series of highly regulated cytological and biochemical events that coordinate the degradation of macromolecules among other activities (Kaur &Mukherjee, 2012). Other studies have indicated that the contribution of starch to the total carbohydrate content of open *Gladiolus* florets was minimal (Waithaka *et al.*, 2001). It is recorded that the predominant sugars in the *Gladiolus* perianth are glucose and fructose and it was considered that starch in the florets was the primary source of soluble carbohydrate that contributed to early stages of flower expansion (Yamane *et al.*, 1991). However, according to their study there was only 2 mg per perianth rise in starch content between the stages of the bud showing color and corolla exertion while the sugar content of the perianth was 15 mg. Hence this observation indicated some other storage carbohydrate could be hydrolyzed during *Gladiolus* flower opening other than starch (Waithaka *et al.*, 2001). It is postulated that there is export of radioactive sugar from wilting florets to younger buds, raising the possibility that the drying florets could be the major source of carbohydrates for acropetal opening in spike-like flowers (Yamane *et al.*, 1995). In a comparative study between the postharvest

characteristics of two rose cultivars (‘Audio’ and ‘Black Magic’) the concentration of starch in the petals did not correlate with the corresponding vase life (Nabigol *et al*., 2014).

Table 4.9: The effect of pulsing with 600 ppm 8- HQS plus 5% sucrose (P) versus distilled water (NP) and wet cold storage duration on vase life and total soluble starch (µg/mg dry weight) during post-harvest life of cut *Gladiolusgrandiflorus* cv 'Fado'

Days of cold storage	**Total starch (µg/ mg dry weight)**				**Vase life (days)**	
	Third day in vase		Senescence			
	Pulsed	No pulsing	Pulsed	No pulsing	Pulsed	Nonpulsed
0	25.75 h	20.74 i	14.10 f	10.33 g	8.75 b	4.75 c
1	34.41fg	33. 56 g	19.29 de	18.58 e	10.25 ab	9.50 ab
2	36.70 de	35.26 ef	21.28 ed	19.93 de	10.00 ab	9.50 ab
3	42.29 a	37.62 d	24.05b	22.55 bc	11.50 a	9.75 ab
4	41.96 ab	40.59 bc	27.98e	24.00 b	11.25 ab	11.00ab
5	40.92abc	39.47 c	22.95 bc	23.99 b	10.25ab	10.25 hi
Mean	37.00a	34.54 b	21.60 a	19.90 b	10.10 a	9.13 b
C.D at 5%	0.136	0.136	0.209	0.209	0.148	0.148
Pulsing (P)	< 0.0001		< 0.0001		0.003	
Period of storage (S)	< 0.0001		< 0.0001		< 0.001	
P*S	< 0.0001		< 0.0001		< 0.0041	

Means followed by the same letter within evaluation period are not significantly different according to Tukey's test at 5 % level of confidence

4.2.2 Influene of pulsing and wet cold storage on subsequent total soluble sugars in cut *Gladiolus grandiflorus* L. cv. 'Fado'

This study established pulsing cut *Gladiolus grandiflorus* L. cv. 'Fado' spikes with 600 ppm 8- HQS in combination with 5% sucrose solution had significant effect ($P < 0.0001$) on the total soluble sugars both on the third day in the vase and at senescence (**Table 4. 10; Appendices A16; A17**). The mean concentration for 0 day stored and pulsed *Gladiolus* cut spikes was higher (31.00 ± 0.16 µg/mg dw.) compared with that(24.01 ± 0.16 µg/mg dw.) of non -pulsed spikes.The storage durations of 0 - 5 days also significantly ($P < 0.0001$) affected the mean concentrations of total soluble sugars on the third day in the vase and at senescence. The interactive effect of pulsing the cut *Gladiolus* with 600 ppm 8- HQS in combination with 5% sucroseand the cold storage durations of 1-5 days also significantly ($P < 0.0001$) influenced the total soluble sugars in the cut *Gladiolus*. The levels of total soluble sugars in the cut spikeson the third day in the vase and at senescence progressively increased as the storage duration increased from 0-5 days (**Table 4.10**). The levels of total soluble sugars in the cut spikes were higher at senescence compared on the third day of the *Gladiolus* vase life for both the pulsed and non-pulsed spikes (**Table 410**). The increase in total soluble sugars correlated with the enhanced vase life of cut *Gladiolus* flowers compared with the control.

These results are in agreement with observations on cultivars of *Gladiolus grandiflora* cv. Hort in which maximum vase life correlated with increased levels of total soluble sugars (da Costa & Finger, 2016). The additive effect of sucrose upto 20 per cent to pulse solution of *Leucadendron* leaf samples derailed postharvest decline of soluble starch after storage (Jones, 1995).The trend is however contrary to the results of postharvest studies on red gerbera (*Gerberajamesonii* cv. Intenza) in which unstored cut flowers registered higher levels of total soluble sugars (Muniz *et al*., 2016). The preservation of dry matter and retardation in starch hydrolysis has been reported in cut rose flowers treated with carbohydrates and biocidesolutions (Reid, 2009). This beneficial effect of sucrose in combination with the biocidal, chelating and stomatal closing properties of 8-HQS may have promoted the cut flower cellular integration.

The trend in the reduction in TSS sugars compared to the control signifies the probable decrease in the rate of breakdown of carbohydrates due to the effect of lowered temperatures, leading to reduced cellular metabolic reactions and respiration. According to a study done on cut spathes of calla lily (*Zantedeschiaaethiopica*) flowers, the addition of carbohydrates in storage solutions did not amount to increase in total soluble sugars (Sales *et al*., 2018). For the pulsed spikes, this could also be indicative of the improved quality of the cut spikes because of

the effect of sucrose in the pulsing treatment that could have enhanced osmotic turgidity and translocation of nutrients in the cut flower thus maintaining the flower integrity. Sucrose metabolite prevents osmotic stress in cut flowers thereby promoting hydration and thus keeping quality of cut flowers (Mukherjee & Mukherjee, 2017).

The results obtained in this study are synonymous with the study done on cut *Ranunculus asiaticus* L cut flowers in which increased concentrations of cycloheximide not only perpetrated senescence but aided in elevation of total soluble sugars in petals and sepals (Shahri & Tahir, 2010). Snapdragon (*Antirrhinum majus* L. cv. Yellow Butterfly) pulsed for 12 hours with 200-ppm 8-HQS in combination with 2% sucrose solution projected extension of the vase life and retardation in the degradation of chlorophyll and carbohydrates (Asrar, 2012).Many species of cut flowers display extension of the vase life when an exogenous carbohydrate source is supplied (Ichimura *et al*., 2005).Increased activity of the enzyme invertase in developing buds shows raised levels of sucrose hydrolysis, a requisite for maintaining osmotic changes needed for cell expansion in opening *Gladiolus* florets (Yamane *et al*., 1991). In contrast, the use of sucrose in vase solution in *Lilium* 'Stargazer'studies did not improve its vase life (Han, 2003). In another study, the use of a vase solution containing either trehalose or sucrose significantly affected the total carbohydrates in the petals of cut *Alstomeria* cv. Mayfair (Hatamzadeh *et al*., 2012). In another study on cut *Gerbera*, long-time preservation in 200-ppm 8-HQS had the potential to improve flower quality negating the need for the pulsing treatment (Jafarpour *et al*., 2015). Accumulation of soluble sugars under low water availability maintains and protects the membrane stability and protein functions (Liepiec *et al*., 2013). The role of soluble sugars in cut flowers is four faceted: supply of substrates for respiration; maintenance of an adequate water balance; decrease in sensitivity to ethylene and delay in climacteric ethylene biosynthesis (Pun & Ichimura, 2003).

However, research on two cultivars of cut rose flowers showed accumulation of soluble carbohydrates in the stems and petals translated into enhanced vase life in HQC plus sucrose treated stems (Nabigol *et al*., 2014). Petals from cut spikes of *Gladiolus* cv. Peters Pears placed in vase solution enriched with 50 mg l^{-1} GA3 + 50 g l^{-1} sucrose solution also registered highest concentrations of both reducing and non-reducing sugars in comparison with the control (Singh *et al*., 2008). This observation is comparable to another study on cut *Gladiolus* in which a vase solution containing 2 Mm calcium acetate promoted an upsurge of total soluble sugars in the petals compared with water control (Ji-gang *et al*., 2009).

Table 4.10 The effect of pulsing with 600 ppm 8- HQS plus 5% sucrose (P) versus distilled water (NP) and wet cold storage duration on vase life and total soluble soluble sugars (µg/mg dry weight) during post-harvest life of cut *Gladiolus grandiflorus* cv 'Fado'

Days of cold storage	**Total soluble sugars (µg/ mg dry weight)**				**Vase life**	
	Third day in vase		Senescence			
	Pulsed	No pulsing	Pulsed	No pulsing	Pulsed	Non pulsed
0	31.00 j	24.01k	39.66 1	33.83 j	8.75 ^{b}	4.75 ^{c}
1	35.71 h	33. 54 i	47.42 h	42.56 i	10.25 ^{ab}	9.50 ^{ab}
2	42.19 f	38.95 g	58.54 f	53.94 g	10.00 ^{ab}	9.50 ^{ab}
3	48.30 d	45.67 e^{c}	73.62 d	67.34 e	11.50 ^{a}	9.75 ^{ab}
4	65.04 b	58.58 c	86.40 b	79.148 c	11.25 ^{ab}	11.00 ^{ab}
5	74.57 a	75.64 a	94.40 a	93.53 a	10.10 ^{ab}	10.25 ^{ab}
Mean	49.47 a	46.07 b	66.67 a	61.72 b	10.333 ^{a}	9.125 ^{b}
CD at 5 %	0.16	0.16	0.34	0.34	0.21	0.21
Pulsing	< 0.0001		< 0.0001		< 0.0003	
Period of storage	< 0.0001		< 0.0001		< 0.0001	
Treatment*Storage	< 0.0001		< 0.0011		< 0.0041	

Means followed by the same letter within evaluation period are not significantly different according to Tukey's test at 5 % level of confidence

4.2.3 Effect of pulsing and wet cold storage duration on subsequent total free amino acids in cut *Gladiolus* (*Gladiolus grandiflorus* L. cv. 'Fado')

There was a significant effect (P<.0001) on the concentration of total free amino acids (TFAA) in cut *Gladiolus grandiflorus* cv. 'Fado' as a result of the pulsing and wet cold storage duration and their interactive effects on the third day in the vase as well as at senescence (**Table 4.11; Appendix A20**). The pulsing treatment of 600 ppm 8- HQS in combination with 5% sucrose resulted into improved vase life in this cultivar of *Gladiolus* compared with the non - pulsed spikes. Elevated levels of TFAA corresponded with reduced vase life. In addition, as the storage duration increased from 0 to 5 days, the levels of total free amino acids on the third day of the vase life and at senescence increased in the cut *Gladiolus* spikes. The best vase life (11.50 ± 0.21 days) was obtained in cut *Gladiolus* flowers pulsed with 600 ppm 8- HQS in combination with 5% sucrose and wet cold stored for 3 days before vase study. The TFAA concentrations in the pulsed cut spikes on the third day (19.39 ± 0.11 µg / mg dw.) and at senescence (21.00 µg / mg dw.) were significantly different compared with the control (**Table 4.11**). The control (0 day stored and non-pulsed spikes) registered the lowest vase life (4.75± 0.21 days) which was significantly different from that of non-stored but pulsed cut flowers (8.75 ± 0.21 days) in comparison.

It is noteworthy that cut *Gladiolus* wet cold stored after pulsing with 600 ppm 8- HQS in combination with 5% sucrose registered lower mean levels (19.35 ±0.14 µg / mg dw)of total free amino acids and better vase life compared with the non- pulsed spikes (22.02 ±0.14 µg / mg dw). A similar study on cut *Dianthus* sprays (*Dianthus chinensis* L). by varying the time of pulsing with cycloheximide before vase study in distilled water and sucrose solutions displayed raised levels of α-amino acids as the storage duration progressed (Dar *et al*., 2015). Cut anthurium (*Anthurium* a*ndraeanom* L. cv. Sirion) stored at 4°C, also registered accumulation of the amino acid proline (Aghdam *et al*., 2015). Qualitative analysis of amino acids from samples by thin layer chromatography (**Plate 4.1**)showed the predominant amino acids from senescent *Gladiolus* spikes pulsed with 600 ppm 8 – HQS plus 5% sucrose solution and cold stored for one day prior to vase study were Lysine, isoleucine and proline (**Table 4.12**). The separations from the baseline 7- 16 represent *Gladiolus* petal samples spotted on the base line (**Plate 4.1**). The numbers 1 – 6 represent standard amino acids Lysine, proline, glutamic acid, aspartic acid, isoleucine, and glutamine.*Gladiolus* spikes subjected to the same pulsing treatment but cold stored for 2 and 5 days registered the amino acids Lys, Cys, Leu, Ileu and Thr (at senescence); Cys, Gly (third day in vase) and Glu plus unknown amino acids respectively (**Table 4.12**). This observation was comparable to non-pulsed and wet cold stored

spikes for 2 and 5 days respectively. The former *Gladiolus* spikes registered Pro and Glu at senescence while the latter had Lys, Ileu apart from other unknown amino acids just after the cold storage period (**Table 4.12**). Accumulation of pools of all amino acids would be indicative of autophagy that is associated with stress-induced senescence (Savoure, 2010; Watanabe *et al*., 2013).

The pulsing treatment could have the role of inhibiting the buildup or biosynthesis of some amino acids such as cysteine, whose accumulation is toxic as earlier postulated in catabolism of plants (Hilderbrandt *et al*., 2015). This trend is synonymous to that displayed in anthurium (*Anthurium* a*ndraeanom* L. cv. Sirion) stored at 4 °C, which registered accumulation of the amino acid proline in γ – aminobutyric acid treated flowers (Aghdam *et al*., 2015).

This difference in *Gladiolus* subjected to the same period of storage of 5 days could be attributed to biotic stress in *Gladiolus* pulsed with the 600-ppm 8-HQS plus sucrose in which the exogenous carbohydrate source could attract microbial proliferation in vase water. The rise in some amino acids during senescence or carbohydrate starvation predisposes them as alternative respiratory substrates (Hildebrandt *et al*., 2015). The induction of acetyl CoA after application of amino acid mixtures supports this hypothesis (El-Naggar & Swedan, 2009). Branched chain and aromatic amino acids increase during stress (Fernie& Stitt, 2012). The pool of free amino acids range in accordance with respective functions: protein biosynthesis, fine tuned metabolic pathway involvement and signal transduction processes (Hildebrandt *et al*., 2015). The upsurge of proline relates to the oxidative stress a plant is encountering (Aghdam *et al*., 2015). On the other hand, application of aromatic amino acids induces vegetative growth, improves chlorophyll a and b and promotes early flowering in Amaryllis (*Hippeastrum vittatum*, Herb) and *Gerbera* j*amesonni* (El - Nagar &Swedan,2009; Geshnizjani & Khosh-Khui, 2016).

The biocides 8-HQS and 8-HQC are the most commonly used of the 8-HQ compounds in flower handling (Lobaud & van Doorn, 2004). These two compounds of 8-HQ have biocidal activity and act to influence enzyme activity by reducing the solution pH. (Damunupola & Joyce, 2008). The solution of 8-HQS @ 600 ppm in combination with 5% sucrose had a pH of 2.78, which was within the range that inhibited microbial proliferation. 8-HQS has been reported to promote stomata closure and also exhibits chelating activity that renders it biologically active (Burge *et al*., 1996). It has been reported that a combination of the biocide 8-HQS @ 100 ppm with sucrose @ 3% solution improved the vase life in cut carnation *(Dianthuscaryophyllus*) flowers (Elgimabi & Yagi, 2016). This could be due to the dual action of the biocide and the respiratory carbohydrate source needed to sustain the cut flowers.

Plants may be exposed to a number of abiotic stresses including osmotic stress, salinity, excess or limitation of essential ions, heavy metals, high temperature, freezing, ozone, excess light, nutrient deficiency and many others that affect their growth and development (Barth *et al*., 2006; Claeys *et al*., 2014; Rani & Singh, 2014; Subbash & Rakesh, 2008). Studies done on plant physiology, biochemical and molecular changes have indicated that plants may respond to abiotic stresses in a variety of ways. Accumulation of a variety of compatible solutes and amino acids including proline (Rai, 2002; Sabados& Savoure, 2009), and their derivatives such as γ-aminobutyric acid (Signorelli *et al*., 2015), and polyamines (Rakesh *et al*., 2014) are some of the responses of plants to abiotic stresses. Another study ameliorated changes in the cellular inorganic ion contents of the plants and the metabolism of growth regulators, particularly ABA in response to abiotic stress (Barth *et al*., 2006; Borochov *et al*., 1976; Cocklin & Barth, 2004). There has also been a report in changes in the activities of enzymes involved in the production and scavenging of reactive oxygen species, and changes in the expression of hundreds of genes, some of which are either up regulated or down regulated. Senescence is the final stage that marks the hallmark of programmed cell death on a cell, tissue, organ or organizational level (Shahri & Tahir. 2010; Yamada *et al*., 2009). Protein degradation is one of the most important processes concomitant with the visible signs in petal senescence (Wagstaff *et al*., 2002). There has also been a report on the decrease of proteins mediating transport and redox reactions during flower senescence (Borochov *et al*., 1994). Moreover, increased activity of protease activity has been reported in cut *Dianthuschinensis* L. as senescence progressed (Dar *et al*., 2014).

The results from the study on the effect of pulsing cut *Gladiolus* with 600 ppm plus 5% sucrose solution are in agreement with the study done on postharvest performance of isolated flowers of *Ranunculus asiaticus* that had been pre-treated with low concentration of cycloheximide (CHI) (Shahri & Tahir, 2010). In their study, protein degradation in *Ranunculus asiaticus* was up regulated when higher concentrations of CHI was used for pulsing of the flowers which generated higher levels of α-amino acids. In the mentioned study, lower concentrations of CHI increased the vase life of cut *Ranunculus asiaticus* flowers and led to release of less value of α-amino acids. A double increase in total free amino acids was reported in broccoli plantlets stored for 8 days at 20°C in anaerobic/ aerobic conditions (Derbali, 1998).

Improved protein retention was among the quality parameters observed in cut Chrysanthemum(*Denderanthema grandiflorus L. cv. Purple*) subjected to the treatments of either 20% tea extract or 100 mg l^{-1} 8- hydroxyquinoline sulphate (Hashemabadi & Bagheri, 2014). Low temperature storage has been associated with a decrease in metabolic processes in

cut flowers and hence aids in improving flower quality depending on the flower type (Gupta & Dubey, 2018). A study on peppermint (*Mentha piperita* L. indicated the improved shelf life and quality of this medicinal plant when cold stored at 5 °C (Barbosa *et al*.,2016). That increase in temperature promotes flower opening and shortens the vase life was proved by work done on cut rose pulsed with 8-HQS and sucrose solution (Ichimura *et al*., 1999). This observation is in agreement with the work done cut *Gladiolus* spikes whereby the use of 1-methyl cyclopropene and salicylic acid retarded senescence (Hassan & Ali 2014).

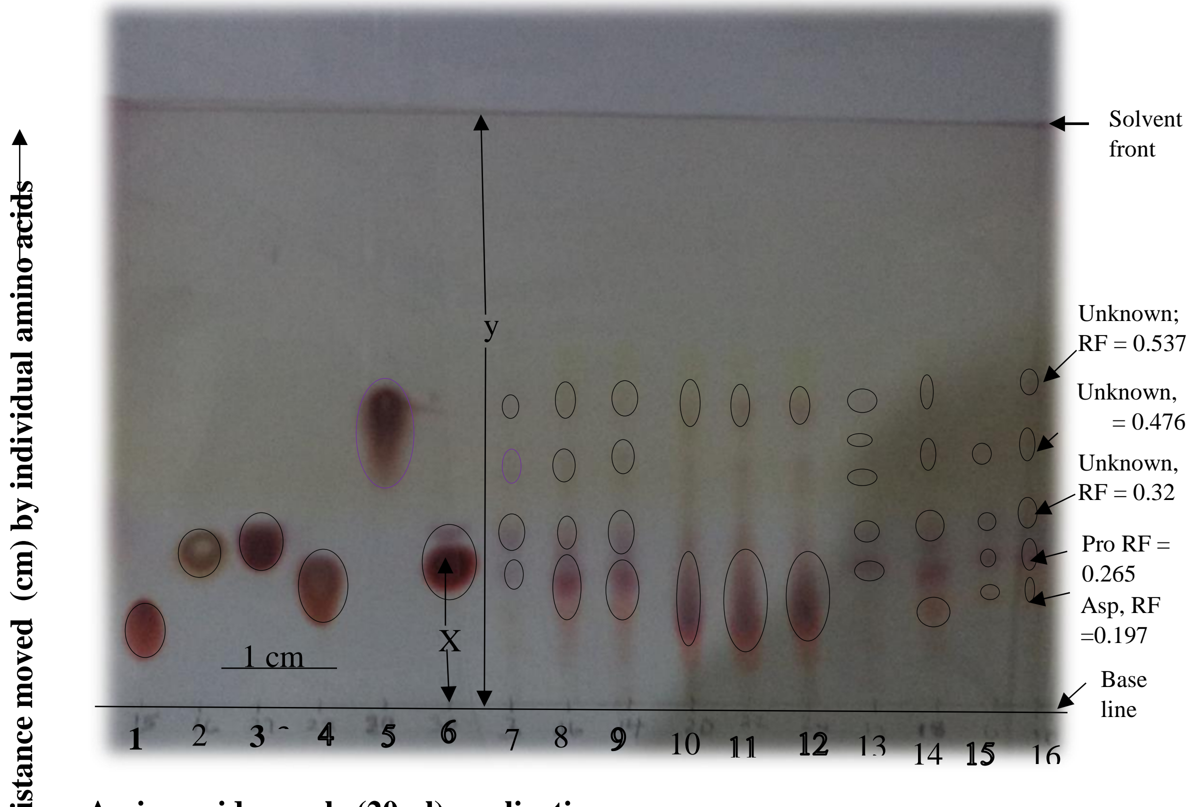


Plate 4.1: The thin layer chromatographic separation of amino acids from cut *Gladiolus grandiflorus* cv. 'Fado'petals using the mobile phase butanoic acid: acetic acid : water in the ratio 4: 1: 4 respectively and stained with ninhydrin.

Legend: The separations from the baseline 7, 8, 9, 10, 11, 12, 13, 14, 15, and 16 represent *Gladiolus* petal samples spotted on the base line. The numbers 1, 2, 3 , 4, 5, and 6represent standard amino acids lysine, proline, glutamic acid, aspartic acid, isoleucine, and glutamine.

Table 4.11: The effect of pulsing with 600 ppm 8- HQS plus 5% sucrose (P) versus distilled water (NP) and wet cold storage duration on vase life and total free amino acids (µg/mg dry weight) during post-harvest life of cut *Gladiolus grandiflorus* cv 'Fado'

Days of storage	Total free amino acids (µg/ mg dry weight)				Vase life (days)	
	Third day in vase		Senescence		Senescence	
	Pulsed	No pulsing	Pulsed	No pulsing	Pulsed	No pulsing
0	15.01 ^{i}	25.33 ^{a}	25.34 ^{j}	36.50 ^{a}	8.75 ^{b}	4.75 ^{c}
1	17.89 ^{h}	19.60 ^{f}	26.73^{i}	32.05 ^{ef}	10.25 ^{ab}	9.50 ^{ab}
2	18.80 g	20.48 ^{e}	27.52 ^{hi}	32.23 ^{de}	10.00 ^{ab}	9.50 ^{ab}
3	19.39 ^{fg}	21.00 ^{de}	2848 ^{gh}	33.64 ^{cdc}	11.50 ^{a}	9.75 ^{ab}
4	21.72 ^{cd}	22.03 ^{c}	29.75 ^{fg}	34.26 ^{bc}	11.25 ^{ab}	11.00 ^{ab}
5	23.30 ^{b}	23.02 ^{b}	35.11 ^{b}	35.47 ^{ab}	10.10 ^{ab}	10.25 ^{ab}
Mean	19.35 a	22.02 b	28.82^{a}	33.87 b	10.333 ^{a}	9.125 ^{b}
CD at 5%	0.14	0.14	0.006	0.006	0.21	0.21
Pulsing	< .0001		< .0001		< 0.0003	
Storage	< .0001		< .0001		< 0.0001	
Treatment*Storage	< .0001		< .0001		< 0.0041	

Means followed by the same letter within evaluation period are not significantly different according to Tukey's test at 5 % level of confidence

Table 4.12: Thin layer chromatographic identification of amino acids from petal and leaf samples of *Gladiolusgrandiflorus* cv. 'Fado' using RF values of standard amino acids

Material chromatographed	Source	R_f values	Inference
Lysine	Standard amino acid	0.145	Lys
L- Proline	Standard amino acid	0.265	Pro
Glutamic acid	Standard amino acid	0.272	Glu
Aspartic acid	Standard amino acid	0.197	Asp
Isoleucine	Standard amino acid	0.503	Ileu
Glutamine	Standard amino acid	0.238	Gln
Cysteine	Standard amino acid	0.329	Cys
Tyrosine	Standard amino acid	0.552	Tyr
Tryptophan	Standard amino acid	0.617	Trp
Arginine	Standard amino acid	0.187	Arg
Glycine	Standard amino acid	0.287	Gly
Histidine	Standard amino acid	0.160	His
Leucine	Standard amino acid	0.573	Leu
1P	Senescent *Gladiolus* petals	0.145; 0.222; 0.503; 0.422	Lys; Ileu and unknown amino acids
2P	Senescent *Gladiolus* petals	0.145;0.205;0.26;0.34;0.573	Lys; Cys; Leu; Ileu and Thr
2P	Third day in vase *Gladiolus*	0.34;0.28	Cys; Gly
2NP	Senescent *Gladiolus* petals	0.265;0.53;0.663	Pro, Glu;
5P	Third day in vase *Gladiolus* petals	0.238; 0.306; 0.415; 0.530	Glu; and unknown amino acids
5NP	Just after 5 days cold storage	0.145; 0.150; 0.503; 0.568	Lys; Ileu and unknown amino acids

The letters P and NP designate the pulsing with 600 ppm 8-HQS+5% sucrose and distilled water respectively while the numbers 1, 2 and 5 represent the respective cold storage durations in days. Amino acids with similar RF values are qualitatively similar

4.2.4 Influence of pulsing and wet cold storage treatments on subsequent total phenol levels in cut *Gladiolus*(*Gladiolus grandiflorus* L. cv. 'Fado')

Pulsing cut *Gladiolus grandiflorus* L. cv. 'Fado' with 600 ppm 8-HQS and wet cold storage durations of 0, 1, 2, 3, 4 and 5 days and their interactive effects significantly (P < 0.0008; P < 0.0001 and P < 0.0235 respectively) affected concentrations of total phenols on the third day in the vase **Table 4.13, Appendices A21; A22**). The mean total phenol concentrations in the cut pulsed *Gladiolus* spikes were significantly higher (38.647 ± 0.517 µg /mg dw) than in the non pulsed flowers whose average was 35.958 ± 0.517 µg /mg dw. (**Table 4.13**). Pulsed cut *Gladiolus* wet cold stored for 3 days prior to vase study registered the highest concentration of total phenols (49.502 ± 0.895µg /mg dw) andvase life (11.50 ± 0.21 days).These values were not significantly different from those recorded in pulsed cut *Gladiolus*wet cold stored for 4 days prior to vase study. They were however significantly different from the levels of total phenols in cut 5 days' wet cold stored spikes whose mean total phenols were lower (40.106 ± 0.517 µg /mg dw and 39.927 ± 0.517 µg /mg dw) for pulsed and non pulsed *Gladiolu*s. Unstored cut *Gladiolus* spikes registered lower values (27.485 ± 0.517 µg /mg dw and 24.367 ± 0.517 µg /mg dw) for pulsed and non pulsed flowers respectively) on the third day in the vase. Pulsed unstored cut spikes had a vase life of 8.75 ±0.21 days while that for non pulsed spikes was 4.5 days ±0.21 days. Cut *Gladiolus* spikes wet cold stored for 1 and 2 days prior to vase study registered mean total phenols and vase life that was higher than that of the control.

The concentrations of total phenols at senescencevaried considerably between the pulsed and the non-pulsed *Gladiolus grandiflorus* cut flowers in regard to the pulsing treatment of 600 ppm 8-HQS plus sucrose versus the non- pulsed spikes (P = 0.0090) (**Table 4.13 Appendix A22**). There was also significant difference (P <0.001) between the levels of total phenols in the pulsed and non- pulsed *Gladiolus* as a result of the wet cold storage durations of 0, 1, 2, 3, 4, and 5 days. Cut *Gladiolus* pulsed with 600 ppm 8-HQS plus 5% sucrose registered higher mean cumulative total phenols (17.67 ± 0.922 µg /mg dw.) at senescence that was significantly different from that of the non-pulsed flowers (15.592 ± 0.922 µg /mg dw.). The mean cumulative levels of phenols for both the pulsed and non- pulsed spikes increased from the lowest mean (7.034 ± 0.922 µg /mg dw.) for unstored *Gladiolus* upto the highest mean (24.095 ± 0.922 µg /mg dw) for spikes wet cold stored for three days prior to vase study. The levels of total phenols however increased with the increase in the storage duration (1 – 3 days) for cut *Gladiolus* pulsed with 600 ppm 8-HQS plus sucrose as well as for the non-pulsed flowers at senescence. The highest mean concentration of phenols (27.513 ± 0.922 µg /mg dw)

at senescence was recorded in cut *Gladiolus* pulsed with 600 ppm 8-HQS plus 5% sucrose that had been wet cold stored for 3 days' duration. Incidentally, these cut *Gladiolus* registered the highest vase life (11.5 ± 0.21 days) compared with the control (4.75 ± 0.21 days).The highest total phenols in the non pulsed*Gladiolus* was recorded in spikes wet cold stored for 4 days prior to vase study and registered a vase life of 11.00 ± 0.21 days. There was no significant difference in the levels of total phenols in non pulsed spikes subjected to 3, 4, amd 5 days' wet cold storage duration prior to vase study. These total phenol levels were however significantly different and higher than those of cut *Gladiolus* wet cold stored for 0, 1and 2 days which registered 5.466 ± 0.922 µg /mg dw.; 9.65 ± 0.922 µg /mg dw. and 15.948 ± 0.922 µg /mg dwrespectively (**Table 4.13**). These results are comparable to those on a study on selected *Iri*s species which displayed progressive increase in the concentration of phenolics in samples dried for 15 days in comparison to freshly harvested samples (Kaŝŝăk, 2012). In this experiment high content of total phenols during the flower development and senescence was associated with longer vase life. Other studies have also indicated that raised levels of phenols was associated with increase in vase life, floral attributes and flower quality parameters (Mwangi & Bhattacharjee, 2004).

The total phenols also significantly reduced at senescence for non- stored spikes irrespective of the type of pulsing employed during this study. The same trend is depicted for levels of phenols at senescence over the range of storage durations of 1- 5 days. There has been a report that a decrease in natural levels of antioxidants occurs during the process of senescence (Bartoli *et al*., 1997). A similar trend was observed in a study on groundcover rose (*Rosa* x *hybrida*) in which seven of the eight cultivars under investigation showed least amounts of total phenol contents at the senescence stage (Schmitzer *et al*., 2010). Conversely, a study done on cut *Dianthus chinensis* L. showed an increase in the levels of phenols in flowers pre-treated with sugars and sugar alcohols during the second and the fifth days of the vase study (Dar *et al*., 2014). Another study on cut rose cv. 'KORcrisett' indicated a reduction in phenolic compounds in senescent petals compared to the buds (Schmitzer *et al*., 2010). A linear significant correlation was established between phenolic compounds and antioxidant activity in methanollic extracts of the whole herb of *Marrubium peregrinum* L. (Lamiaceae) (Stankovic, 2011). Phenolic secondary metabolites have been reported in many plant functions including defense mechanisms and stress responses (Cavaiuolo *et al*., 2013). However, the levels of phenolic compounds is variable in different species and cultivars during the stages of flower development (Ahmad & Tahir, 2017; Cavaiuolo *et al*., 2013). An elevation in the concentration of lipoxygenase activity has been reported to promote senescence in ethylene insensitive flowers: daylily and *Gladiolus* species (Tripathi & Tuteja, 2007).

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Table 4.13: The effect of pulsing with 600 ppm 8- HQS plus 5% sucrose (P) versus distilled water (NP) and wet cold storage duration on total phenols (µg/mg dry weight) during post-harvest life of cut *Gladiolus grandiflorus* cv. 'Fado'

Days of storage	**Total phenols µg/ mg dry weight**				**Vase life (days)**	
	Third day		Senescence			
	Pulsed	No pulsing	Pulsed	No pulsing	Pulsed	No pulsing
0	27.49 ^{gh}	24.37 ^{h}	8.60 ^{g}	5.46 ^{g}	8.75 ^{b}	4.75 ^{c}
1	33.64 ^{efg}	29.27 ^{fgh}	11.20 ^{efg}	9.66 ^{fg}	10.25 ^{ab}	9.50 ^{ab}
2	36.66 ^{cde}	34.59 ^{def}	16.48 ^{cde}	15.95 ^{def}	10.00 ^{ab}	9.50 ^{ab}
3	49.50 ^{a}	41.96 ^{bc}	27.51 ^{a}	20.68 ^{bcd}	11.50 ^{a}	9.75 ^{ab}
4	44.49 ^{ab}	45.64 ^{ab}	23.72 ^{ab}	22.80 ^{abc}	11.25 ^{ab}	11.00^{ab}
5	40.11 bcd	39.93 ^{bcd}	18.51 bcd	19.01 ^{bcd}	10.25 ^{ab}	10.25 ^{hi}
Mean	38.64 ^{a}	35.95 ^{b}	17.67 ^{a}	15.59 b	10.333	9.125
CD at 5 %	0.34	0.34	0.53	053	0.21	0.21
Pulsing	0.0008		< 0.0090		0.0003	
Storage	< 0.0003		< 0.001		< 0.001	
Treatment* Storage	0.0235		< 0.0981		0.0041	

Means followed by the same letter within evaluation period are not significantly different according to Tukey's test at 5% level of significanc

Lipoxygenases also promote senescence in ethylene sensitive flowers such as carnations and rose flowers through oxidative cell damage (Thompson *et al*., 1998). The use of the phenolic salicylic acid derivative 5- sulfosalicylic (SSA) @ 100ppm plus 4% sucrose had a positive influence on oxidative stress and antioxidative system during *Gladiolus* cut flower senescence (Ezhilmathi *et al.,* 2007). The treatment of spikes with 5-SSA promoted lower respiration rates, lipid peroxidation and lipoxygenase (LOX) activity, higher membrane stability, soluble protein concentration, and activity of superoxide dismutase (SOD) and catalase. (Eizhilmathi *et al.,* 2007). Treatment of cut *Gladiolus grandiflorus* cv. White prosperity with 1-MCP was found to regulate floret senescence not only through its effect on ethylene action, but also by other mechanisms including proline accumulation, maintaining membrane stability and reducing lipid peroxidation (Hassan & Ali, 2014). The accumulation of phenolic compounds has been hypothesized to be a flower response to cell disruption (Cavaiuolo *et al*., 2013). Application of growth stimulants (SA, GA_3 and BA significantly minimized accumulation of phenolic compounds in aster (*Symphyotrichum novibelgii* L.) cv. Purple Monarch (Mohamed*,* 2017).

4.3.1 Influence of pulsing and wet cold storage on subsequentspectrophotometric and HPLC determination of anthocyanins in cut *Gladiolus* (*Gladiolus grandiflorus* L. cv. 'Fado')

In this study the effect of pulsing cut *Gladiolus grandiflorus* cv. 'Fado' with 600 ppm 8-HQS significantly ($P < 0.0001$) affected the levels of anthocyanins on the third day of the vase study and at senescence. The effect of wet cold storage durations of 1, 2 3, 4, and 5 days also had significant effect $P< 0.0001$) on the anthocyanin concentrations in both the pulsed and the non pulsed *Gladiolus* (**Table 4.14., Appendices A23; A24**). The interaction effects of the pulsing treatment and the cold storage durations of 0, 1, 2, 3, 4 and 5 days also significantly ($P < 0.0001$) affected the levels of anthocyanins in the cut *Gladiolus*.The cumulative mean concentration of anthocyanins in the pulsed *Gladiolus* spikes was higher (0.248 ± 0.004 µg / mg. dw) compared with that (0.180± 0.004 µg / mg. dw.) in the non pulsed flowers. Cut *Gladiolus* spikes unstored but pulsed with 600 ppm 8-HQS plus5% sucrose solution registered enhanced anthocyaninlevels (0.16 ± 0.007 µg / mg. dw) compared with the control (0.11± 0.007µg / mg. dw) µg / mg. dw).The lowest levels of anthocyanins (0.126 ± 0.007 µg / mg. dw) were recorded in cut *Gladiolus* wet cold stored for 5 days before vase study. This was lower than the mean anthocyanin concentration (0.135 ± 0.007 µg / mg. dw) in unstored cut

Gladiolus. The cut *Gladiolus* stored for 1, 2, 3, and 4 days prior to vase study registered higher concentrations of anthocyanins compared with unstored spikes 0.306± 0.007 µg / mg. dw., 0.404 ± 0.007 µg / mg. dw. 0.16± 0.007 µg / mg. dw., and 0.146± 0.007 µg / mg. dw respectively). A similar trend was observed in the concentrations of anthocyanins in spikes subjected to high performance liquid chromatography separation (**Table 4.14**., **Appendix 24**). High performance analysis registered the highest concentration (8.88 ± 0.041 µg / mg. dw) that was significantly different from that of the control (**Table 4.14., Figure 4.1, Plate 4.2).** According to this , the pulsing treatment maintained higher anthocyanin concentrations for spikes wet cold stored for 0, 1 and 2 days compared with the non-pulsed flowers. A drop in the anthocyanins in the cut *Gladiolus* pulsed and wet cold stored for 3 and 4 days (inspite of displaying other superior quality parameters) may be due to differential vacuole pH changes in the cutflowers, leading to changes in chromocity.

The same trend was observed in cut *Gladiolus* pulsed with 600 ppm 8-HQS plus5% sucrose solution and wet cold strored for one day whose anthocyanin content (0.357± 0.007 µg / mg. dw.) was statistically different from that of the non pulsed spikes subjected to a similar storage duration before vase study whose mean was 0.256 ± 0.007 µg / mg. dw.).The highest anthocyanin concentration (0.569 ± 0.007 µg / mg. dw.) on the third day in the vase was recorded in pulsed cut *Gladiolus* spikes which was statistically different from that of the control. Non pulsed cut *Gladiolus* subjected to a similar wet cold storage duration prior to vase study registered lower concentration of anthocyanins 0.240 ± 0.007 µg / mg. dw).

The variable trends in the levels of anthocyanins on the various days of storage after pulsing with 600 ppm 8-HQS plus 5% sucrose or distilled water could be attributed to differences in types and stability of anthocyanins expressed in the respective vase solutions during the study. Anthocyanins are flavonoid compounds that impart the blue, purple, red and orange colors in flowers, fruits and vegetables (Miguel, 2011).This class of polyphenols have bioactive activities including the action as antioxidants (Ponmozhi *et al*., 2011). Other naturally occurring pigments that impart color to fruits and petals are the carotenoids (Wang *et al*., 2018). The use of sucrose in vegetative tubers of *Petunia* plants was associated with a need for anthocyanin regulating transcription factors (Ai *et al*., 2016). The effect of sucrose in the pulsing solution could be responsible for differences in variable anthocyanins in the 600 ppm 8-HQS plus 5% sucrosepulsed spikes versus the non-pulsed *Gladiolus*. Stability and existence of type of anthocyanins is pH dependent (Miguel, 2011). The pH range could be a factor in variable levels of anthocyanins in the600 ppm 8-HQS plus 5% sucrose pulsed spikes versus the non- pulsed spikes.

Table 4.14: The effect of pulsing with 600 ppm 8- HQS plus 5% sucrose (P) versus distilled water (NP) and wet cold storage duration on anthocyanins (µg/mg dry weight) during post-harvest life of cut *Gladiolus grandiflorus* cv. 'Fado'

Days of cold storage	**Anthocyanins µg/ mg dry weight (HPLC)**				**Anthocyanins µg/ mg dry weight (spectrophometry)**				**Vase life (days)**	
	Third day		Senescence		Third day		Senescence		Vase life	
	Pulsed	Non pulsing	Pulsed	No pulsing	Pulsed	No pulsing	Pulsed	No pulsing	Pulsesd	Non Pulsed
0	7.76^{c}	6.93^{d}	7.23^{a}	6.25^{cd}	0.15^{de}	0.11^{e}	0.13^{c}	0.078^{ef}	8.75^{b}	4.75^{c}
1	8.25^{b}	7.20^{d}	7.50^{a}	$6.9\ 8^{ab}$	0.36^{b}	0.25^{c}	0.28^{a}	0.19^{b}	10.25^{ab}	9.50^{ab}
2	8.88^{a}	7.03^{d}	7.52^{a}	6.57^{bc}	0.57^{a}	0.24^{c}	0.27^{a}	$0.2\ 0^{b}$	10.00^{ab}	9.50^{ab}
3	5.76^{fg}	6.55^{e}	4.91^{fg}	5.73^{de}	0.14^{de}	0.18^{d}	0.09^{b}	0.077^{ef}	11.50^{a}	9.75^{ab}
4	5.75^{fg}	6.00^{f}	4.77^{fg}	5.33^{ef}	0.14^{de}	0.16^{de}	0.089^{def}	0.072^{f}	11.25^{ab}	11.00^{ab}
5	5.25^{h}	5.71^{g}	4.38^{g}	4.60^{t}	0.12^{e}	$0.\ 13^{e}$	0.083^{def}	0.10^{b}	10.25^{ab}	10.25^{hi}
Mean	6.94^{a}	6.57^{b}	6.05^{a}	5.91^{a}	0.24^{a}	0.17^{b}	0.152	0. 119	10.333	9.125
C.D at 5 %	0.041	0.041	0.052	0.052	1.326	1.326	0.002	0.002	0.21	0.21
Pulsing (P)	<.0001*		0.0586		0.0586		<.0001		0.0003	
Period of storage (S)	<.0001*		<.0001*		<.0001		< 0.001		< 0.001	

P*S	<.0001*	<.0001*	<.0001	< 0.001	0.0041

Means followed by the same letter within evaluation period are not significantly different according to Tukey’s test at 5% level of significance

In the petunias it has been reported that anthocyanins reduced as the flower advanced in longevity. Conversely the levels of anthocyanins in cut *Alstromeria* cv. Mayfair flowers was highest in leaves and petals when vase solution containing 40nM trehalose solution was used compared with a similar concentration of sucrose and the control (Asil & Roein, 2012). Higher levels of carotenes were also observed in cut rose (var. 'Sun King' flowers that had not been subjected to any cold storage treatment in comparison with the packaged and aluminium sulfate treated stems (Makwana *et al*., 2015). The contents of anthocyanin in aging *Digitalis purpurea* L. cv. Foxy has been reported to vary greatly between spikes but there is a very small decrease at senescence (Stead & Moore, 1977).

In a study on Bird of paradise (*Strelitzia reginae*) cut flower spikes with chemical preservatives and ethanol extracts from sweet basil (*Ocimum basilicum*) before vase study, levels of anthocyanins significantly increased in the petals as compared with the untreated control (Gendy & Mahmoud, 2012). An accumulation of anthocyanins, a subclass of flavonoid phenolic compounds in long life and ephemeral *Hibiscusrosa-sinensis* L. flowers has been linked to senescence processes (Trivellini *et al*., 2007).

In the petunias it has been reported that anthocyanins reduced as the flower advanced in longevity. Conversely the levels of anthocyanins in cut *Alstromeria* cv. Mayfair flowers was highest in leaves and petals when vase solution containing 40 nM trehalose solution was used compared with a similar concentration of sucrose and the control (Asil & Roein, 2012). Higher levels of carotenes were also observed in cut rose (var. 'Sun King') flowers that had not been subjected to any cold storage treatment in comparison with the packaged and aluminium sulfate treated stems (Makwana *et al*., 2015). The contents of anthocyanin in aging *Digitalis purpurea* L. cv. Foxy has been reported to vary greatly between spikes but there is a very small decrease at senescence (Stead & Moore, 1977).

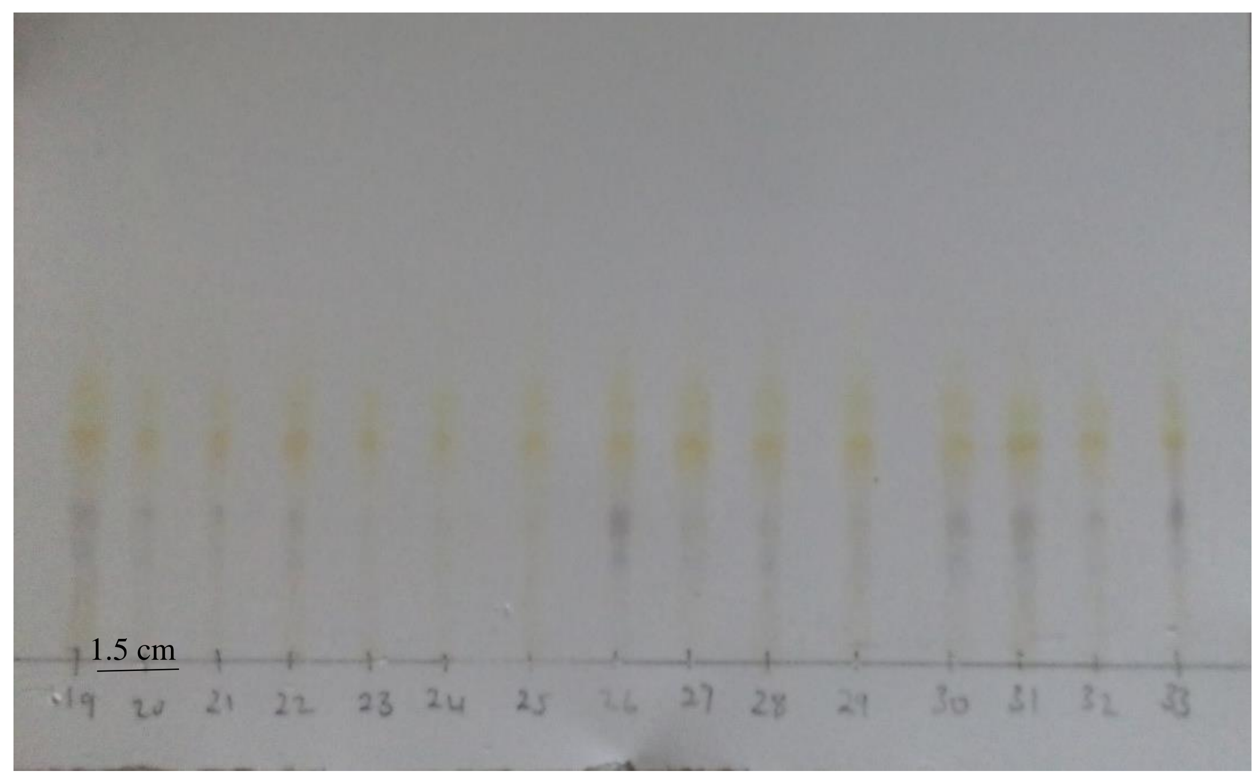
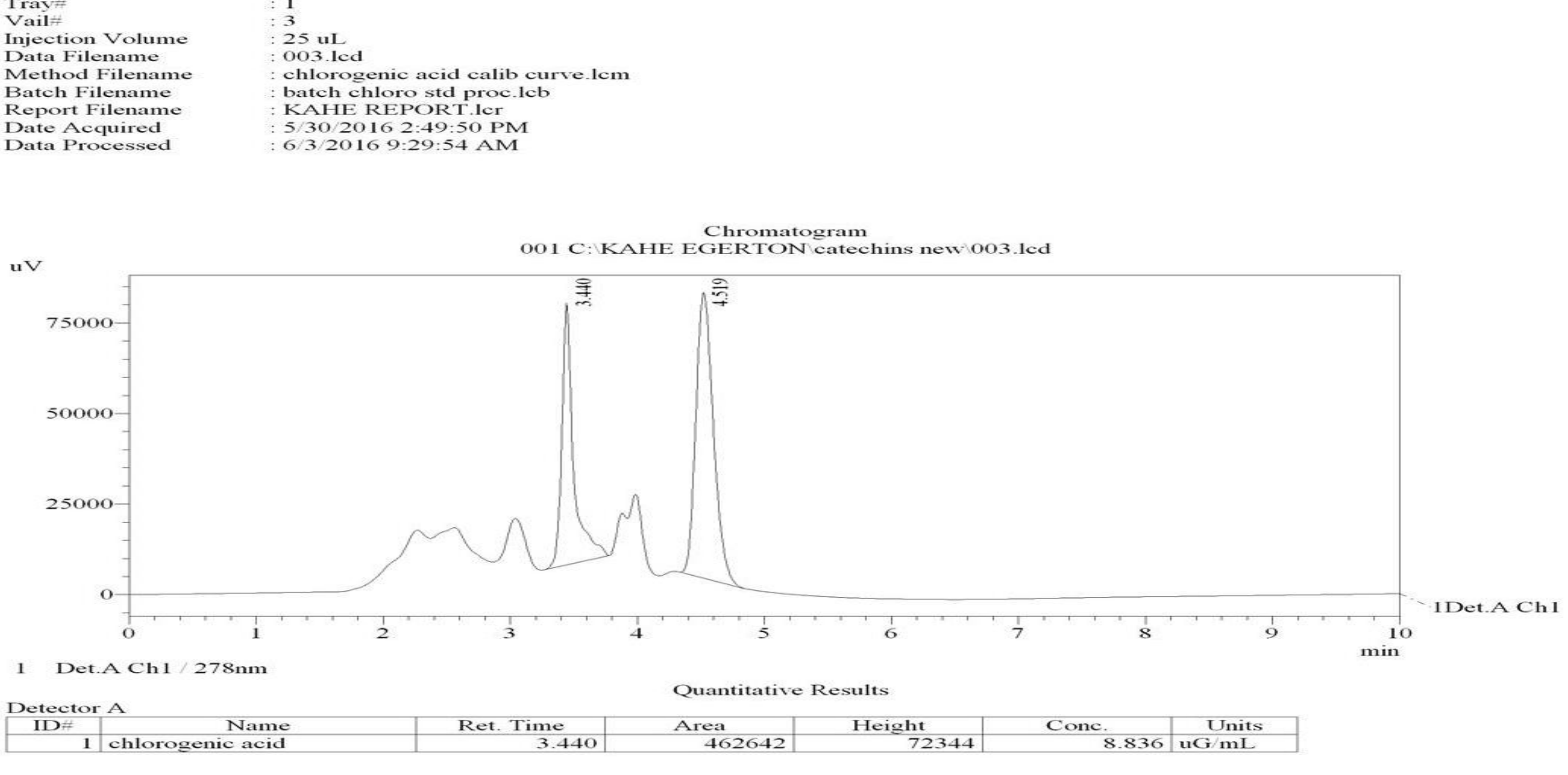


Plate 4.2: The thin layer chromatographic qualitative analysis of petal anthocyanin profiles in cut *Gladiolusgrandiflorus* cv. 'Fado'.

Legend*:* 19 = pulsed in distilled water and cold stored for four days; 20 = Senescent *Gladiolus* pulsed with 600 ppm 8- HQS plus 5% SUC and cold stored for 1 day; 21 = Pulsed *Gladiolus* at day three in vase after two days cold storage; 22 = Pulsed with distilled water and cold stored for three days; 23 = Senescent *Gladiolus* Pulsed with distilled water and cold stored for 5 days;24 = Pulsed with 600 ppm 8- HQS plus 5% SUC and stored n cold for two days; 25 = Pulsed and not cold stored 26 = Pulsed with $Al_2(SO_4)_3$ before three days' cold storage; 27 = Senescent Gladiolus pulsed and cold stored for 1 day; 28 and 29 = *Gladiolus* harvested and dried immediately; 30 = *Gladiolus* pulsed with $Al_2(SO_4)_3$ and cold stored for 5 days; 31 and 32 = Pulsed with 600 ppm 8- HQS plus 5% SUC and cold stored for 5 days



ID#	Name	Ret. Time	Area	Height	Conc.	Units
1	chlorogenic acid	3.440	462642	72344	8.836	uG/mL

Figure 4.1: High performance liquid chromatography profile for anthocyanin content of *Gladiolus grandiflorus* cv. 'Fado' pulsed with 600 ppm HQS plus 5% sucrose and cold stored for two days before vase study

4.4.1 Influence of pulsing and wet cold storage on microbial isolates in cut *Gladiolus* (*Gladiolus grandiflorus* L. cv. 'Fado')

Pulsing and wet cold storage treatments had an effect on the microbial profiles in vase water of cut *Gladiolus grandiflorus* L. cv. 'Fado'during different days in the vase (**Table 4.15**). It can be observed that the maximum number of genera of the fungal and bacterial flora (*Streptomyces albus,Dreschlera specifera, Cladosporium oxysporum, Alternaria alternata, Aspergillus flavus andStaphylococcus aureus)* were from *Gladiolus* that were neither stored nor pulsed before vase study (**Table 4.15**). These spikes also registered lower days of vase life compared to those pulsed with 600 ppm HQS plus 5% sucrose (**Table 4.15).** Asimilar study showed that a series of preservatives including 200 ppm 8-HQS in combination with 2% sucrose and citric acid at 100 ppm improved the quality and vase life of cut chrysanthemum (*Dendrantherma grandiflorum* kitam (Abou El Ghait *et al*., 2012).During the 1st day of the vase life of non-pulsed and unstored cut *Gladiolus*, the microbial species isolated were *Dreschlera specifera*and *Alternaria alternata*. On the 2nd day in the vase, *Cladosporum oxysporum*was observed alongside the two previous isolates. On the third day in the vase *Staphylococcus aureus* was added to the three previous isolates. The species of *Aspergillus flavus* was observed in the vase water in addition to fungal species isolated on the former days of the vase study for thecut *Gladiolus* spikes on the fourth day in the vase.

The maximum number of fungal genera appeared on the 5th day of the the vase study of the cut *Gladiolus* when *Streptomyces albus* and *Talaromyces* species were also isolated which culminated into senescence of the cut spikes. In the case of non-pulsed and unstored *Gladiolus* the baterialisolate from the vase water immediately on commencement of display was *Aeromonas hydrophila.* On the 1st day in the vase the bacteria of *Vibrionaceae* genus and *Staphylococcus warneri*were isolated. On the second day in the vase, apart from the previous isolates, a *Penicillium* species and *Shewanella putrefaciens* were observed. The same microbial profiles were recorded in the vase water of the cut *Gladiolus* on the third day of the vase study. On the 4th day in the vase the microbial profiles consisted of *Aspergillusniger*, *Penicillium* species and *Staphylococcus warneri*; the previous bacteria species were not isolated, probably due to depletion/competion for nutrients in vase water. On the 5th day in the vase the fungi species and bacteria isolated were *Aspergillus niger*, *Penicillium* species, *Alternariaaltanata*, *Aspergillus flavus* and *Staphylococcus warneri* respectively.

The second batch of cut *Gladiolus* consisted of spikes that were not wet cold stored but they were pulsed with 600 ppm 8 –HQS plus 5% sucrose solution. On the 1st day in the vase

the microbes isolated were *Proteus vulgaries,Serratia mercescens*, *Pantoea agglomerans* and *Bacillus* species. On the 2nd day in the vase a *Staphylococcus* spp alongside the previous isolates were observed. On the 3rd day in the vase*Pseudomonus putida*; *Pantoea agglomerans*, *Bacillus* species, *Aspergillus* species, *Ulocladium, Staphylococcus aureus* isolates were observed. On the 4th day in the vase the species*Emericela nidulans*, *Ulocladium*, *Aspergillus* species, *Trichophyton interdigitale* and *Pseudomonus putida* were recorded. On the 5th day in the vase the microbial flora included *Trichophyton interdigitale*, *Trichophyton verrucosum*, *Acremonium* species, *Ulocladdium*, *E. nidulans* species and this marked the maximum number of fungi in unstored 600 ppm 8-HQS and 5% sucrose pulsed *Gladiolus.*

Bacillus bacteria were identifiedfrom non- pulsed cut *Gladiolus* spikesafter wet cold storage for one day. The same bacterium was observed after 1st day of vase study at ambient temperatures. On the 2nd day of vase study *Serratia mercecens* bacterium was recorded alongside the first isolate. On the third day in the vase a *Penicillium* species was isolated together with the previous microbial flora. On the 4th day in the vase the microbial profiles included *Penicillium* species. *Aspergillus niger*, *Alternaria* species, *Aspergillus flavus* and *Acremonium* species. On the 5th day in the vase *Aspergillus fumigatus, Serratia mercescens*, *Pantoea agglomerans* and *Rhizopus* species were isolated.*Gladiolus* pulsed with 600 ppm 8 – HQS plus 5% sucrose and subjected to the same storage duration registered one bacterium isolate (*Serratia mercescens*). On the second day one more bacterium was isolated (*Pantoea agglomerans*). On the 3rd day in the vase the same two bacteria species were recorded; which were also identified in the vase water on the 4th day of the display life. *Aspergillus fumigatus*, *Serratia mercecens, Pantoea agglomerans*and *Rhizopu*s species isolates were observed on the 5Th day of the cut *Gladiolus* vase life.

Aresearch study on eight species of cut flowers reported the presence of *Escherichia coli* and *Aeromona*s *hydrophila* as among the bacteria species recorded in the control, purpoted to have originated from the water (Marousky *et al*., 1977). In another study, it was reported that the composition of the bacteria flora had no effect on the longevity of cut flowers (van Doorn *etal*., 1995). For *Gladiolus* cold stored for 2 days and non-pulsed, the bacterium *Hafnia alvei was isolated*. The same isolate was observed upto the 4th day of the vase study, when another isolate (*Penicillium* species) was recorded. On the 5th day of the vase study three more fungal isolates were registered (*Alternata alternaria*, *Aspergillus niger* and *Aspergillus flavus*). *Gladiolus* pulsed with 600 ppm 8-HQS plus 5% Sucrose registered *Staphylococcus aureus* bacterium just aftterthe two days cold storage duration. Then the following 1st and2nd days' vase study the microbial sepcies isolated were *Staphylococcus aureus* and *Albugo* species. On

the third day of the vase study another species (*Aspergillus niger*) was observed. The same fungal species were isolated on the 4th and 5 th days of the vase study of the cut *Gladiolus*.

At the end of three days' cold storage duration after the pulsing treatment with distilled water the isolate identified through 0, 1st, 2ndupto the 3rd days of vase study was *Pantoea agglomerans*, when *Alternaria alternata*was identified. Comparatively, *Gladiolus* subjected to a similar cold storage duration after prior pulsing treatment with 600 ppm 8-HQS plus 5% Sucrose resulted in *Pantoea agglomerans*, *Proteus vulgaries* and *Staphylococcus* species proliferation on the zero upto the 2nddays of the vase study. From the 3rd upto the 5th days of vase life of cut *Gladiolus*vase study the isolates identified were *Acremonium* species, *Paecilomyces species*, *Phytophthora*specie*s* and *Alternaria* species. *Pantoea agglomerans* (formerly called *Enterobacter agglomerans*) and *Shewanella putrefaciens* were among the bacteria isolates from the secateur's blade for cutting Sonia roses while *Bacillus* and *Staphylococcus* species were isolated from the flower leaves and stems (van Doorn *et al.,*1995).

Cut *Gladiol*us spikes cold stored for 4 days after non-pulsing treatment registered *Bacillus* species upto 2nd day in the vase life. On the 3rd day two more species were identified (*Candida albicans* and *Uromyces species*). On the 4thand 5th days of the vase study *Acremoniu*m and *Aspergillus niger* species were registered, alongside the previous fungal isolates respectively. *Gladiolu*s pulsed with 600 ppm 8-HQS and 5% Sucrose treatment but wet cold stored for a similar duration presented with*Bacillus* species for 0 and 1stdays' periods of the vase study. On the 2nd upto the 4th days of the vase life of cut *Gladiolus,Bacillus* and *Cladosporium* species were identified. On the 5th day in the vase the isolates identified were *Rhizopus*, *Aspergillus nidulans* and *Cladosporium* species.

Lastly, *Gladiolus* cold stored for five days after pulsing with distilled water regisrered *Fusarium* species just after low temperature exposure period. This was followed by *Exophiala* secies on the 1st upto the 4th days of the vase study. On the 5th day of the vase life *Cephalostricum* was identified alongside the two previous isolates. For the spikes subjected to 24 hours pulsing with a solution of 600 ppm 8-HQS plus 5% Sucrose prior to 5 days' cold storage resulted in *Staphylococcus aureus* being identified on zero just after low temperature exposure. This was followed by the isolates *Madurell*a, *Tricothecium roseum* and *Exophiala jeanselmei* besides *Stsphylococcus aureas* which were identified from the vase solution on the 1stday of display life of the cut spikes. On the 2nd day in the vase the isolates were fungal floraZygomycete, *Madurella*, *Tricothecium roseum* and *Exophiala jeanselmei.* On the 3rd day of the vase study the fungi isolates identified were of the *Madurella species*, *Tricothecium roseum*, *Exophiallajeanselmei*and *Staphylococus* aureas. On the 4th day of vase study another

fungal isolate (a Zygomycete) was identified beside the three previous fungi. Alichen was isolated on the 5th day of the vase study beside the previous fungi species isolated from the *Gladiolus* vase water.

4.4.2 The effect of wet cold storage and pulsing with 600 ppm 8-HQS plus 5% Sucrose on the microbial profiles of cut gladioli (*Gladiolus grandiflorus* L. cv. 'Fado' at senescence

The highest fungal profiles (*Streptomyces albus, Dreschlera specifera, Cladosporium oxysporum, Altenaria altanata, Aspergillus flavus, Taloromyce and Acremonium* species) were recorded at senescence in cut *Gladiolus* spikes' vase solution that had neither been pulsed nor cold stored. Incidentantally, unstored *Gladiolus* pulsed with distilled water for 24 hours had only five fungal species: *Aspergillus niger,Penicillium species, Cladosporum oxysporum, Alternaria altanata, andAspergillus flavus.* Incidentally, this was in contrast with fungal isolates from unstored but 600 ppm 8-HQS plus 5% sucrose pulsed spikes which comprised of *Syncephalastrum Trichophyton verrucosum, Epidermophyton, Nigrospora, E..nidulans, Acremonium species and Ulocladium*. Subsequent storage durations of 1,2,3,4 and 5 days at 3 ± 1^{0}C registeredcomparatively less fungal isolates than the control (**Table 4.15**). Many studies have implicated microbial proliferation that occasions vascular occlusion for the poor quality and water relations in cut flowers (Bhat & Sheikh, 2015). The use of a carbohydrate for energy needed in respiration together with biocides and sometimes in combination with acidifiers plus ethylene inhibitors improve the keeping quality of cut flowers (Damunupola & Joyce, 2008; Elgimabi & Ahmed, 2009; Rani & Singh, 2014).

The fungal species isolated from the vase solution the cut non-pulsed *Gladiolus*cold stored for one day were *Penicillium species, Aspergillus niger, Alternaria species, Aspergillus flavus and Acremonium species.* Similarly stored *Gladiolus* pulsed with 600 ppm 8-HQS plus 5% sucrose registered *Aspergillus fumigatus,Rhizopus species, Talaromyces* and *Aspergillus parasiticusspecies. Gladiolus* spikes cold stored for two days after the pulsing treatment of distilled water had the isolates: *Alternaria alternata, Aspergillus niger, Alternaria* species, *Aspergillus flavus*and*Acremonium species. Gladiolus* cold stored for a similar period but pulsed with600 ppm 8-HQS plus 5% sucrose contained the isolates: *Alburgo* species, *Aspergillus* species and *Alternaria* species in vase water at senescense. *Gladiolus* spikes cold stored for three days after the pulsing treatment of distilled water containes the isolates *Alternaria species, Aspergillus nidulans and Acremonium* species in the vase water at

senescence. This was comparable to cut *Gladiolus* that had been subjected to a similar storage duration after the pulsing treatment of 600 ppm 8-HQS plus 5% sucrose from whose vase water the following isolates were identified: *Alternaria species sAcremonium, species,Phytophthora* and *Paecilomyces* species.

Spikes cold stored for four days after the pulsing treatment of distilled water registered the microbial isolates *Candida albicans, Acremonium species, Aspergillus niger, Aspergilus flavus and uromyces* species.Similarly stored spikes after600 ppm 8-HQS plus 5% sucrose had thefollowing isolates in vase water at senescence*: Rhizopus, Aspergillus nidulans,Aspergillus flavus and Cladosporium* spp. Cold storage for 5 days of cut *Gladiolus* spikes after the pulsing treatment with distilled water resulted in the identification of the following microbial flora*: Cephalotricum, Fusarium, Exophiala jeanselmei, Candida albicans and Tricothecium.* This was comparable to similarly stored spikes which were pulsed with a solution of 600 ppm HQS plus 5% sucrose from whose water at senescence the microbial flora were*Madurella, Tricothecium roseum, Exophiala jeanselmei, Zygomycete, Lichen and Madurela* species.The mean vase life of these cut flowers was 10.33 ± 0.148 days, which was significantly different (P <0.0003) from that of similarly stored *Gladiolus* but non-pulsed, whose mean was lower (9.5 ± 0.148 days).

Prolonged storage duration has been associated with inferior keeping qualities of cut *Gladiolus* (Bhat & Sheikh, 2015).These results can be compared with the study done on cut *Dendranthema grandiflorus* Kitam in which the cold storage for seven days at 2 ± 1°C followed with use of a holding solution composed of 100 ppm HQS, 2% sucrose and 100 ppm citric acid enhanced the quality parameters compared with the control (Rani& Singh, 2014). The use of 8- HQS at concentrations of 200 – 600 ppm has been habitually used to improve the keeping qualities of cut flowers (Senapati *et al.,* 2016). Hydroxyquinoline sulphate has other effects on the cut flower physiology apart from its biocidal action (Asrar, 2012; Nabigol *et al*., 2014). Incidentally in this study the number of fungi isolates at senescence in *Gladiolus* pulsed with600 ppm 8-HQS plus 5%b sucrose were six. Similarly stored *Gladiolus* spikes pulsed with distilled water registered five different fungi species. This difference could be attributed to the role played by exogenous sucrose as a substrate for fungi (Gupta *et al*., 2018).

The ability of fungi to proliferate inspite of the presence of a biocide could be due to the ability of filamentous fungi to biodegrade and transform phenolic compounds (Enguita & Leitao, 2013). There are reports that cold storage makes cut flowers to be prone to fungi, bacteria and yeasts (Abou El Ghait *et al*., 2012). Appropriate storage temperatures (4 -7°C for cut *Gladiolus*) for typical cut flower species have been adopted for bulking and orderly

marketing of cut flower commodities (Senapati *et al*., 2016).However, other studies have reported the potency of 8-hydroxyquinoline as a scaffold for drug design to get novel microbial bioactive derivatives (Pippi *et al* ., 2019). It is hypothetica that the key proteins and signal transduction pathways in plant pathogenic fungi could be the targetsprone to preservatives such as 8-HQSin microbial inhibition (Zhang *et al*., 2016). A comparative study on cut rose (*Rosa* × *hybrida* cv. Cherry Brandy) reported the ability of the biocide aluminium sulfate to contain microbial proliferation upto the 4th day in the vase of this cut flower (Jowkar*et al.,* 2012).

The results in this study are comparable with the study on cut tuberose (*Polianthes tuberosa* L.) in which cold storage without pre-storage pulsing with 20% sucrose in combination with the biocide HQS had significant effect on the vase life and quality of the spikes. (Waithaka *et al*., 2001).The pulsing treatment of 8-HQS plus 5% sucrose and the period of storage at 3 ± 1 ^{o}C may have had an added effect on the vase life and quality of the cut *Gladiolus* in this study.

Table 4:15: Effect of Pulsing and Wet cold Storage Duration on the Occurance of Microbes in the Vase Water of Cut Gladioli (*Gladiolus grandiflorus* cv. 'Fado')

Treatment / Storage	**0 day**	**1st day**	**2nd day**	**3rd day**	**4th day**	**5th day**	**At senescence**
No pulsing + No cold storage		*Alternaria alternata* *Dreschlera specifera*	*Cladosporium oxysporum* *Altenaria alternata* *Dreschlera specifera*	*Cladosporium oxysporum* *Alternaria alternata* *Dreschlera specifera* *Staphylococcus aureus*	*Aspergillus flavus* *Alternaria alternata* *Cladosporium oxysporum* *Dreschlera specifera*	*Streptomyces albus* *Dreschlera specifera* *Cladosporium oxysporum* *Alternaria alternata* *Aspergillus flavus* *Staphylococcus aureus*	*Streptomyces albus* *Dreschlera specifera* *Cladosporium oxysporum* *Alternaria alternata* *Aspergillus* *Talaromyces* *Acremonium species*
Pulsing with distilled water for 24 hours + No cold storage	*Aeromonas hydrophilla*	*Aeromonas hydrophilla* *Vibrionaceae* *Staphylococcus warneri*	*Shewanella putrefaciens* *Salmonella species* *Vibrionaceae*	*Penicillium species* *Shewanella putrefaciens*	*Aspergillus niger* *Penicillium species*	*Aspergillus niger* *Penicillium species* *Alternaria alternata*	*Aspergillus niger* *Penicillium species* *Cladosporum oxysporum* *Aspergillus flavus*

			Aeromonas hydrophilla	*Salmonella species Vibrionaceae Aeromonas hydrophilla*	*Staphylococcus warneri*	*Aspergillus flavus Staphylococcus warneri*	
Pulsing with 600 ppm 8-HQS plus 5% Sucrose + No cold storage	*Proteus vulgaries*	*Proteus vulgaries Serratia mercescens Pantoea agglomera Bacillus species*	*Staphylococcus aureus Bacillus species Proteus vulgaries Serratia mercescens Pantoea agglomerans*	*Pseudomonus putida Pantoea agglomerans Bacillus species Aspergillus species Ulocladium Staphylococcus aureus*	*Emericela nidulans Ulocladium Aspergillus species Trichophyton interdigitale Pseudomonus putida*	*Trichophyton interdigitale Trichophyton verrucosum Acremonium* species *Ulocladdium E. nidulans*	*Syncephalastrum Trichophyton verrucosum Epidermophyton Nigrospora E..nidulans, Acremonium species Ulocladium*
Pulsing with distilled water + Cold storage for 1 day	*Bacillus* specis	*Bacillus* specis	*Serratia mercescens Bacillus* specis	*Penicillium* species *Bacillus* specis *Serratia mercescens*	*Penicillium* species *Pantoea agglomerans Serratia mercescens*	*Penicillium species Aspergillus flavus Alternaria alternata*	*Penicillium secies Aspergillus niger Alternaria secies Aspergillus flavus Acremonium species*

						Acremonium species	
Pulsing with 600 ppm 8-hqs plus 5% Sucrose + Cold storage for 1 day	*Serratia mercecens*	*Serratia mercecens* *Pantoea agglomerans*	*Serratia mercecens* *Pantoea agglomerans*	*Aspergillus fumigatus* *Serratia mercecens* *Pantoea agglomerans*	*Aspergillus fumigatus* *Serratia mercescens* *Pantoea agglomerans*	*Aspergillus fumigatus* *Serratia mercecens* *Pantoea agglomerans* *Rhizopus speciesx*	*Aspergillus fumigatus* *Rhizopus species* *Talaromyces* *Aspergillus parasiticus*
Pulsing with distilled water + Cold storage for 2 days	*Hafnia alvei*	*Hafnia alvei*	*Hafnia alvei*	*Hafnia alvei*	*Hafnia alvei* *Penicillium species*	*Alternata alternaria* *Aspergillus niger* *Aspergillus flavus*	*Alternaria alternate* *Aspergillus niger* *Alternaria secies* *Aspergillus flavus* *Acremonium species*

Pulsing with 600 ppm 8-HQS plus 5% Sucrose +Cold 2 days storage	*Staphylococcus aureus*	*Alburgo species Staphylococcus aureus*	*Alburgo species Staphylococcus aureus*	*Alburgo species Aspergillus* species *Staphylococcus aureus*	*Alburgo species Aspergillus* species	*Alburgo* species *Aspergillus* species	*Alburgo* species *Aspergillus* species *Alternaria* species
Pulsing with 600 ppm 8-HQS plus 5 % Sucrose + Cold storage for 3 days	*Pantoea agglomerans Proteus vulgaries Staphylococcus species*	*Pantoea agglomerans Proteus vulgaries Staphylococcus species*	*Alternaria* species *Proteus vulgaries Staphylococcus species*	*Acremonium species Paecilomyces Phytophthora Alternaria species*	*Alternaria* species *Acremonium* specie*s* *Paecilomyces Phytophthora*	*Alternaria* species *Acremonium species Paecilomyces Phytophthora*	*Altanaria species sAcremonium species Paecilomyces Phytophthora Paecilomyces species*
Pulsing with 600 ppm 8-hqs plus 5 % Sucrose + Cold storage for 4 day	*Bacillus* species	*Bacillus* species	*Bacillus* species *Cladosporium*	*Bacillus* species *Cladosporium*	*Bacillus* species *Cladosporium*	*Rhizopus Aspergillus nidulans Cladosporium*	*Rhizopus Aapergillus nidulans Aspergillus flavus Cladosporium sp.*

Pulsing with 600 ppm 8-hqs plus 5% Sucrose + Cold storage for 5 days	*Staphylococcus aureus*	*Madurella Tricothecium roseum Exophiala jeanselmei Staphylococcus aureus*	*Madurella Tricothecium roseum Exophiala jeanselmei Staphylococcus aureus*	*Madurella Tricothecium roseum Exophiala jeanselmei Staphylococcus*	*Zygomycete Madurella Tricothecium roseum Exophiala jeanselmei*	*Lichen Madurella Tricoth,ecium roseum Exophiala jeanselmei Zygomycete*	*Madurella Tricothecium roseum Exophiala jeanselmei Zygomycete Lichhen Madurela spp.*
Pulsing with distilled water+ Cold storage for 5 days	*Fusarium*	*Fusarium Exophialla*	*Fusarium Exophialla*	*Fusarium Exophiala*	*Fusarium Exophialla*	*Fusarium Exophiala jeansmei Cephalotricum*	*Cephalotricum Fusarium Exophiala jeanselmei Candida albicans Tricothecium*

4.4.3 Inluence of pulsing and wet cold storage on subsequent microbial proliferation in vase water of cut *Gladiolus* (*Gladiolusgrandiflorus* L. cv. 'Fado')

In this experiment the numbers of quantified microbes were significantly different (P = ≤ 0.0001) in vase water of *Gladiolus* spikes pulsed with 600 ppm 8 – HQS plus 5% sucrose solution compared with the control. The initial microbial load from vase water of the control washigher (2.22 ± 0.007 log10 cfu ml $^{-1}$) and significantly different (1.93 ± 0.007 log10 cfu ml^{-1}) from that of 600 ppm 8-HQS plus 5% sucrose pulsed unstored spikes (**Table 4.16**). The lowered microbials enumerated in *Gladious* spikes pulsed with 600 ppm 8 – HQS plus 5% sucrose solution may be responsible for enhanced vase life (8.75 days) compared with that control (4.75 days) for unstored spikes. The microbial proliferation on the third day in the vase for the control was higher (3.387 ± 2.028 log cfu ml^{-1}) compared with the unstored *Gladiolus* pulsed with 600 ppm 8-HQS plus 5% sucrose whose microbial load was 3.208 ± 2.028 log cfu ml^{-1}. This observation is comparable to other experiments invoking the biocidal influence of preservatives and pulsing agents to inhibit microbial proliferation in the stems and / or vase water of many cut flower and foliage plant species (Damunopola & Joyce, 2008;Idris *et al*., 2012; Naing *et al*., 2017; Wijayabandaria *et al*., 2018). As shown in **Table 4.16**, wet cold storage duration significantly affected microbial proliferation (P value < 0.0001) in the vase water of the spikes pulsed with 600 ppm 8–HQS plus 5% sucrose solution compared with the control.

The interactive effect of the pulsing treatment and the cold storage duration also significantly (P value < 0.0001) affected the number of colony forming units in the *Gladiolus* vase water. The pulsing solution negatively affected the mean (3.208 ± 2.028 log cfu-ml^{-1}) microbial counts, compared with the control (3.3± 2.028 87 cfu ml^{-1}) on the third day of the vase study. The same trend was observed on the third day of the the vase life in the microbial counts from vase water of cut *Gladiolus* pulsed with 600 ppm 8 – HQS plus 5% sucrose solution **(Table 4.16**) and wet cold stored for upto 4 days before display life. However, *Gladiolus* cut flowers subjected to 5 days' wet cold storage before vase study had higher mean microbial counts compared with the control (4.720 ± 2.028 log cfu-ml^{-1}; 3.387± 2.028 log cfu ml^{-1}) respectively. The pulsing treatment of 600 ppm 8-HQS plus 5% sucrose, the wet cold storage duration and their interactive effects significantly (P value 0.020; < 0.000 and < 0.0001 respectively) affected the means of the colony forming units in the vase water of cut *Gladiolus* at senescence (**Table 4.16**). The cut *Gladiolus* pulsed with 600 ppm HQS plus 5% sucrose and not stored had lower microbial load (6.093 ± 3.008 log cfu-ml^{-1}) in comparison with the

control(6.738 ± 3.008 log cfu ml^{-1}) at senescence. The same trend at senescence was observed in the mean colony forming units in vase water of *Gladiolus* pulsed with 600 ppm HQS plus 5% sucrose and wet cold stored for 2,3,4 and 5 days before the study of vase life at ambient temperatures.

Spikes wet cold stored for one day after the pulsing treatment of 600 ppm HQS plus 5% sucrose had a higher bacterial load (7.25 ± 3.008 log cfu ml^{-1}) in comparison with that of the control (6.738 ± 3.008 log cfu ml^{-1}) (**Table 4.16., Plate 4.3**) but still depicted a better vase life (10.25 ± 0.21 days). This could imply that the rate of microbial proliferation may not be the sole motivating factor behind the postharvest performance of cut *Gladiolus grandiflorus* L. cv. 'Fado'. The use of the pulsing treatment of 600 ppm HQS plus 5% sucrose instead of distilled water invariably increased the vase life of the cut *Gladiolus* compared to the control (**Table 4.16**). In this study, it was observed that irrespective of the mode of pulsing undertaken, the microbial load in spikes wet cold stored for 5 days before vase study was invariably lower at senescence compared with the control (**Table 4.16**). This could be attributed to the inhibitory effect of lowered temperatures on microbial proliferation coupled with competition for available nutritive resourses. It is presumed that the enhanced vase life could be associated with improved keeping quality of the dual effects of HQS in enhancing water uptake and biocidal roles that minimize plant xylem vessel emboli. These results are in agreement with the work done on cut tuberose (*Polianthes tuberosa* L) in which keeping quality of the flowers significantly decreased as a result of cold storage without application of a preservative treatment (Waithaka *et al*., 2001).

Moreover, a study on cut rose cv. First Red showed that the use of a carbohydrate source without a biocide increased the bacterial growh in the vase solution (Bhaskar *et al*, 2017). This observation is in contrast to another reported study done on cut *Gladiolus* cv. American Beauty in which spikes pulsed with different concentrations of sucrose elevated their vase life. Comparable results attained by the use of 300 mg l^{-1}aluminium sulphate solutiom showed the complete inhibition of microbial growth upto the fourth day of the vase life of cut rose (*Rosa* x *hybrida* cv. Cherry Brandy) (Jowkar *et al*., 2012). According to a study done on cut rose cvs. Akito and Grand Prix the cold storage treatment only affected stomatal opening in the former cultivar, and not the latter and it was further postulated that bacterial contamination was not related to lowered temperatures (Woltering & Pililart, 2018). Asimilar observation done on cut gerbera flowers indicated that bacterial interactions in the postharvest life was strongly genotype dependent (Schouten *et al*., 2018). Studies done on cut *Dianthuscaryophyllus* L. cv. Meddei; *Iris* L; *Alstromeria* L. and *Tulipa* L. highlighted their ability to tolerate bacterial counts

of upto 10^8 cfu ml^{-1} which negated the postulation that wilting and shortened vase life in these cut flowers was primarily due to these microbial interactions. Roses flowers inoculated with 10^7 cfu ml $^{-1}$ of several different strains of bacteria reduced the water intake on the first day in the vase (De Witte & Van Doorn, 1988). Another study on cut rose flowers found that their vase life was reduced by concentrations of bacteria as low as 10^5 cfu ml $^{-1}$counts (Put & Jansen, 1989). Other studies done on the interaction ot microorganisms and and cut flowers have pointed to the positive effects of isolates such as *Pseudomonas putida* and *Enterobacter cloaca* which increased the vase life of cut carnation cv. Omea flowers (Naing *et al*., 2017). Biological control of soil borne plant pathogens in the rhizosphere by the use of bacteria has been repoterd (Weller, 1988).

Other bacterial species that have been employed in biocontrol of plant are *Pseudomonas flourescens*, *Bacillus subtilis, Serratia liquefaciens and Hafnia alvei* among others (Snen *et al*., 1995; Weller, 1988). The addition of probiotic bacteria to vase solution can improve the vase life of cut flowers and plants for they exert positive effects by preventing growth and proliferation of phytopathogensvia: production of toxic metabolites, competing for nutrients and trigerring defense-response – related genes (Carlson, 2014; Gao, 2012). Salts of hydroxyquinoline used as cut flower preservatives have been reported to enhance water uptake and inhibit proliferation of microbes such as bacteria and fungi (Nabigol *et al*., 2014; Saader *et al*., 2020). A similar study done on cut rose (*Rosa hybrida* CV. Black magic) showed improvement in vase life of the flowers preserved in a solution of 200 gl^{-1}sucrose plus 200 ppm HQS (Sudaria *et al*., 2017). Pulsing of cut *Gladiolus* (*Gladiolus grandiflorus*) with 3% sucrose in combination with 250 ppm silver nitrate solution improved the post harvest qualities in two cultivars (Alexandria and White prosperity) under study (Manzoor *et al*., 2018).

A study done on the effect on the postharvest life of cut *Zinnia elegans* L. "Benery" Giant Wine by pure isolates of bacteria from the stems showed differential results depending on the species, incidentally *Escherichia coli* (*E.coli* 12 and *Pseudomonas fulva* improving their vase life. ThepH (2.78) of the pulsing solution of 600 ppm 8 – HQS plus 5% sucrose solution used in this study may have played a role in lowering microbial growth on the third day in the vase. Lowered pH of substrate composition was hypothesized to be among the factors that led to reduced growth of *Botrytis*propagules in a study on *Fusarium* wilt in carnation cut flowers (Duskova and Prokinova, 1989). Low pH (3.0 – 4.0) was used to improved the vase life of some cut flowers such as Rosa L., *Dendranthema* L, and *Helianthus* L. (Carlson & Dole, 2013; Stevens *et al*., 1993; Regan &Dole, 2010). However, lowering the pH of cut *Zinna* flowers did not improve the vase life of cut *Zinna elegans* L.'Benary" Giant Wine flowers. (Carlson &

Dole, 2013). While not all bacteria are killed by lowerd pH,there are other advantages associated with acidified conditions which include the prevention and repair of xylem air occlusion and promoting higher stem hydraulic conductance (Carlson & Dole, 2013; Hosein *et al*. 2011: Marousky, 1971; Sperry *et al*., 1988). While treatments constituting of a biocide, a carbohydrate source and acidifier solutions improve the vase life of commercial cut flowers, a case study on Alexandria and White prosperity cultivars of *Gladiolus grandiflorus'* differential vase life results to such preservatives is a pointer to the molecular involvement aspect apart from pH and microbial load among others (Carlson & Dole, 2013; Manzoor *et al*., 2018).

Table 4.16: Quantification of microorganisms in vase water of cut gladioli (*Gladiolus grandiflorus* L. cv 'Fado') as affected by wet cold storage and pulsing with 600 ppm 8- HQS plus 5% Sucrose

Days of storage	**Initial log 10 cfu ml^{-1}**		**Third day in vase log 10 cfu ml $^{-1}$**		**Senescence cfu ml $^{-1}$**		**Vase Life (days)**	
	Non-pulsed	Pulsed	Non-pulsed	Pulsed	Non- pulsed	Pulsed	Non- pulsed	Pulsed
0	2.228^{e}	1.935^{g}	3.387^{bcd}	3.208^{cd}	6.738^{abc}	6.093^{bcde}	4.75^{c}	8.75^{b}
1	2.215^{e}	1.833^{h}	2.584^{ef}	3.539^{bc}	5.579^{def}	7.255^{a}	9.50^{ab}	10.25^{ab}
2	2.178^{ef}	1.925^{g}	2.359^{ef}	2.215^{f}	6.784^{abc}	6.402^{abcd}	9.50^{ab}	10.00^{ab}
3	1.253^{i}	2.102^{f}	4.696^{a}	3.251^{bcd}	7.103^{ab}	5.331^{ef}	9.75^{ab}	11.50^{a}
4	2.46c	2.336^{d}	3.696^{b}	2.637^{e}	5.914^{cdef}	4.973^{f}	11.00^{ab}	11.25^{ab}
5	2.734^{b}	3.264^{a}	3.097^{d}	4.720^{a}	5.258^{ef}	5.506^{def}	10.25^{hi}	10.25^{ab}
Means	2.178^{a}	2.232^{b}	3.288^{a}	3.261^{a}	6.229^{a}	5.926^{b}	9.13^{a}	10.33^{b}
CD at 5%	0.007	0.007	2.028	2.028	3.008	3.008	0.21	0.21
Pulsing (P)	<.0001		0.5453		0.0209*		0.003	
Period of Storage	<.0001		<.0001*		<.0001*		<.0001*	
Treatment * Storage	<.0001		<.0001*		<.0001*		0.0041	

Means followed by the same letter within evaluation period are not significantly different according to Tukey's test at 5 % level of significance

4.4.4 Effect of pulsing with 600 ppm 8-HQS plus 5% Sucrose and storage duration on subsequent bacterial isolates from vase water of cut *Gladiolus*(*Gladiolus grandifloru*s L. cv. 'Fado')

The bacteria isolated from the vase solution were characterized using their morphological appearance on nutrient agar and on selective and differential media (**Plates 4.3 and 4.4).** The bacteria isolated were further subjected to the Gram's stain before subjecting them to the VITEK machine. Gram negative bacteria isolated included *Serratia marcescens*,*Pantoear agglomerans*, *Hafnia* a*lvel*, *Pseudomonas putida,*Gram positive *Bacillus* species, *Shewanella putrefaciens*, *Aeromonas hydrophilla*and *Salmonella species*. The gram positive bacteria isolated included S*taphylococcus warneri* and *Stahylococcus aureus* (**Plate4.3**). These were after isolates from vase water of cut *Gladiolus* spikes previously pulsed with 600 ppm 8- HQS plus 5% sucrose and wet cold stored for 3 days before vase study; grown for 24 hours on at 27± 2 ºC. The medium of growth was cysteine lactose electrolyte deficient (CLED) agar. Among these bacterial species, *Pseudomonas putida*, *Serratia mercescen*s *Haffniaalvei*, *Pantoea agglomerans* and gram positive *Bacillus*have been used in biological control of plant pathogenic microorganisms (Gunes, 2015: Lecomte e*t al*., 2016; Opelt 2007; Shariati *et al*., 2017; Sneh, 1985; Tan *et al*., 2018).

Staphylococcus warneri is one of the endophytes that resides in rice tissues without causing any evident damage on the host and also plays a crucial role in plant growth, development and protection (Chaudhry*et al*., 2017).*Staphylococcus, Hafnia, Yersinia and Pantoea* genera endophyte isolates from the bryophyte species of *Sphagnum* (*S. magellanicum* and *S. fallax*) had antagonistic activity against soil-borne fungi *Verticillium dahlia* and *R. solani*(Opelt, 2007). Different species of bacteria isolated from vase water and stems of cut flowers have different impacts on the postharvest life of ornamental plants (Carlson, 2014).The interaction of bacteria with cut flowers is dependent on the flower genotype and also the temperature of the vase water relative to the atmospheric temperature (Schoulten *et al*., 2018).

The performance of cut rose cv. Akito and Grand Prix after cold storage indicated that bacterial contamination was not the reason for flower failure (Woltering & Pillart, 2018). Indeed, some bacteria serve to promote plant growth by resisting pathogenic bacteria, fungi and viruses (Weller, 1988). For instance, *Pseudomonas putida* and *Enterobacter cloaca* isolated from cut carnation cv. Omea actually increased the vase life of these flowers (Nang *et al*., 2017). Probiotic bacteria are known to exert positive effects by preventing growth and proliferation of phytopathogen producing toxic metabolites, competing for nutrition and

colonization sites thereby triggering defense –related genes in plants (Gao *et al*., 2012).It has been hypothesized that bacterial products rather than their live cultures play effective role on plants' quality (Carlson, 2014). It could be postulated therefore that the bacteria species *Serratia marcescens*, *Pantoea agglomerans*, *Hafnia* a*lvel*, *Pseudomonas putida,* Gram positive *Bacillus* species and *Aeromonas hydrophilla*may have had beneficial effects on the quality of cut *Gladiolus*. It is reported that bacteria isolates belonging to the genera of *Acinetobacter, Bacillus and Pantoea* populations of upto 10^5 cfu g^{-1} stem weight caused the stem break in *Gerbera jamesonii* cut flowers (Belestra *et al*., 2005).

The slight yellow colonies grown on cysteine lactose electrolyte deficient agar (CLED) could either belong to the bacteria *Escherichiacoli* or *Staphylococcus aureus*. CLED agar supports growth of both Gram positive and Gram negative bacteria, on which *E. coli* presents with large elevated yellow colonies; while *Staphylococcus aureus* has deep yellow colonies(Falton *et al*., 2002).However, for proper identification of the bacteria, growth on mannitol salt agar (**Plate 4.3**) showed colonies characteristic of *Staphylococcus aureus.* Only *Staphylococci* bacteria are able to grow on mannitol salt agar which inhibits the growth of most other bacteria because of its constituent 7.5% Na Cl (sodium chloride) (Shittu *et al*., 2006). The acid produced from mannitol, the sugar alcohol of mannose results into change of colour of the phenol red indicator from pink to yellow.The colonies isolated were further subjected on the Gram's staining and they retained the initial cystal violet stain(Beveridge, 2001). So the colonies in **Plate 4.3)** could be presumed to be those of *S. aureus.*The inclusion of 8-Hydroxyquinoline sulphate, one of the derivatives of 8-hydroxyquinoline in the pulsing solution before wet cold storage may have hindered the biofilm formation of *Staphylococcus aureus*. It is also indicated in another study that tannic acid from tea plant extract inhibited aggregation of *Staphylacoccus* bacteria thus minimized its virulence (Payne, 2015),

It is documented that 8- hydroxyquinoline is effective against several bacterial pathogens including *Clostridium difficile*, *Enterococcus faecalis* and *Staphylococcus aureus*(Novakova *et al.,*2014:Prachayasittikul *et al*.,2014; Srisung *et al*., 2013).This could be the reason behind improved vase life in spikes pulsed with 600 ppm plus 5% sucrose versus distilled water (11.50 ±0.21 days versus 9.75 ±0.21 days) before wet cold storage duration of three days prior to display (**Table 4.17)**.

The capability of the animal pathogen *Staphylococcus aureus* being infective to plants was positively demonstrated using the model plant Arabidopsis in which the same modes of virulence and pathogenicity were shared between both kingdoms (Prithviraj *et al*., 2005). It is envisaged that the virulence of this Gram positive cocci could have affected the quality of the

cut *Gladiolus* spikes. The source of bacteria isolated from cut rose flowers was suspected to be tap water (van Doorn & de Witte, 997). *Proteusvulgaris*that shows good growth after 24 hours on CLED agar (**Plate4.3**) and *Salmonella*species wereisolated from vase water of *Gladiolus* pulsed with 600ppm 8 HQS plus 5% sucrose priorto display may have originated from irrigation or surface water during cultivation (Liu *et al*., 2018).This could have been the case in the field grown *Gladiolus* in this study, where surface run-off rain and irrigation water sources were used. Contaminated soil may have been the source of *Salmonella entericca* isolated from tomatoes (Barak & Liang, 2009).

Bacteria belonging to Enterobactericeae (e.g. *Salmonella* and *E. coli*) can survive in diverse environments including their interaction with plants as their alternative hosts (Ávila-Quezada *et al*., 2010). The Gram negative *Bacillus* rod (*Salmonella*) has pathogenic tendency towards both plants and animals by employing the same mechanisms of infection (Schikora, 2011). Necrosis and water soaking induced by pathogens increses the plant pathogenesis by *Salmonella enterica* (Neha *et al*., 2015). *Salmonella enterica* serovar *Typhymurium* which is a human enteric pathogen has the ability to survive endophytically in plants (Chalupowiz *et al*., 2018).Two strains of *Proteus vulgaris* isolated from tea plantations had fungicidal and insecticidal properties (Barthakur & Bezbaruan, 1999). It can be hypothesized that the interactive effect of *Proteus vulgaris* from vase water of cut *Gladiolus* pulsed with 600 ppm 8-HQS and wet cold stored for three days before the study may have affected the quality of these flowers. The Genus *Proteus* is widely distributed in the environment, including polluted water, soil and manure (Kadhim*et al*., 2016).*Aeromonashydrophila* (**Plate 4.3**) a member of the family *Vibrionaceae* was isolated from the vase water of cut *Gladiolus* that were pulsed with distilled water but not subjected to the wet cold storage treatment before vase study. TheseGram negative rods have previously been isolated from aquatic sources including tap and mineral water (Scoaris *et al*., 2008).

The possible source of the *Aeromonas hydrophila* isolated from the vase water solution of the cut *Gladiolus* could be the water used in keeping the flowers.*Aeromonas hydrophila* was originaly associated with its pathogenesis of cold-blooded animals and was isolated from soil and water; but now its virulence in humans is documented (Gilard, 1967; Hasan *et al*., 2013). *Aeromonas hydrophila* can grow in both aerobic and anaerobic environments. These bacteria are also capable of resisting cold temperatures, refrigeration and chlorine.

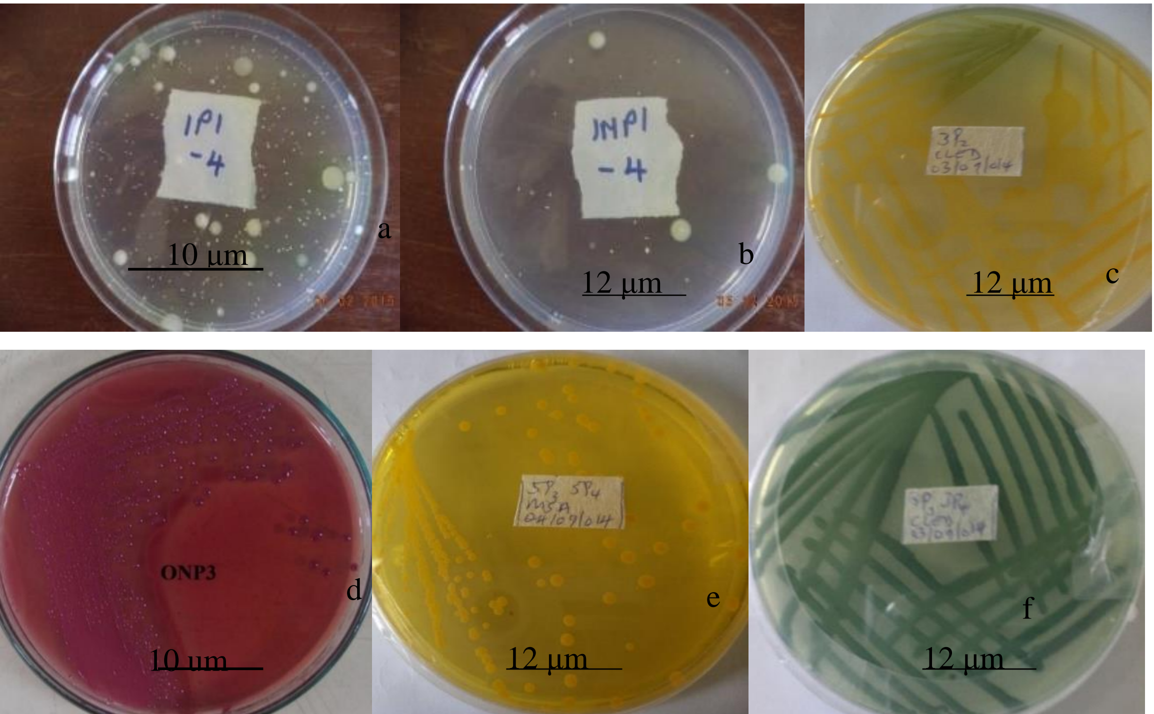


Plate 4.3: Colony forming units on nutrient agar isolates at senescence from vase water of *Gladiolus* cold stored for one day after pulsing with 600 ppm 8-HQS plus 5% sucrose (a) and disilled water (b) respectively., Yellow colonies of *Staphylococcusaureus* on CLED agar (c)., *Aeromonas hydrophila* on deoxycholate hydrogen sulphide agar (d)., *Staphylococcus aureus* yellow colonies on mannitol salt agar (after 24 hours growth at 27 ±2 °C)., (d) *Staphylococcus aureus* colonies on CLED agar (e) and blue-green colonies of *Proteus vulgaries* after 27±2 °C on CLED.

Another Gram negative bacterium, *Pantoea agglomerans*, (**Table 4.17.,Plate 4.4 (a)**) was isolated in *Gladiolus* vase water after pulsing treatment with 600 ppm 8- HQS plus 5% sucrose before wet cold storage of 1 and three days respectively prior to flower display at ambient temperatures. It is reported that a strain of *Pantoeaagglomeran*s that was used to control bacterial diseases on the onion plant.*Aeronomads* have been isolated from myriad water bodies including chlorinated water, drinking water and water distributing systems among other other sources (Sadik *et al*.,2016; Tomás, 2012). Gram negative reaction of a bacterium isolated in vase water that has comma shaped form probably belongs to the *Vibronacea*e group (**Plate4.4 (b)**).

Differantiation between *Entobactericeae* bacteria was done using the biochemical characterization (**Plate 4.4**). The growth of *Aeromonas hydrophila* on Triple Sugar Iron (TSI) agar gave results of of an acidic slant and acidic butt, (A/A) showing fermentation of glucose

plus lactose and / or sucrose. Neither hydrogen sulfide nor CO_2 /H_2 gas production was observed. This bacterium isolate also gave a positive reaction with the Voges – Proskauer (VP) test indicating fermentation of sugars via the butanediol pathway leading to production acetoin, which in the presence of KOH and under catalysis of α- naphthol reacts with guanidine associated with molecules contributed by peptone in the medium forms a pinkish-red-coloured complex. *Aeromonas hydrophila* alsoshowed capacity to utilize citrate as the sole carbon source (**Plate 4.4**).

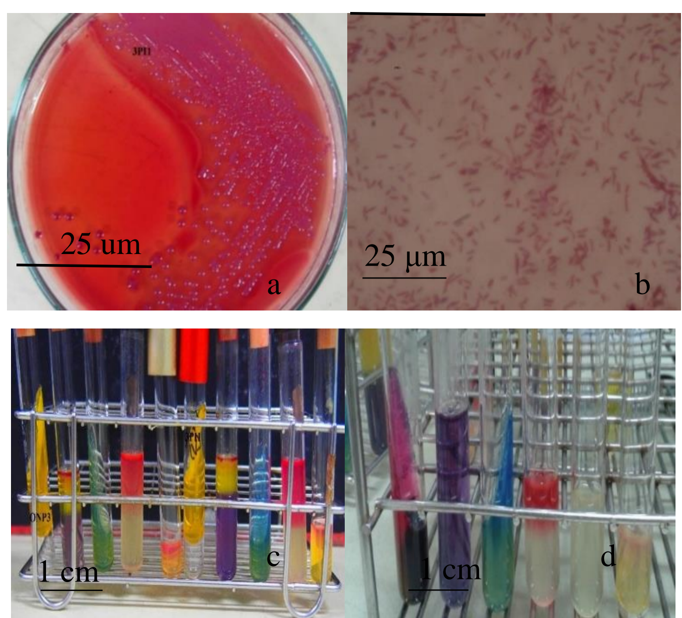


Plate 4.4: Pinkish colonies with a red center (a)., Gram negative curved rod bacilli (probably a member of Vibronaceae (b)., duplicate biochemical tests of TSI, IMVIC reactions for *Aeromonashydrophila* bacteria (c). and TSI, lysine decarboxylase, citrate, indole production and VP tests for *Salmonella* species (d).

This bacterium gave a positive indole test and also a positive methyl red test. The Indole, methyl red, Voges-Proskauer and citrate reactions (IMVIC) are as shown in **Plate 4.4 c**). Similar results on the identification schemes based on biochemical characteristics on the Genus of *Aeromonas* have been rccorded (Abbott, 2003). These tests were however inconclusive in determining the *Aeromonad* species and strain. In this study, morphological, cultural and biochemical characterization tests were coupled with the biotyping of the isolated microbes using standard charts for identification. In this study isolated *Salmonella* grown on triple sugar iron showed an alkaline slant and an acid butt, H_2S production, gas in the butt, citrate positive, lysine decarboxylase positive, indole negative, Voges – Proskauer negative

and methyl red positive (**Plate 4.4**). Similar biochemical test results for *Salmonella* have been recorded (Hui, 2015).

These results were validated by the use of the Vitek 2 compact biomerieux model VK209938 analyzer for validation of the identification. The Vitek 2 analyzer has been used for identification of Gram negative bacilli and cocci; Gram positive cocci and bacilli; anaerobes and *Corynebacteria* and Neisseria and *Haemophilus* and yeast groups of microbes comprehensively. The same procedure was adopted for identification of the other isolated genera including *Salmonella.*This bacterium (*Pantoea agglomerans*) is also documented to be a clinically important plant pathogen, which also affects humans , and is found prevalent in the environment (Deletoile *et al*., 2009; Fujikawa & Akimoto, 2011; Geere, 1977; Rezzonico *et al*., 2009) *Pantoea agglomerans* is an epiphyte found living on plant surfaces and also an endophyte residing inside the plants (Dutkiewicz *et al*., 2016).

Table 4.17: Morphological characterization of bacteria using differential media

Code / Media	**SS**	**DHL**	**BA**	**Gram's stain**	**Species**
1P1	Non lactose fermenter	Non lactose fermenter, mucoid	Non – heamolytic	Gram's negative	*Pantoea agglomerans*
1P2	Late lactose fermenter	Non lactose fermenter	Non – heamolytic	Gram's negative	*Serratia mercecens*
2NP3	Non-lactose fermenter	Non-lactose fermenter	Non haemolytic	Gram's negative	*Hafnia alvei*
0NP0	No growth	No growth	Beta heamolytic	Gram's positive	*Staphylococcus warneri*
0P13	Lactose fermenter	Lactose fermenter	Non heamolytic	Gram's negative	*Serratia mercescens*
0NP1	No growth	No growth	No growth	N/A	N/A
0P11	No growth	No growth	No growth	N/A	N/A
5P4-5P5	No growth				*Staphylococcus aureus*
3P11	Non-lactose fermenter	Non-lactose fermenter, mucoid	Non heamolytic	Gram negative	*Pantoea agglomerans*
0NP4	Non-lactose fermenter	Non-lactose fermenter	Non heamolytic	Gram's negative	*Pseudomonas putida*
4P11	No growth	No growth	Beta heamolytic	Gram's positive rods	*Bacillus* species
0P3	Black	Black	Haemolysis	Gram's negative	*Salmonella* species
0NP12	Black	Black	Alpha haemolysis	Gram's negative	*Shewanella putrefaciens*

Legend: First number refers to storage duration in days; letters N and P refer to pulsing for 24 hours in distilled water and 600 ppm HQS plus 5% sucrose respectively

Table 4.18: Macroscopic and microscopic identification of fungi isolates from *Gladiolus* vase solution after 7 days growth on potato dextrose agar at 28 ±2 °C

Isolate	Front surface	Reverse surface	Diameter (mm)	Microscopic appearance	Inference
1P13	Suede –lke white to pink conidia with green spots	Creamy yellow to red; diffusible wine red pigment	4.8 – 7.3	Hyaline smooth biverticillate terminal verticils of three to four metulae, bearing three to seven phialides	*Talaromyces* species (Tsang *et al*., 2018)
2P12	Striated blue green conidia with white margin	Wrinkled orange reverse	2.5 – 5.3	Charactteristic globular oospores oogonia and hyphae	*Albugo* species (Pandey *et al*., 2013)
3P3	Blue-green concentric rings, grayish to olive conidia with white margin	Yellow green reverse to brown	7 – 8	Branched acropetal chains of dictyoconidia produced from elongate conidiophores	*Altanari*a species (Woudenbert *et al.,* 2013)
ONP15	Blue green conidia, white margin mycelia	Cream to light yellow	4.5 – 5	Biverticillate,erect conidiophore; phialides producedfrom branched metulae, single –celled basocatenate conidia	*Penicillium* species (Ellis *et al*, 2007)
5P2	Yellowish – brown colonies with brown diffusible pigment dearing white margin mycelia	Cream to pink	4.8 – 5.5	Both simple and branched conidiophores; pyriform conidia with truncated bases	*Madurela* species (Kidd *etal*., 2016)

Continuation Table 4.18

Isolate	Front surface	Reverse surface	Diameter (mm)	Microscopic appearance	Inference
3P17B	Dense blue green conidia with concentric rings	Yellow green reverse with concentric rings	Full plate-8.5	Club –shaped anthredia; aseptate hyphae, terminal pyriform sporangium	*Phytophthora* species (Christova *et al*., 2018)
OP15B	Densely packed blue green conidia	Dark brown to black	4.0 – 4.5	Conidiophores reduced to conidiogenous cells that are monoblastic, discrete, solitary , determinate and hyaline	*Nigrospora* species Abass &Mohammed, 2014
ONP1	Sparsely white colonies with brown spots	White reverse	1.0	Presence of tuberculate macroconidia with microconidia and mycelia could signify *Histoplasma* species	Probably belonging to *Sepedonium*; *Pythium* or *Histoplasma* species Kidd *et al*., 2016
3P15	Slow growing compact whitish-grey colonies	Brown reverse	1.4	Erect phialides with inconspicuous collaretes bearing hyaline one celled globose conidia clusterd at the apex	*Acremonium* species Webster & Weber, 2007

2P15	Plain green colonies. With red brown cleidtothecia developing within and upon the conidial heads	Brown reverse	3 -4	Biseriate spatulate vesicle, short brown stipe, green globose conidia, Hürle cells present	*Emericelanidulans* (*Aspergillusnidulans*) (Gautam & Bhadauria, 2012)
3P12	Blue-green colonies, white margin mycelia with concentric rings	Light brown to yellow with yellow margin	6 – 7	Erect conidiophores bearing branches with phialides' chlamydospores absent	*Paecilomyces* species
0NP16	Blue green colonies with yellow exudates, white margin mycelia	Wrinkled cream to yellow reverse	5.1 – 5.3	*Bru*sh - like verticillate head	*Penicillium* species (Mushimiyimana *et al.*, 2016)
1P16	Wrinkled blue green conidia with white margin mycelia	Cream to yellow with yellow margin, wrinkled	4.9 – 5.2	Brush – like head	*Penicillium* species (Ilhan *et al.,* 2006)
2P6	Brown – reddish with creamish – yellow margin, pink pigmentation	Pink to red reverse; pink effusion	5.5	Broad hyphae; clamp connections	Unidentified filamentous fungus
0P16	Greenish-brown colonies with a suedel like surface; raised and folded in the center, flt periphery and submerged fringe of growth	Dark brown to almost black; yellowishish-brown reverse pigmentation	4.7 – 6.2	Smooth, thin –walled macroconidia growing directly from the hyphae	*Epidermophyton*

0P18	Fast frowing cottony to fluffy , white to light grey colonies	Red exudate on reverse	4.5 – 5.1	Erect-stolon –like sporangiophore bearing merosporangia producing sporangiospores	*Syncephalastrum* species Hoffman *et al*., 2013)

Legend: First number refers to storage duration in days; letters N and P refer to pulsing for 24 hours in distilled water and 600 ppm HQS plus 5% sucrose respectively

4.4.5 Influence of pulsing and wet cold storage on the fungal isolates in vase water of cut *Gladiolus* (*Gladiolus grandiflorus* L. cv. 'Fado'

In this study, a fungus belonging to the genus *Puccinia* (**Plate 4.5**) was isolated from vase water of the cut *Gladiolus grandiflorus* cv. 'Fado'. The *Gladiolus* spikes had been pulsed with 600 ppm 8-HQS plus 5% sucrose and then cold stored at 3 ± 1°C for three days before vase study at ambient temperatures. This fungus is characterized for having broad hyphae, single and multicelled telliospores and aeciospores.

This fungus is an obligate rust causing plant pathogen which has multiple life stages including different spore types (Ellison, 2016). .*Puccinia* is listed among regulated plant pests (Cline & Farr, 2006). It is documented that the *Gladiolus* Genus is prone to infection by the rust fungi *Uromyces transversalis*which is closely associated with *Puccinia* species (Beilharz *et al*., 2001). This could be the isolate in **Plate 4.5** above which may have negatively affected the vase life and quality of cut *Gladiolus*. The species of*Puccinia pelargoni – zonalis* which causes geranium rust can bring about huge economic losses to commercial flower growers as a result of unmarkartable infected plants (Scocco, 2011). Another rust fungus, *Puccinia horiana*, causes major damage to *Chrysanthemum x morifolium* ornamental flowers (De Becker, 2011).

Fungi of the Genus *Puccinia* have been isolated from some ornamental plants (Trkulja *et al*., 2018). The macroscopic morphological features of *Puccinia* isolate are as shownin the plate above (**Plate 4.5**). This isolate was cultured from vase water of *Gladiolus* spikes pulsed with 600 ppm 8- HQS plus 5%sucrose solution and subjected to wet cold storage for three days prior to vase study. It is probable that these treatments did not deter the growth of this fungus.

The fungus, *Scopulariopsis* (**Plate 4.5**) was isolated from vase water of cut *Gladiolus* that were neither pulsed nor cold stored before vase study. The conidiophores of *Scopulariopsis* terminate into basocatenate annellidic conidia as shown in **Plate 4.5**. This fungus forms chains of conidia, which are hyaline and truncated.The isolate in this study had the chatacteristic greyish-brown colour of *Scopulariopsis* (**Plate 4.5** after seven days growth at 28 ±2°C) on Sabouraud's agar (SA). This fungus is cosmopolitan, found in indoor and clinical places (Swick *et al*., 2010; Woudenberg *et al*., 2017). Fungi belonging to this genus are saprobes commonly isolated from plant debris, soil, paper and indoor environments (Sandoval- Denis *et al*., 2013). The genus *Scopulariopsis* has its teleomorphs that belong to the genus *Microascus* (Sandoval-Denis *et al*., 2013). *Scopulariopsis gossypii* species of this genus is an opportunistic plant pathogen of cotton plants (*Gossypium hirsutum*) (Xiao-Lin *et al*., 2017). The fungus was found in the vascular bundles of cotton plant infected with *Verticillium dahlia*, in which severity of leaf interveinal chlorosis and vascular browning was witnessed.

The cotton plants that were not infected had no *Scopulariopsis* infiltration, hence the preposition of opportunistic attack. It is probable the *Scopulariopsis* species identified in the vase water could have had a negative impact on the vase life and quality of the cut *Gladiolus* spikes. Plant pathogens exploit the host plant by extracting nutrients from it and hence this could lower quality parameters such as fresh weight, dry weight and vase life of ornamentals (McDowell, 2011). The most common species of *Scopulariopsis* genus is *S. brevicaulis* whose colonies are tan with a brown center. The teleomorphs of the genus *Scopulariopsi*s are included in the genus *Microascus* (Braddly *et al*., 2000).These saprobes are commonly isolated from plant debris, soil , paper and indoor environments (Sandoval-Denis *et al*., 2013).

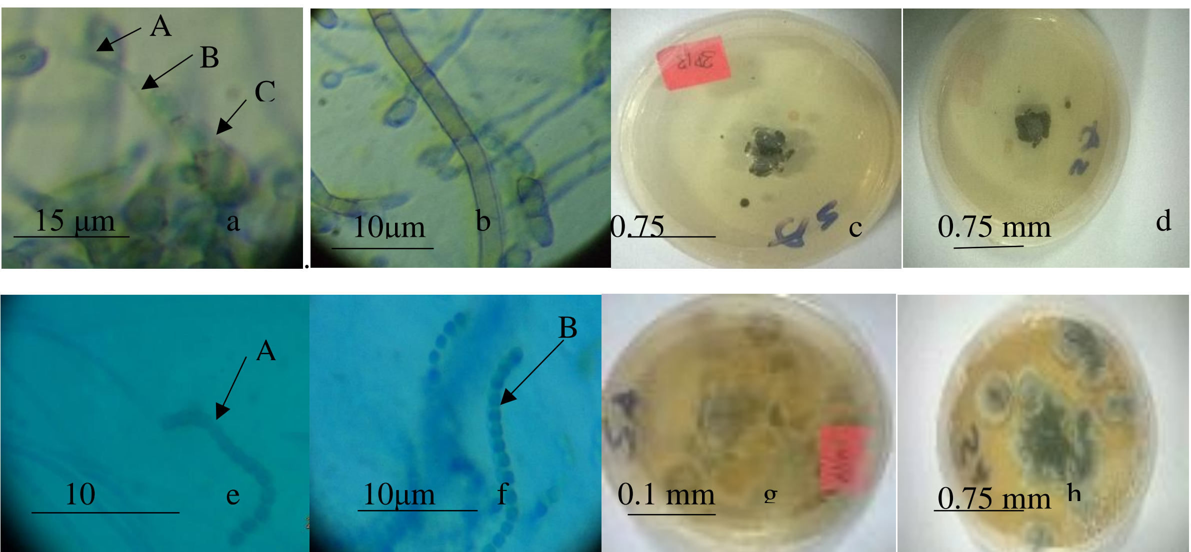


Plate 4.5: Basidiospore (pointed arrow from A); sterigma (poited arrow from B); hypobasidium (pointed arrow from C) of *Puccinia* species with reddish orange uredospores (a)., telliospores (arrow from A), aeciospores and broad septate hyphae (arrow B) of *Puccinia* (b)., front (c) and (d) the reverse surfaces of *Puccinia* species colonies on SA after seven days' incubation at 28 ± 2 ^{o}C., (e) *Scopulariopsi*s Bain and conidiogenous head (A)., (f) basocatenate annellidic conidia (B)., (g) the front and (h) reverse surfaces of *Scopulariopsis* species brownish grey colony on SA

The fungus *Aspergillus nidulus* (teleomorph *Emericela nidulans*); (**Plate 4.6** (**a**)) was isoalted from vase water of cut *Gladiolus* pulsed with 600 ppm 8- HQS but not subjected to any wet cold sorage treatment. (**Plate 4.6 (b)**)shows the teleomorph (*Emericela nidulans*)with abundant thick walled Hürlle bodies, yellowish soluble pigment and spherical conidia with

smooth hyphae on Sabouraud's media.The hyphal wall of *Emericella nidulans* has a characteristic yellow to buff colour after staining with lactophenol blue.**Plate 4.6 (c)** shows the anamorph, *Aspergillus nidulans* with hemispherical vesicle, mettulla and phialides. The metullae cover only the upper half of the vesicle. The conidia are smooth globose and green in colour.The cleistothecia is dull yellow. The fungus in this was an isolate from vase water of cut *Gladiolus* pulsed andwet cold stored for two days prior to vase study. It presented with characteristic*Emericella nidulans* deep green coloured conidia with cream / greyish reverse with brownish orange front (**Plate 4.6 (d., e)**) after 7 days'growth on SA at 28 ±2 ^{o}C. This fungus is a saprobe *Ascomycete* belomging to the *Aspergillus* genus of over 200 species (Taylor *et al*., 2009).

.*Aspergillus nidulans* (*Emericella nidulans,* the telemorph) is a potential phytopathogen owing to its production of plant cell wall degrading enzymes (de Vries*et al*., 2001). *Aspergillus nidulans* produces polygalacturonase and pectatenase enzymes that degrade the polysaccharides of the plant cell wall that enables penetration and colonization by the fungal pathogen (Dean & Timberlake, 1989). Since excised plant tissues inoculated with *A. nidulans* conidia developed necrotic water soaked lessions (Dean &Timberlake, 1989), it is probable that this effect in cut *Gladiolus*spikes under this study must have undermined the quality of the flowers.

Aspergillus nidulans isolates in this study were cultured on SA from vase water of *Gladiolus* spikes pulsed with 600 ppm 8- HQS plus 5% sucrose and immediately taken for vase study at ambient temperatures. Other studies indicated that inclusion of glucose and other sugars from wounded tissues repressed pectolytic enzyme production thereby deterring disease development in the plant (Dean &Timberlake, 1989). The inclusion of sugar in the pulsing solution could be the reason behind the improved vase life of the cut flowers compared with the control (8.75 ± 0.21; 4.75 ± 0.21 days respectively). The majority of species in the genus of *Aspergillus* are non-pathogenic (Paulussen *et al*., 2017). The smally percentage of *Aspergillus* species that are pathogenic to animals and plants include: *Aspergillus fumigatus*, *A. niger, A. nidulans, A. flavus and A, terreus* among others (Enock, 2006). Generally the *Aspergillius* are found growing on plants, decaying matter, in soils, in air/aerosols, in animal systems, in fresh and marine water systems (Cray *et al*., 2013). They have asexual conidia that readly become airborne, are highly stress tolerant and they produce environmentally persistent ascospores (Stevenson *et al*., 2015).

The spores produced in the environment are readily airborne due to their hydrophobicity and small size (Taha *et al*., 2005). *Aspergillus nidulans* is one of the moulds whose transmission

in controlled enironments such as hospitals with immunocompromised patiennts could be through substrates such as cut flowers (Lopez- Moya *et al.,* 2019).

Aspergillus niger **(Plate 4.6 (f))** on theother handis also widely distributed, and colonizes a wide range of substrates. This fungus microscopic appaearance depicts thick hyaline yellowish smooth walls that turn brownish at the apex. The vesicle is spherical and the conidia are globose, the metullae covering virtually the entire surface of the vesicle. It exists as a saprophyte on dead leaves, stored grain, compost piles and other decaying vegetation, *Aspergillus niger* is a common laboratory contaminant and it has been isolated in plant products such as smokeless cigaretes (Saleem *et al*., 2018). *Aspergillus niger* complex species have microscopic features that depict uniseriate and biseriate heads; black spherical to pyriform conidia (Kidd *et al*., 2016).

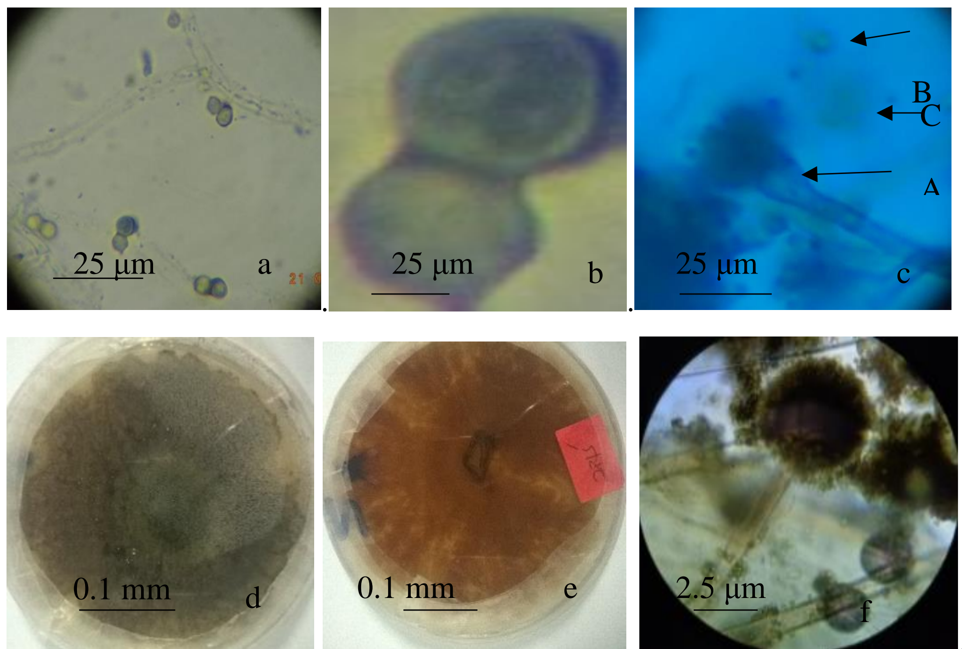


Plate 4.6: Two celled conidium of *Emericela nidulans* species stained with lactophenol blue dyea (a).,)., the dull yellow cleistothecia of *Emericela nidulans* and purple staining ascospores., (b)., *Aspergillus nidulans* pyriform vesicle (A), globose conidia (B) and cleistothecia (C)., (c)., reverse (d) and front surfaces of *Emericela nidulans* (e)., *Aspergillus niger* conidial head with spherical vesicle with globose conidia (f)

This fungus is termed as the black aspergilli, belonging to *Aspergillus* section Nigri (Varga *et al*., 2011). Morphological characterization of *Aspergillus niger* is hinged on its large biserita conidial head which is globose, dark brown radiating with phialides that are bourne on the metulae (Diba *et al*., 2007). *Aspergillus niger* has a variety of hydrolytic and oxidative enzymes which are involved in the breakdown of plant lignocelluloses (Sharma, 2012). It is a

plant pathogen of various plant species causing infections such as the black mould and rot diseases (Sharma, 2012). It was isolated from *Gladiolus* cold stored for 3 days after pulsing with 600 ppm 8 – HQS plus 5% sucrose solution before vase study at ambient temperatures. *Aspergillus niger* is associated with postharvest decay of various substrates hence its presence could have played a role in the vase life and quality of the cut *Gladiolus*(Perrone *et al*., 2007).

Aspergillus fumigatus. (**Plate 4.7 (a)**) was isolated from vase water of *Gladiolus* cut flowers pulsed with 600 ppm 8- HQS plus 5% sucrose solution and then wet cold stored for one day prior to vase study at ambient temperatures. The microscopic appearance has characteristic uiseriate row of phialides on the upper two thirds of the vesicle.This fungus encodes an external arsenal of degradative enzymes that makes it possible to grow on plant matter and in the environment (Taylor *et al*., 2006). This may explain it's ability to withstand the biocidal effect of the pulsing treatment containing 8- HQS.Possibility of cut flower contamination with aspergillosis causative fungi including *Aspergillus fumigatus* in controlled hospital enviroments has been indicated (Patterson *et al*., 2017; Peterson *et al.,* 2016).A study on clinical implications of the sources of pathogenic invasive aspergilli molds indicated fresh or dried flowers, potted plants and fruits as some of the possible substrates (Warris & Verweij, 2005).

In another study, azole resistant *A. fumigatus* was isolated from patiets with previous exposure to azole infested habitats such as flower beds, compost plant seeds among others. The pathogenicity nature of biofilm formation by the filamentous fungus *Aspergillusfumigatus* is inhibited by the polymer, chitosan (Lopez-Moya *et al.,* 2019).The hyphomycete in (**Plate 4.7 (c)**) could belong to the sub-genus Section *Nidulantes* because of the existence of a short stipe that is brown in colourand smooth-walled. The genus is characterized by the possesiion of green colonies with a reverse that could be olive to drab-grey or purple in colour (Kidd *et al*., 2016). Aspergilli of sections Flavi, *Fumigati* and *Nidulantes* have characteristic yellow green, green and deep green colonies. The aspergilli in section *Ndulante*s include *A. unguis*, *A.rugulovalvus*, *A. sydowii* and *A. tetrazonus* (Nyongesa *et al*., 2015). Fungi in the section *Nidulantes* have biseriate conidial heads and they may be pigmented (Al-Sheikh & Abdelzaher, 2012). Some species may contain pigmentation, red ascospores and ascomata (Chen *et al*., 2016). Sequencing of the internal transcribed spacer region (ITS) is sufficient in identification of the section *Nidulantes* (Ellis *et al*., 2007).

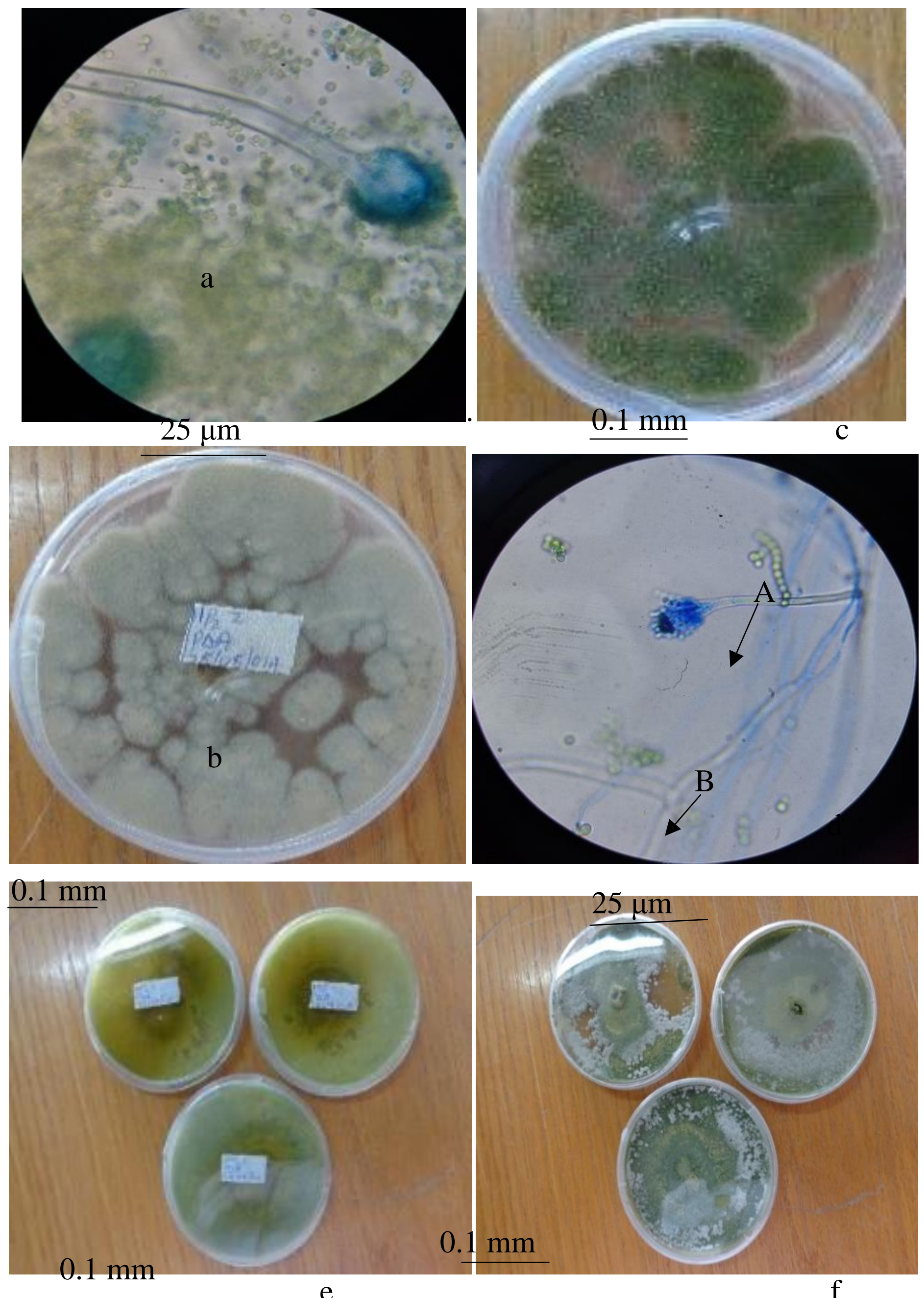


Plate 4.7: *Aspergillus fumigatus* with unseriate row of phialides on the upper two thirds of the vesicle (a)., front (b) and reverse (c) surfaces of *Aspergillus* species with cleistothecia (B), cracked conidiophore (A), hyaline conidia in chains and Hürlle bodies (d)., front (e) and reverse (f) surfaces of the *Aspergillus* after 14 days growth on PDA at 28 ±2ºC

Distinction of *A. nidulans* from other *Nidulantes* members is accomplished by sequencing analysis of β-tubulin, calmodulin and actin genes (Chen *et al*., 2016). *Aspergillus* species are able to survive in a wide range of climatic conditions (Mousavi *et al*., 2016).

Members in the *Nidulantes* subgenus were frequently present at latitudes in the subtropical/ warm temperate zone between 26 and 35ºC (Klich, 2002). Conversely, another study has reported that genera such as *Aspergillus, Penicillium* and *Talaromyce*s are able to thrive in extreme ecological niches including low/high temperatures, high salt/sugar concentrations , low acidities and low oxygen tensions (Barbosa *et al*., 2018; Tsang *et al*., 2018). A study done in the Arctic and Antarctic regions revealed that fungi adopt physiological tactics including increased intracellular trehalose, polyols, unsaturated membrane lipids as well as secretion of antifreeze proteins and viable enzymes enabling survival to negative zero degrees centigrade (Robinson, 2001). Pathogenicity of these fungi could be by the nature of production of mycotoxins, aflatoxins are acting as saprophytes (Kidd *et al.,* 2016). The fungus in **Plate 4.7 (d))** was isolated from cut *Gladiolu*s that had been pulsed in distilled water for 24 hours at ambient temperatures and then cold stored for one day at 3 ± 1 ºC before vase study at room temperature. It is probable the presence of the fungus belonging to section *Nidulantes* affected the postharvest quality of the cut *Gladiolus*.

The fungi in (**Plate 4.8 (a))** were isolates belonging to the genus *Acremonium* and unidentifiedspecies of *Aspergillus.* This*Acremonium* species was isolated from vase water of *Gladiolus* pulsed with 600 ppm 8-HQS + 5% Sucrose and wet cold stored for 3 days prior to vase study at ambient temperatures. The isolate in this study possess the characteristic ball of pores produced at the apex of tapering conidiophores that arise at right angles to the vegetative hyphae. (**Plate 4.8 (b., c**)) represent the macroscopic appearance of *Acremonium strictum* after growth at 27 ± 2 ºC on SA for7 days with characteristic white and brown colonies,The genus *Acremonium* has 100 species, most of which are saprobes isolated from plant material and soil. Some of the species are parasitic on living fungi (Hong *et al*., 2000). *Acremonium* species belong to the non- clavicipitaceous endophytes inhabiting non-grass host plants (Petrini, 1992). The mode of action of *Acremonium* species include the production of mycotoxins and altering the host plant (Finlay *et al*., 2003; Gaylord, 1996). Non- clavicipitaceous endophytes have also been implicated in disease resistance increased abiotic tolerance and enhancement of plant growth (Arnold *et al*., 2003; Ernst, 2003). However, some studies have shown that this perceived mutualism is questionable (Arnold, 2008; Rodriguez *et al*., 2008; Sieber, 2007).

The use of the non–clavicipitaceous endophytes *Stemphylium globulliferuum*, *Embellisia leptinellae* ans *S. globuliferum* reduced nutrient deficiencies in *Poa pratensis* fungal leaf endophytes were defined as as non-pathogenic microfingi that live within plant leaves and can assist in host defence against pathogens (Busby *et al*., 2013; Lledó*et al*., 2015). The effects of fungal endophytes have since been termed as ubiquitous owing to their variable effects on

pathogen symptom severity, the plant genotype seemingly determining the overral outcome (Vardhana *et al*., 2017).

The role of endophytes as antagonists of plant pests and parasites has been affirmed (Clay, 1991; Dong & Zhang, 2006). The genus *Acremonium* has been credited with both desirable and undesirable effects on plants. In perennial grass, *Acremonium* has the protective effect against attack by some insects, nematods and plant diseases, but produces alkaloids, that harm herbovores (Latch, 1994). Likewise, *Acremonium strictum* Gam (*Cephalosprium acremonium* Corda) together with *Fusariummoniiliforme* Shield, cause stalk rot in maize (King, 1981). This species of *Acremonium* also causes strawberry disease in strawberry plants (*Fragaria ananessa*) (Racedo, 2013). On the other hand, *Acremonium zeae* endophyte in maize produces antibiotics that are inhibitory to *Aspergillus flavus* and *Fusarium verticilloids* (Shoshannah *et al*., 2005)

The desirable aspect of *Acremonium strictum* as a mycoparasite of *Helminthosporum solani* has been reported (Rivera-Varas *et al*., 2007).In a case study of a a nematode isolated from ornamental plants (*Gardenia jasminoides* and *Euonymus hamiltonianus*), there was possible incidence of action of *Acremonium* on parasites in flowers(Maria *et al*., 2018). Given the variable pathogenic and mutualistic nature of *Acremonium* genus, the effect of the isolated species on the quality and vase life of the cut *Gladiolus* spikes could be ubiquitous.

The role of endophytes as antagonists of plant pests and parasites has been affirmed (Clay, 1991; Dong & Zhang, 2006). The genus *Acremonium* has been credited with both desirable and undesirable effects on plants. In perennial grass, *Acremonium* has the protective effect against attack by some insects, nematods and plant diseases, but produces alkaloids, that harm herbovores (Latch, 1994). Likewise, *Acremonium strictum* Gam (*Cephalosprium acremonium* Corda) together with *Fusariummoniiliforme* Shield, cause stalk rot in maize (King, 1981). This species of *Acremonium* also causes strawberry disease in strawberry plants (*Fragaria ananessa*) (Racedo, 2013). On the other hand, *Acremonium zeae* endophyte in maize produces antibiotics that are inhibitory to *Aspergillus flavus* and *Fusarium verticilloids* (Shoshannah *et al*., 2005).

The macroscopic morphology in plate **(48 (d,. e))** refers to the macroscopic appearance of a uniseriate unidentified *Aspergillus* species isolated from cut *Gladiolus* spikes that had been pulsed with a solution of 600 ppm 8- HQS plus 5% sucrose solution before cold storage period of two days prior to study of vase life at ambient temperature. Uniseriate species of *Aspergillus* include *A. parasiticus* which is toxin producing and grows on any crop or food (Vanga *et al*.,

2011). The growth of this fungus species may have affected the quality and vase life of the cut *Gladiolus* flowers.

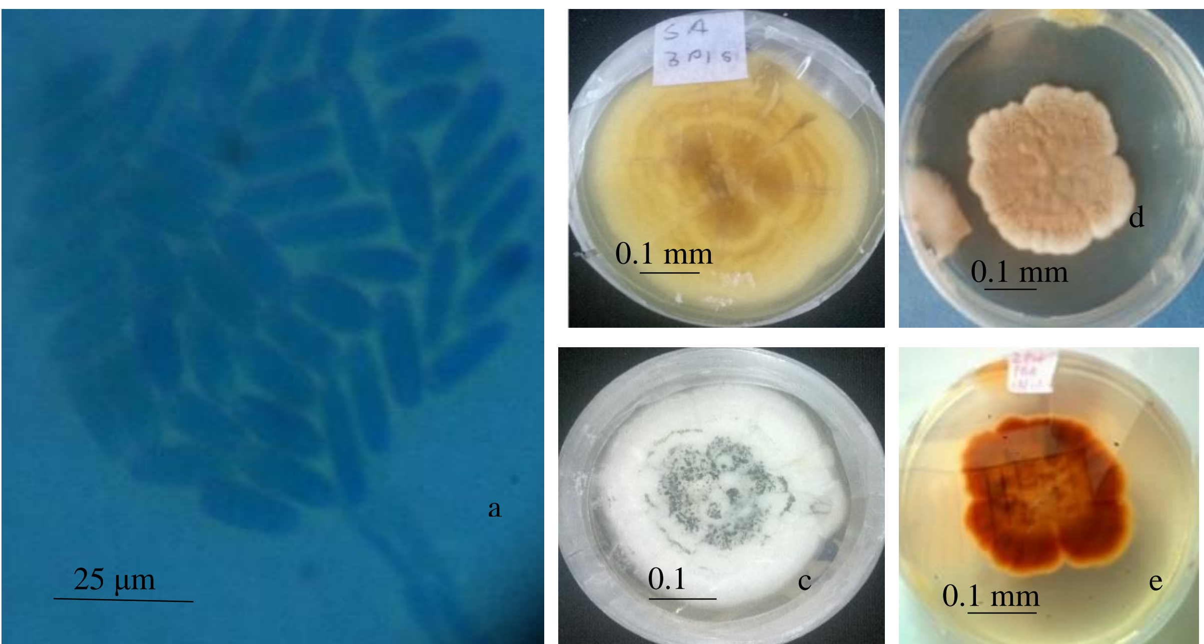


Plate4.8: *Acremonium strictum* showing a ball of spores at apex of tapering solitary conidiophore arising at rght angles to the vegetative hyphae (a)., reverse (b) and front (c)surfaces of *Acremonium strictum* growth at 27 ± 2°C on Sabouraud's agar after seven days., front (d)and reverse surface (e) of unidentied *Aspergillus* species isolated from *Gladiolus* vase water

The fungi in **Plate 4.9** are the microscopic and macroscopic morphologies respectively of the fungus *Trichophyton verrucosum and T. interdigitale.***Plate (4.9 (a., b))** represent the microscopic and macrospic appearance of the fungus *Trichophyton interdigitale*. The fungus is chatacterized by yellowish-brown pigment effusion showing nodular organs, spiral hyphae, smooth walled macroconidia, spherical chlamydospores and few hyaline globose microconidia (**Plate 4.9 (b**)). This fungus is anthropophillic with a worldwide distribution. Morphological characterization is based on front surface containing flat white to cream colonies. The reverse presents with a pinkish brown pigmentation. Like *Trichophyton verrucosum,* this fungus could have had an effect on the *Gladiolus* cut flower by competing for available nutrients and space for colonization (Carlson, 2014).

Microscopically, *Trichophyton verrucosum* Bodin has clavate microconidia, terminal vesicles at the tips of the hyphae in young colonies' rat tail or string bean – shaped macroconidia and chains of chlamydospores. The chlamydospores may be intercalary as shown in **Plate 4.9**

(**c., d**)). The image was taken at × 1000 magnification. The meandering paths of the hyphae this dermatophyte, rather than being progressive in the same plane or in a straight line may be an avenue to aid in penetration of host tissue (Kaufman *et al.,* 2007). *Trichophyton verrucosum* cream colored colonies with a raised center on the front surface after five days growth at 27 ± 2ºC are as shown in **(4.9 (g))**

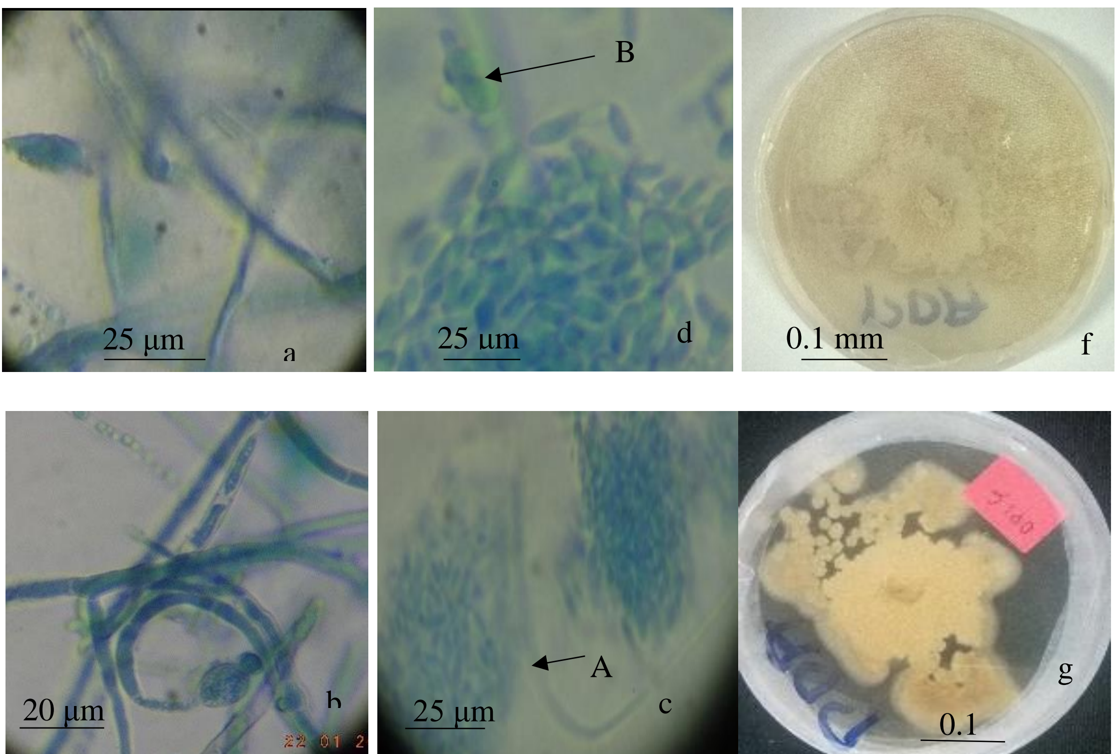


Plate 4.9: *Trichophyton interdigitale* with yellowish-brown pigment effusion with nodular (a) organs, spiral hyphe (b), smooth walled macroconidia, spherical chlamadospores and few hyaline globose macroconidia. Macroscopic appearance of *Trichophyton verrucosum* bodin with spiral hypha (arrow A) with clavate to pyriform microconidia, terminal vesicles at the tip of hyphae in young colonies , rat tail or string bean-shaped macroconidia and chains of chlamydospores (c); and intercalary chlamydospores (arrow B) (d)., Front (g) and reverse (f) macroscopic surfaces of *Trichophyton verrucosum* cream colonies after five days' growth at 27 ± ºC on potato dextrose agar

Dermatophytes are able to occupy a variety of ecological niches including soil, skin, and hair among others. Their hosts include plants and animals apart from capability to cohabit with different microbes (Martinez *et al*., 2012). The secondary metabolite ergot, whose secretion is encoded by fungus genes is an alkaloid mycotoxin of indole derivative with a variety of

biological activities (Coyle & Punaccione, 2005; Wang *et al*., 2018). The dermatopytes are enriched with clusters of genes encoding classes of proteases, kinases, secondary metabolite toxins and host evasion components that permit their growth and virulence (Dal *et al*., 2019; Martinez *et al*., 2012). *Trichophyton verrucosum* is a zoophile. It is probable that by adopting any one of the fungal modes of virulence, its presence and interation with the cut *Gladiolus* may have affected the quality of the cut spikes

Plate 4.10 refers to fungal isolates belonging to *Ulocladium*, A*lternaria* and *Candida* spicies.Ulocladium species was isolated from vase water of cut *Gladiolus* subjected to pulsing solution of 600 ppm 8 HQS plus 5% sucrose solution and displayed immediately to vase study at ambient temperatures. **Plate 4.10 (a)** exhibits characteristic phaedictyospores produced from simple, short elongated conidiophores of *Ulocladium* species. Macroscopic appearance in **Plate 4.10 (c., d)** shows characteristic brownish black colonies of *Ulocladium* after growth on SA at 28 ± 2 ºC for 14 days,*Ulocladium* genus, like *Alternaria*, *Embellisia* and *Stemphylium* fungi are all morphologically phaedictosporic airbourne *Hyphomycetes* (Xiue & Zhang, 2007). Fungi of these genera are common plant saprobes on plant debris, timber, paper and water damaged buildings (Runa *et al.,* 2009). The morphology of the conidia in *Ulocladium* is subject to environmental conditions such as substrate composition, humidity, light cycles and temperature; which impact on their growth and development (Xiue & Zhang, 2007). *Ulocladium atrum* species has been used in the biological control of the gray mould disease caused by *Botrytis cinerea*infecting grapevines (Elmer & Kohl, 1998; Kohl *et al*., 2003). It is also documented that *Ulocladium atrum* can suppress sporulation on *Botrytis* species by competitive saprophytic colonization on leaf tips of onion or necrotic lily canopies (Kohl *et al*., 2003).

Ulocladium species have been isolated from several plant species (Wang *et al*., 2008). Some species of *Ulucladium* (for instance; *U. chartarum* and *U. cucurbitae*) are plant pathogens, causing a range of diseases on important agricultural crops and trees. It is probable that the isolate from the *Gladiolus* vase water could have affected the vase life and quality of the cut flower spikes. Combining morphological and molecular phylogenetic data would have enabled better placement of the isolate species. The species has conidia that have short conical beaks and are smooth walled. The conidia also are pigmented and are multisegemented, thus termed as phaedictyospores

The morphologically closely related genus (*Alternaria*) fungi on (**Plate 4.10 e,f,g,h)** were isolated from *Gladiolus* cut lowers that had been pulsed with 600 ppm 8-HQS plus 5% sucrose solution and then cold stored for three days prior to vase study. Like *Ulocladium*, members of the genus *Alternaria* produce large multicellular dark-coloured melanized conidia

with longitudinal as well as transverse septa (phaedictyospores) (Bensassi *et al*., 2012). The *Alternaria* conidia are characteristically single or gradual chains in short, erect conidiophores (Ellis *et al.,* 2007).*Alternaria* species have been found as saprophytes on many hosts, and they are common laboratory contaminants (Mangwende *et al*., 2018; Thomma, 2003). They are found in diverse ecological niches, including the soil, decaying plant fibres and in the air. *Alternaria* species have been associated with postharvest decay as pathogens (Thomma, (2003).

Alternaria species produce mycotoxins, some of which are host-specific. Alternariol is one of the most important mycotoxins produced by *Alternaria* species (Bensossi *et al*., 2012). *Alternaria* species are known to produce other mycotoxins which act as inhibitors of photophosphorylation through specific binding to the chloroplast ATP synthase. Consequently, this renders ATP hydrolysis and synthesis processes to be inhibited (Steele *et al*., 1978). Their pathogenicity towards some medicinal plants has been documented (Maiti *et al*., 2007). Seed airbourne *Alternaria alternata* isolated from coriander (*Coriandrum sativum*) was associated with the leaf spot disease on this plant.*Alternaria alternata* species that outwinters in sunflowers leaves in Greece was indicted in yearly poor yields of these flowers in Greece (Mangwende *et al*., 2018).

It is probable that the *Alternaria* isolate in this study had an effect on the vase life and qualityof the cut *Gladiolus* flowers.**Plates 4.10 (i)** is the microscopic morphology of yeast- like fungi isolates from cut *Gladiolus* vase water. It displays the germ tube appearance of *Candida* species. *Candida albicans* is tetramorphic whereby it exists as budding yeast-like cells, pseudospore, hypha and chlamydospores (Barnett, 2008). *Candida albicans* is not a true member of the phyllosphrere (Picco *et al.,* 2012). *Candidaalbicans* is listed as a pathogen of both animals and plants (Perez-Nadale *et al*., 2014). Intrinsic biofilm formatiom (biological communities with a high degree of organization, in which microorganisms form structured, coordinated and functional communities) is among mechanisms of *Candida* pathogenesis (Sardi, 2013). Filamentation and quorum sensing are important factors used by *Candida albicans* in biofilm formation (Hornby *et al*., 2001; Mayer *et al*., 2013).

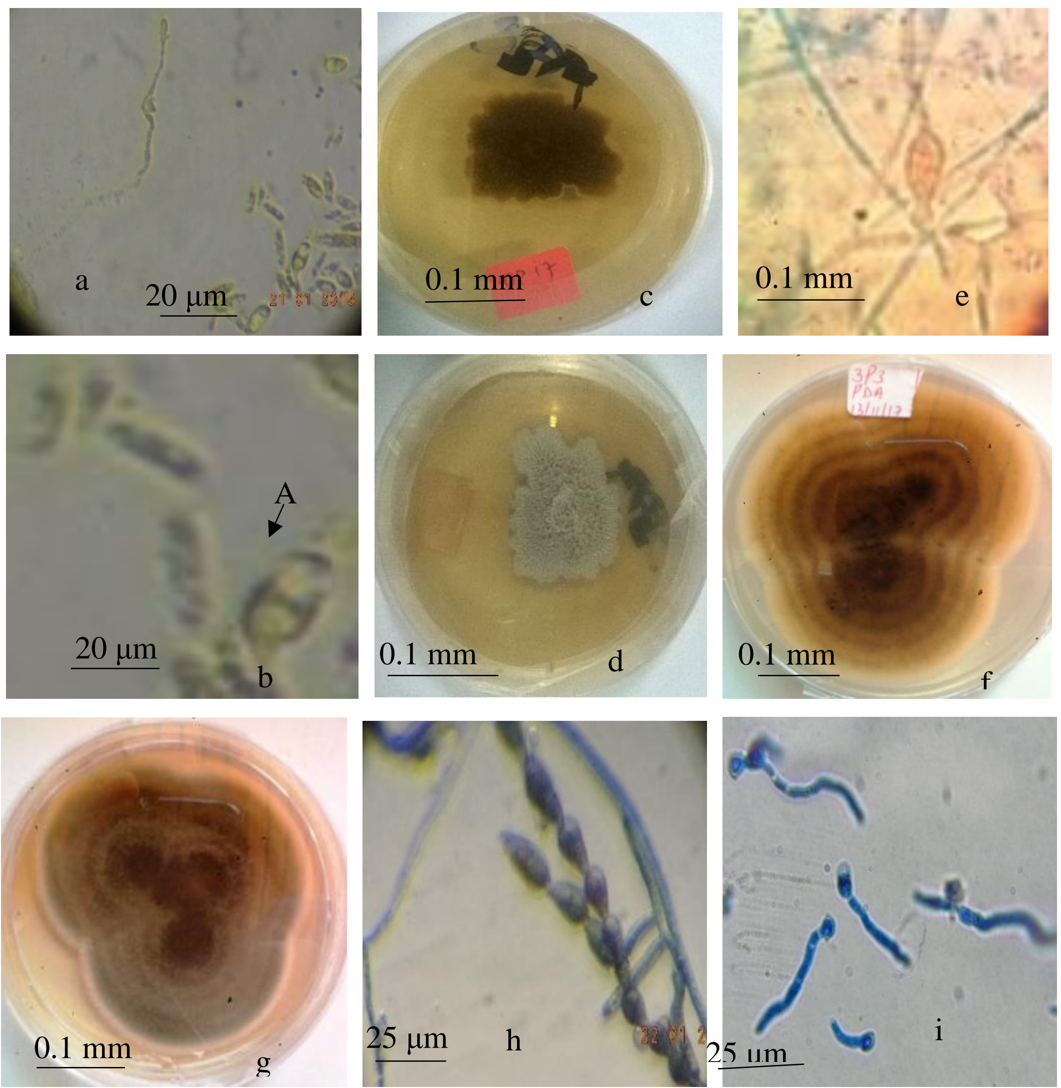
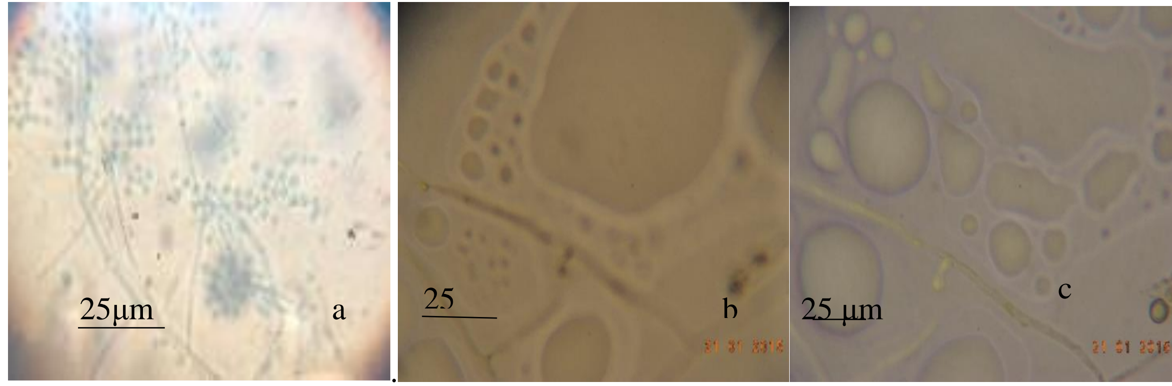


Plate 4.10: *Ulocladium* species with phaedictyospores (A), produced from simple, short elongated conidiophores (a., b)., *Ulocladium* species showing brownish black colonies on (c)front and (d) reverse surfaces., microscopic appearance of the fungus *Alternaria* species with phaedictyospore (A (e). showing the macroscopic front (f) and reverse (g) appearance of *Alternaria* species (h)., *Alternaria* species isolated showing dark coloured conidia, broader at the base than at the tip., demonstrating production of germ tubes by *Candida albicans* (i)

Candidaalbicans is a commensal pathogen, that can adapt to the host's environmental changes very quickly, even under restricted nutrient biovailabilty (Dadar *et al*., 2018). It senses and adapts to environmental pH by modulating the extracellular hydrogen ion concentration by actively alkalinizing its surrounding under nutrient starvation, thereby inducing hyphal formation (Mayer *et al*., 2013). Other strategies used by *Candida albicans* in its virulence include interplay in acquisition of host iron, secretion of hydrolases and aspartic proteases to

facilitate entry / enhance efficieny of extracellular nutrient mooping, and damage of epithelial cells (in human) respectively (Mayer *et al*., 2013; Noble, 2013). Given the strategies adopted by *Candida albicans* as a commensal turned pathogen to sustain itself, is probale that it affected the postharvest quality of the cut *Gladiolus.*

Plate 4.11 refers to the microscopic and macroscopic features of the fungi *Albugo* species.The microscopic and macroscopic features of *Albugo* fungus isolated from vase water of *Gladiolus* flowers that had been pulsed with 600 ppm 8-HQS before wet cold storage at 3 ± 1^0C before vase study at ambient temperatures are shown in **Plate 4.11 (a.,b., c., d., e., f., g**.,)). *Albugo*, like all oomycetes is found colonizing continents and oceans in a variety of habitats (Thines, 2013). *Albugo* is an obligate filamentous (**Plate 4.11 (a**)) biotroph as well as a hemibiotroph plant pathogen characterized by suppression of the host's defence mechanism and also enabling attack of otherwise avirulent pathogen growth (Ruhe, *et al*., 2016). It is the causative agent of the common white rust disease, found attacking a number of plants including cruciferous crops (Pandey, 2013) *Albugo* species cause disease by systemic symptoms of infection which results in abnormal growth and distortion of inflorescence and sterility in rapeseed mustard (Pandey *et al*., 2013). *Albugo* penetrates host tissue via stomata by germtubes arising from asexual inoculum, mesophyll colonization of compatible host tissue and sporulation within spore-bearing pustules (Rimmer *et al.,* 2000). The morphologyof the micro and macro of the *Albugo* species vary from one host to the other and even the ecological location (Lakra & Saharan, 1988). **Plate 4.11 (b)** shows the spherical micro-sporangia and and macro-sporangia that are elongated and globular. *Albugo tragoponis* (Pers) S. F. Gray observed in afew plants of both oilseed and confection sunflowers (*Helianthus annuus* L.) in North Western Kansas is the causal agent of white rust in this ornamental crop (Guliya *et al*., 2002). The white blister rust of the sunflower is caused by *Pustula elianthicola* (Lava *et al*., 2013).



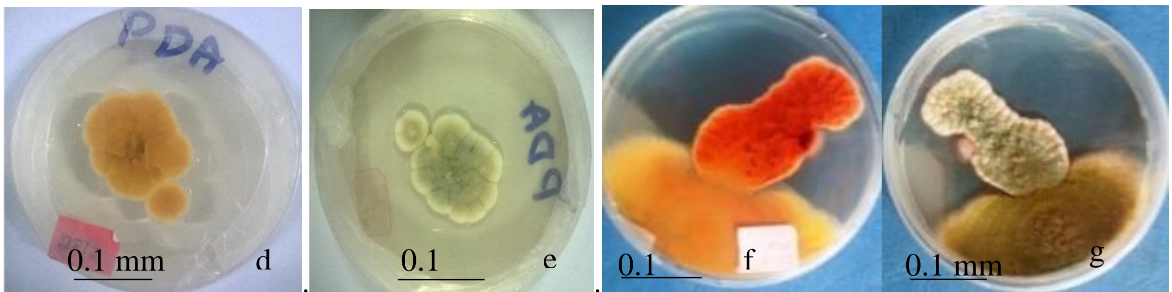


Plate 4.11: *Albugo* species stained with lactophenol blue sporangia and oogonia at × 40 magnification (a)., *Albugo* displaying sporangia, sporangiospores, oogonium, oospores and antheridium (b)., *Albugo* stained with lacto phenol blue displaying sporangia, sporangiospores, oogonium and oospores (c)., the front (a) and reverse (b); surfaces of the fungus *Albugo* cultured on PDA (d)., the front e) and reverse (f); surfaces fungus *Albugo* cultured on PDA

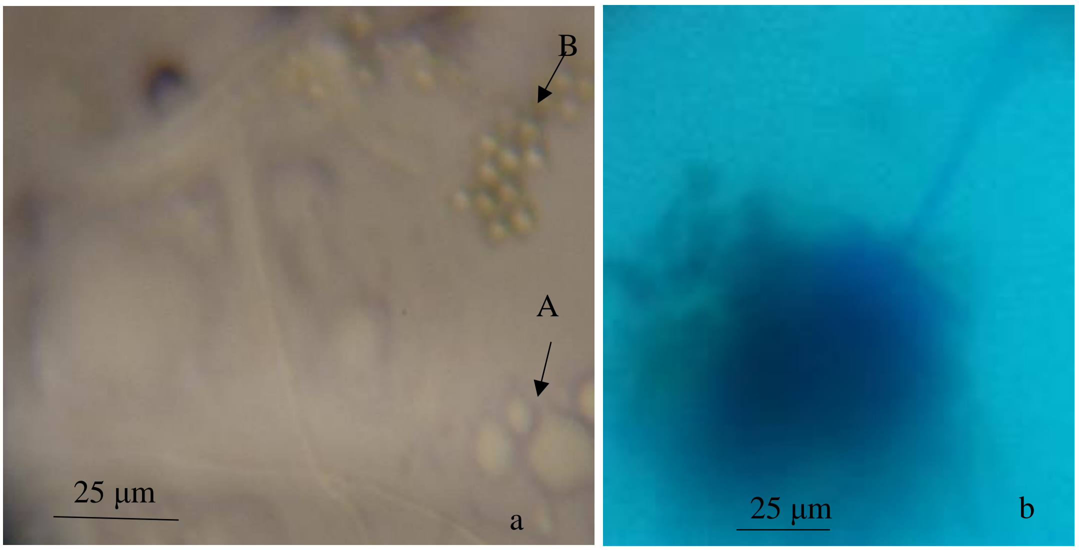
The fungi of the genus *Rhizopus*and a *lichen* (**Plate 4.12 (a., b., c., d., e)**) were isolated from*Gladiolus* vase water. *Rhizopus* was isolated after pulsing with 600 ppm 8- HQS plus 5% sucrose solution and wet cold storage duration of 1 day before vase study. Fungi in the *Rhizopus* genus have been isolated in habitats such as vegetable debris, manure, compost and indoors. Some species may have chlamydospores that are solitary and in chain taking on spherical, ellipsoidal and cylindrical shapes. *Rhizopus* have elliptical conidia presenting in chain s and singly with purple-reddish pigmentation. *Rhizopus* are ubiquitous in their interaction with plants and other microorganisms; releasing mycotoxins and other metabolites including those for evading host defense mechanisms (Van Baarlen *et al*., 2007).

Extracts of *Rhizopus stolonifer* were effective against *Aspergillusniger*, *Aspergillus oryzae*, *Candida albicans*, *Penicillium digitatum, Fusarium oxysporum* and the bacteria pathogens: *Escherichia coli* and *Pseudomonas aeruginosa.* (Radman *et al.,* 2014*).* On the other hand, the bacteria- fungus relationship is such that there is horizontal transfer of metabolite encoding genes, in which the fungus may also introduce or transfer bacteria into novel hosts or niches (Van Baarlen *et al*., 2007). The pathogenicity of *Absidiacorymbifera* and *Rhizopus* species towards plants has been shown to be mediated by the secretion of several proteins and metabolites including auxin (indole acetic acid) which is implicated in the induction of gall and callus formation; also in suppression of plant defences (Shinshi *et al*., 1987).

Several species of *Rhizopus* that produce fumaric acid and other toxins been implicated as the causative agents of the almond hull rot disease characterized by leaf and twig necrosis distant from the actual infection site and without the actual presence of the fungal structures

(Woltz, 1978). Zygomycetes can also employ iron-scanvenging siderophores as a factor of pathogenicity (Holzberg & Artis, 1983). *Rhizopus oryzae* is the causal agent of soft rot in the banana plant, strawberries, other berries, fruits, vegetables and ornamental plants (Kwon *et al*., 2011; Kwon *et al.,* 2012). *Rhzopus oryzae* is a fast growing fungus with white mycelium and black sporangia (Kwon *et al*., 2011). It is probable that growth of the *Rhizopus* species in vase water may have influenced the postharvest quality of the cut *Gladiolus* flowers.A lichen (**Plate 4.12 (e)**) was also isolated from vase water of the cut *Gladiolus*.

Lichens are a polyphyletic taxonomically diverse group existing either as plant pathogens or mycorrhizal fungi (Honegger, 2012). Lichenization comprises of the mutualistic symbiosis of fungi with populations of minute algal and / or cyanobacterial cells (Sanders, 2001). Lichens (such as *Peltigera neckeria* ex. Müll. Arg.) produce secondary metabolites which have negative effects on soil bacteria including *Baccillus* species, *Burkhoderia gladioli*, *Corynebacterium bovis* and *Dermococcus nishinomiyaensis* (Akpinar *et al*., 2009). Lichens are known to produce biologically active metabolites with various effects such as antibiotics, antimycobacterial, antiviral, anti-inflammatory, analgesic, antipyretic, proliferatic and cytotoxic activities (Grube & Boustie, 2005). Endowed with such a myriad of bioactive capacity, lichen interaction with cut *Gladiolus* may have had an effect on the flower postharvest quality.



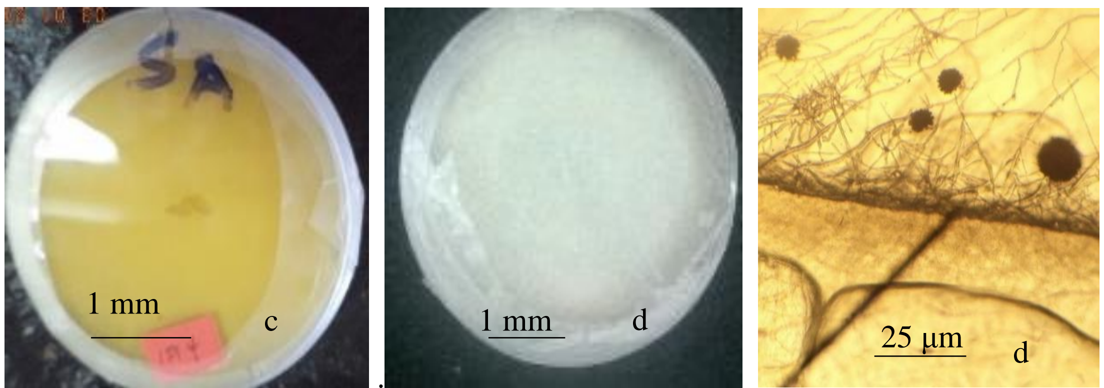


Plate 4.12: Sub- hyaline simple rhizoids of *Rhizopus* species and abundant hyaline chlamydospores (A) and conidia (B).(a)., front (b) and (c) the reverse surfaces of *Rhizopus* grown on SA after 14 days at 28 ± 2ºC showing dehisced sporangium (d);*Lichen* isolated from cut *Gladiolus* showing the fungal hyphae, small algae cells and *Cyanobacteria* association (e)

The **plate (4.13 (a,. b,. c,. d,. e., f., g., h., i))** refers to the microscopic and macroscopic appearance of the fungi *Phytophthora* and *Penicillium* species. *Phytophthora* fungus was isolated from cut *Gladiolus* spikes that had been pulsed with 600 ppm 8- HQS plus 5% sucrose prior to vase study at ambient temperatures. It is probable that the presence of *Phytophthora* species in the cut *Gladiolus* vase water may have affected the postharvest quality of the cut spikes.*Phytopththora* species are widely distributed fungi- like organisms belonging to the Kingdom *Chromista / Stramenopiles* and can infect numerous woody host plants in the natural ecosystem, nurseries and plantings (Mileskovic *et al*., 2018). Fungi in this soil-bourne genus exhibit both sexual and asexual reproduction (chlamydospores and sporangia) stages (Grunwald *et al*., 2012).

One such fungus is *Phytophthora ramorum* which is a water mold that affects Oak species in Europe and North America and it is adapted to cool temperatures with optimal growth at 20ºC (Grunwald *et al*., 2012) . This species of *Phytophthora* also affects woody ornamentals (Grunwald *et al*., 2008).*Phytophthora pluvivora* isolated from Bulgaria has a wide range of host species including ornamental plants (Christova *et al*., 2018). Pathogenicity of fungi belonging to the genus *Phytophthora* (e.g. *Phytophthora tropicalis*) to ornamental plants has been reported, whose origin was the irrigation water used in plant nurseries (Hong *et al*., 2008)

Phytophthora tropicals has pontential hosts of families: *Apocynaceae*, *Astereraceae*, *Begoniaceae*, *Fabaceae*, *Ericaceae*, *Cucurbitaceae* and *Solanaceae* belonging to both ornamental and vegetable crops. The introduction of *Phytophthora* to new hosts and environments is a common mechanism of this pathogen emergence, existence of hybrid species

spanning a greater host range compared with the parental ones (Grunwald *et al*., 2008). *Phytophthoracryptogea* species is infective to tho roots and stems of gladioli plants whereby the plants appear water soaked and black (Singh *et al*., 2012).

The fungiof the genus *Penicillium* (**Plate 4.13 (b., c)**) were isolated from vase water of cut *Gladiolus* flowers that had neither been cold stored nor been pulsed prior to vase study. *Gladiolus*. The macroscopic appearance of this fungus is as shown in **Plate 4.13 (f., g., h., i)** show the grey front and the greyish-blue reverse of the *Penicillium* after 7 days' growth on Sabouraud's dextrose and potatato dextrose media. conidial head and chains of conidia formed with brownish yellow pigmentation. *Penicillium* species are morphologically characterized by fast growing colonies that ar are in shades of green (Kidd *et al.,* 2016).The conidia produced from specialized conidiogenous cells termed as phialides are single celled and in basipetal succession (Webster & Weber, 2007). **Plate 4.13(b., c)** refers to the microscopic appearance of this fungus with a verticillate head. These plates display the characteristicfeature of *Penicillium* with phialides emanating from branched metulae, giving a brush-like appearace.

The two Penicilli isolates could belong to the same species, gauging from the type of macroscopic appearance. *Penicillium* species are *Deuteromycetes* that are extensively spread in nature ranging from diverse soil environments such as cultivated soil, forest soil, desert soil, beach soil and marine environments (Yadav *et al*., 2018). *Penicillum* – like fungi, *Tricoderma*, hyphal fragments, *Aspergillus*, *Cladosporum* and *Bortrytis* are among the top five fungi in green houses (Li & LaMondia, 2010). Many species of *Penicillium* speciesare common contaminants on various substrates (Kidd *et al*., 2016). A recent study established that *Aspergillu*s and *Penicillium* species were isolated in a hazelnut processing plant (Botondi, 2019). *Penicillium* is known for the production of antibiotics (Nigam *et al*., 2018). Fungi in this genus have also been known to produce mycotoxins (Kidd *et al*., 2016). These fungi are are known to have lignocellulase, and cellulase enzymes (Nigam *et al.,* 2018). *Penicillium hirstum* is pathogenic to *Crocus*, *Gladiolus* and *Iris* genera of ornamental plants while *P. tulipae* is pathogenic to the genera: *Narcissus, Crocus*, *Ornothogalum* and *Iris* (Dugan *et al*., 2014).

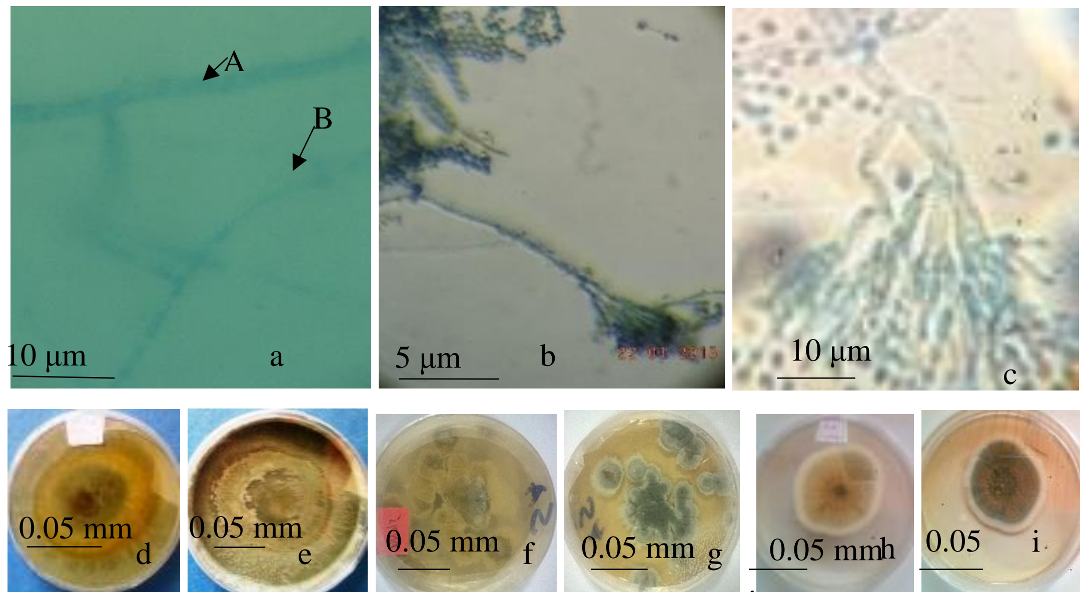


Plate 4.13: *Phytophthora* showing club-shaped antheridia, aseptate (A) hypha and terminal sporangium; (B) some of which are dehisced (a)., *Penicillium* species with conidiophore of asymmetrical branches that form brush-like head (b)Penicillus head of the filamentous fungi after growth at 28 ± 2ºC for 7 days on PDA. (c) Front (d) and cracked dull green-grey reverse (e). of *Phytophthora* species after growth on PDA at 28 ± 2ºC for 7 days ., front surface (f) and (g) the reverse surface of *Penicillium* isolate b exhibiting the green, grey-green, blue-geen and yellow-green colonies on SA *Penicillium* isolate cultured on PDA at 28 ± 2ºC for 7 days., colonies of *Penicillium* isolate c (i)

In contrast, it has been reported that fungal isolates strains of either *Penicillium neoechinulatum* or *Penicillium viridicatum* enhanced and conferred protection against damping off and antracnose in in cucumber plants by destroying the causative agent: *Colletotricum arbiculate* (Hossain *et al*., 2014). *Penicillium nonatum* also improves the yield of Chinese radish (Phuwiwat & Soytong, 2001). Still in another study two species: *Penicillium radicum* and *Penicillium bilalae* are reported to have increased the dry weight of medic and lentil plants (Wakelin *et al*., 2007).It is documented that *Penicillium italicum* together with *Aspergillus niger* and *Moniliniafructicola* are postharvest pathogens (Lazar *et al*., 2008). The use of ascorbic acid and /orsalfosalicylic acid had positive effect on cut *Gladiolus grandiflorus* cv white prosperityflowers, *Penicillium* species and yeast cells being the only microbes that were isolated fromthe vase solution (Khattab *et al*., 2017).The use of silver nitrate/ silver nano particles plus 5% sucrose gave the best results for the vase life and quality parameters of cut *Gladiolus hybridus* Hort, (Sharma, 2017). Flowers of *Gerbera jamesonii* cv. Balance flowers pulsed with

silver nano particles and then stored in a solution of 8- HQS plus 3% sucrose showed maximum vase life. In view of the umbiquitous interaction of *Penicillim* species with other microorganisms and plants, it is possible its presence in vase water affected the quality and vase lfe of the cut *Gladiolus*

Plate 4.14 refers to a *Paecillomyces*and *Talaromyces*species isolated from *Gladiolus* vase water. *Paecillomyces* (**Plate 4.14 (a.,b., d., e**)) was isolated on the third day in the vase from cut *Gladiolus* spikes that had been pulsed with 600 ppm 8-HQS plus 5% sucrose solution and wet cold stored for five days prior to vase study. Fungi in this genus are characterized by having long divergent phialides with colonies that are never typically green (Kidd *et al.,* 2016). Species of soilbourne *Paecilomyces* either as saprobes or pathogens exhibit inhibitory or cytotoxic effects against other microorganisms (Mohammadi *et al*., 2016). These attributes of *Pacilomyces* may have had an impact on the postharvest and vase life of cut *Gladiolus* in this study.

Plate4.14 (c., g., h) refer to the microscopic and macroscopic appearance of a *Talaromyces* species isolated from *Gladiolus* spikes subjected to the pulsing treatment of 600 ppm 8-HQS and cold stored for one day prior to vase study at ambient temperatures. Fungi in this genus are dimorphic, existing as moulds in the environment and yeasts at 37ºC (Kaufman, 2017). *Talaromyces* grow best at 25ºC, forming fast growing colonies which are suede-like to down-white with yellowish-green conidial heads, with a reddish – brown pigment (Kidd *et al*., 2016). The macroscopic appearance of the isolate (**Plate 4.14 (g.,h**) from cut *Gladiolus*from the vase water of cut *Gladiolus* spikes pulsed in distilled water and wet cold stored for five days at day three of display life had glabrous colonies with red-brown pigmentation on the front surface. The reverse surface was brownish green.

Members in this genus have conidiophores that are generally biverciticillate and sometimes monoverciticillate which are hyaline and smooth walled (Ellis *et al.,* 2007). The phialides formed in these species of fungi are normally acerorose to flask-shaped; while they develop conidia that are globose to sub-globose with smooth walls and produced in basepetal succession (Kidd *et al*., 2016). Contrary to the general morphology of members in this genus, *Talaromycesamyrossmaniae* possesess the synnematous structure. (Kunhiraman *et al*., 2019). *Talaromyces* species have been isolated from soil, fresh water and in plants (e.g. maize, cucurbit) among other substances (Dethoup *et al*., 2007; Heo *et al*., 2019;Peterson & Jurjević, 2017; Wang *et al*., 2016).

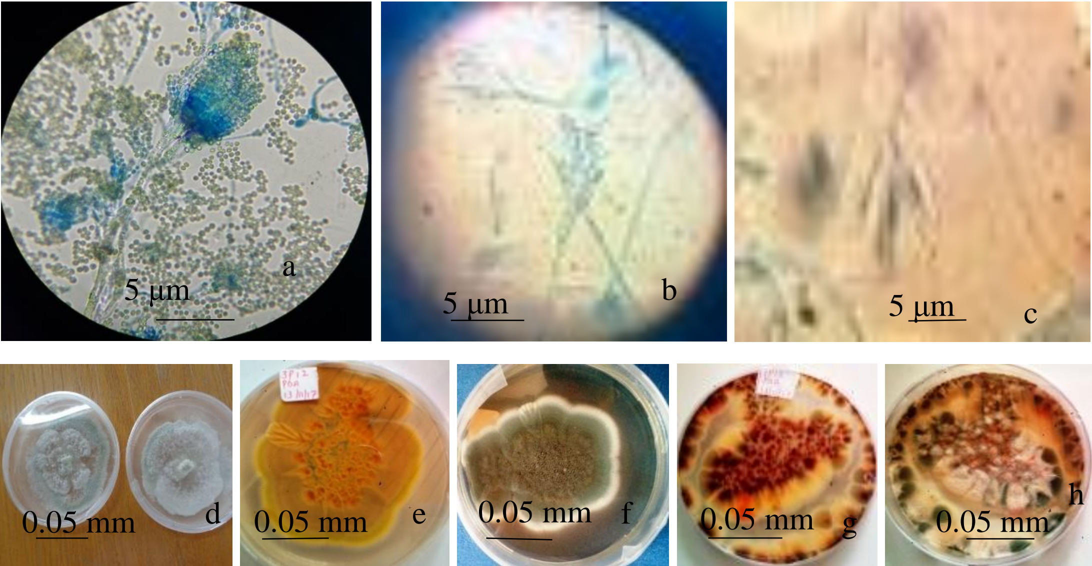


Plate 4.14: *Paecilomyces lilacinus* species isolated from the vase water of cut *Gladiolus* spikes (a)., *Paecilomyces* species showing the verticiliate head (a) and long tapering conidiophores (b)., *Talaromyces* species showing reddish pigmentation with conidiophores bearing flask-like to acerose phialides (c)., front and reverse surfaces of of the fungus of *Paecilomyces* species (d)., Front (e) and the reverse (t) of *Paecilomyces* isolated from vase water of cut *Gladiolus* front (g) and brownish green reverse (h) surfaces of *Talaromyces* species

Actually, some new species of *Talaromyces* and Species in the genus of *Talaromyces* exhibit antagonism against mycelial growth of some plant pathogenic fungi (Dethoup *et al*., 2007; Naraghi *et al.,* 2012). Fungi in the genus of *Talaromyces* possesss the property of pigment production (Lebeau *et al*., 2017: Ogbonna *et al*., 2017; Wang *et al*., 2016). Probably this is the property potrayed by the fungus in **Plate 4.14 (g., h)** which is showing a reddish brown pigmentation. *Talaromyces* are *Ascomycetous* filamentous fungi that are well known as producers of antibiotics as well as various enzymes including cellulases and hemicellulases (Fujii *et al*., 2014). In view of the diversity of the existence of *Talaromyces* species it is probable that the isolate from the *Gladiolus* water in this experiment may have had an effect on the vase life and the quality of the cut flowers.

Plate 4.15 (**a., b., c**)indicates the microscopic characteristics of the fungi *Fusarium*, *Exophiala* and *Tricothecium* genera. The cultural characteristics (**Plate 4.15** (**d., e**)) of a fungus belonging to the genus *Fusarium* which was isolated from vase water of cut *Gladiolus* that neither been pulsed nor wet cold stored is shown (**Plate 4.15** (**c**)). Given the number of symptoms elicited by members of *Fusarium* on agricultural crops, it is probable that their presence in the cut flower vase water affected the postharvest quality of the cut *Gladiolus*. The

member of *Fusarium* exist in respective complex groups of phylogenetically distinct species (Kidd *et al*., 2016).

Fusarium are ascomycetes belonging to the kingdom Eumycota, whose identification is based on the presence of one to two celled microcondia, two to seven septate macrocondia and chlamydospores besides the molecular targets (Kidd *et al*., 2016). *Fusarium* wilt disease has been reported in a number of flowers in the *Gladiolus* species inclusive of *G. psitfacinus* L. cv. White prosperity; King Lear, Friendship, Her Majesty and American Beauty; which were all susceptible (Khan & Mustafa, 2005).

Gladiolus grandiflorus is among the species of the sword lily flowers infected by the *Fusarium* corm rot disease (Riaz *et al*., 2010). A study done on *Gladiolus grandiflorus Hort*. implicated fungal pathogens as disease causing agents: *Fusarium oxysporum* f. sp. gladioli (wilt); *Rhizoctonia solani* (corm rot) and *Alternaria alternata* (leaf spot) (Bhagat&Gupta, 2018). Members of *Fusarium* are soil fungi with worldwide distribution and some species cause storage losses and are important mycotoxin producers (Crember *et al*., 2015). Some of the mycotoxins produced by *Fusarium* species are fumonisin, moniliformin, zearalenone, beauvericin, kumonisin B and deoxynivalenol (Aziliza *et al*., 2014; Crember *et al*., 2015).

Apart from corm rot, *Fusarium* disease on gladioli causes the following symptoms: yellowing and staunting of foliage, occasionally appearance of misshapen and discoloured flowers (Hatai *et al*., 1986). Members of *Fusarium solani* are pathogenic to both humans and agricultural crops such as the pea, cucurbits and the sweet potato among others(Zhang *et al*., 2006). *Fusarium* is also a devastating carnation disease worldwide (Wolcan *et al*., 2018). The most frequently reported *Fusarium* species is *F. solani*, followed by *F. oxysporum* and *F. moniliforme* inthe descending order.

The microscopic appearance *Exophiala* species the isolate of *Gladiolus* pulsed and then wet cold stored for two days before vase study is as shown in (**Plate 4.15**(**b**)**.** *Exophiala dermititidis*, the most frequently isolated speciesis a saprophyte; isolated from hydrocarbon rich or humid ologotrophic environments and is characterized by annelidic condiogenesis (Kirchhoff *et al*., 2019; Seydmousavi *et al*., 2014). *Exophiala* species have been isolated from the environment in habitats such as the soil, decaying wood, pulp, bathwater and in humidifiers (Wang *et al*., 2001).

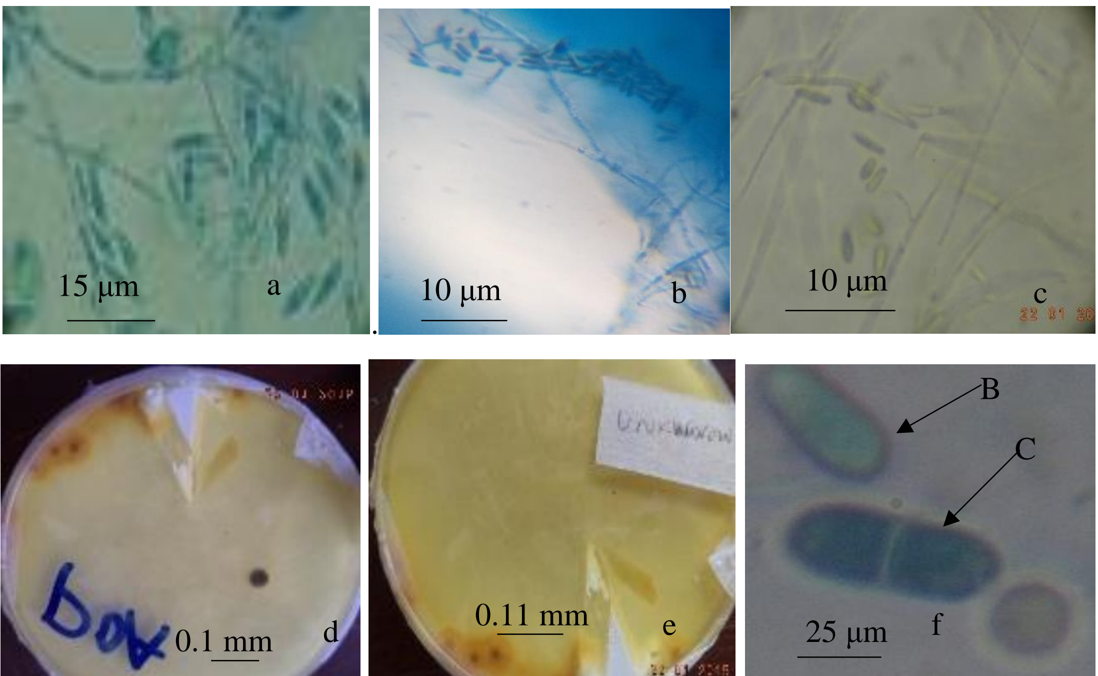


Plate 4.15: *Fusarium* solani showing micro and macroconidia and long phialides (a)., showing microscopic appearance of *Exophiala jeanselmei* with annelids, conidia and conidiogenous pegs on yeast-like cells (b)., Retrogressive conidial growth in *Tricothecium roseum* (c)., front (d) and reverse (f) surfaces of the macroscopic fluffy appearance *Fusarium* species., two celled hyaline conidium (A) and immature spores of elliptical globose (B) conidia of *Tricothecium* (f)

One of the species belonging to the genus *Exophala* , *E. jeanselmei* is characterized as having dark-moist olivaceous black yeast-like colonies at room temperature, whose conidia are clustered at the apices of tapered annelides and long, thick-walled , septate conidiophores (Badali *et al*., 2010). *Exophiala dermatitidis* has several virulence factors including biofilm formation, melanization and dimorphism (Seyedmousavi *et al*., 2014). Production of melanin by this fungus not only increases its virulence and capacity to scavenge reactive species produced during host defence but also enhances fungal cell resistance to lysis and phagocytosis (Van Baarlen, 2006). Unlike the black-yeast *Aureubasidium* which is listed among the phytopathogens, fungi in the genus *Exophiala* are associated with human pathogenicity (Alylward *et al*., 2017; Kirchhoff *et al.,* 2019). However, the saprophytic and biofilm characteristics of this fungus may have affected the postharvest quality and vase life of the cut *Gladiolus* in this study. *Tricothecium* species (**Plate 4.15** (**c**)) was isolated from vase water of cut *Gladiolus*.*Tricothecium* are characterized by the retrogressive conidiophores which are erect

and indistinguishable from the vegetative hyphae and also have two celled conidia. Immature conidia are sometimes one – celled.

A member of this genus, *Tricothecium roseum* is the causative agent of the pink mold rot and is found distributed on decaying vegetables and the soil (Yun *et al.,* 2013). Increase in germination of *Tricothecium* species requires enhanced humidity and exogenous nutrients (Dal *et al*., 2019). It is probable that the growth of this Fungus affected the postharvest quality of cut *Gladiolus* as a result of completion of the same food resources. *Tricothecium* species produce many toxins; being the causative agents of the tomato fruit rot and white stain symptoms in grapes (Yun *et al*., 2014). Roseotoxin B, prodused by *Tricothesium* species is able to penetrate the apple peel and cause chlorotic lesions (Žabka *et al*., 2006). *Tricothecium roseoum* is also one of the fungus associated with the decline in pine trees (Helena, 2015). Identification of these fungi is by nucleotide sequence of the internal transcribed spacer (ITS) region supported by the phylogenetic analysis of the rDNA - ITS regions (Kidd *et al*., 2016).

Plate 4.16 shows the microscopic appearance of a fungi belonging to the genera*Euromyces,* yeast like *Candida,* and a Zygomycete.*Euromyces*(**Plate 4.16** (**a**)) are obligate biotrophs that can be distinguished from each other by the number of germ tubes they possess (Clement*et al*., 1993). The infection structures of *Uromyces* fungi are the haustoria and appressoria (Fawke *et al*., 2015).*Uromyces* species also generate enzymes such as acidic cellulases, extracellular proteases and chitin deacetylases at the infection hypha which break the host cell wall (Voegele, 2006). *Uromyces* thrive under conditions of hydration, temperatures of 3 – 26^{0}C and optimum lighting conditions: factors that all favour their adhesion to the host (Clement*et al*., 1993).*Uromyces transversalis* Thum is the the causal agent of *Gladiolus* rust, which affects members of the Iridaceae family (Bonde *et al*., 2015).

The species of *Uromyces fabae* which has three to four germ tubes has been reported as a pathogen of *Lathyrus* species among other hosts (Clementer *et al*., 1993). *Uromyces transversallis*, as the causal agent of *Gladiolus* rust affected hybrid gladioli house garden species in by inflicting bright orange uredinal lessions on them (Beilharz *et al*., 2001; Blomquest, 2007; Rodriguez- Alvarado, 2006).It is possible that the *Uromyces* isolate in vase water of *Gladiolus* in this current study affected the quality and vase life the cut *Gladiolus*.

Plate 4.16(**b**) belonged a tetramorphic form of yeast like *Candida albicans* that was alsoisolated from *Gladiolu*s vase water.**Plate 4.16** (**c**) refer to unidentified mucorale isolated from *Gladiolus* vase water of cut flower spikes which had been subjected to pulsing treatment of 600 ppm 8-HQS plus 5% sucrose prior to five days storage duration before vase study. The

*Mucorales*comprise of the genera *Rhizopus*, *Mycocladus*, *Rhizomucor*, *Cunninghamella*, and *Apophysomycets*, all identified as zygomycetes (Kidd *et al.,* 2016).

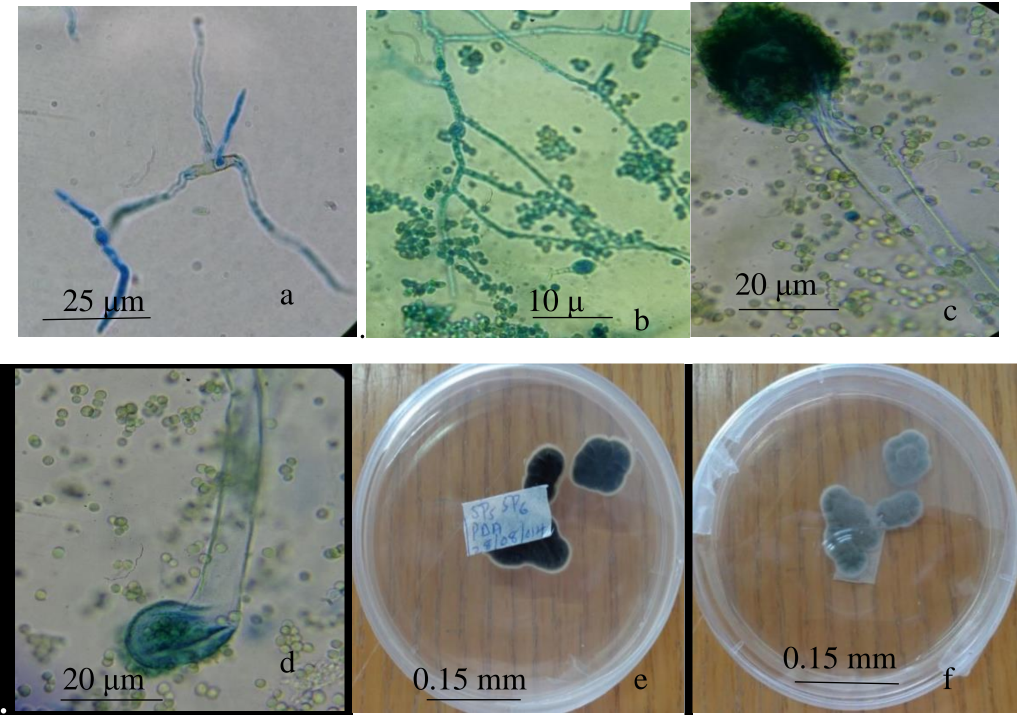


Plate 4.16: Penetrating vesicle, infection hypha, spore and the urenidiophore of *Uromyces* species (a)., Yeast-like species with chlamyospores, (A) hyaline globose spores (B), hypha and pseudohyphae (C) (b)., Zygomycete with collumela, numerous globose conidia existing in clusters, chains, pairs and singly (c)., poorly developed rhizoids of the zygomycete (d)., front surface (e) and reverse surface (f) of the of the zygomycete in c,d

The poorly developed rhizoids in **Plate 4.16** (**d**) plus the conidial microscopic morphology together with the greenish gray appearance of cultural macroscopic appearance could align with features for *Rhizomucor* genus (Kidd *et al*., 2016). *Mucor* and *Rhizomuco*r are members of the Mucorales group containing pathogens of both plants and humans (De Lucca, 2007). The *Mucorales* are causative agents of the fruit rot (Michailidis, 1991). Mucorales are airbourne thermotolerant ubiquitous saprophytic molds widely distributed in nature (Hoffman *et al*., 2013; Richardson, 2009). Species of endophytic *Mucor* were identified to have enabled the adaptation of *Arabidopsis arenosa* vegetation to thrive in a degraded wasteland environment (Rozpadek *et al*., 2018). It is also documented that plant growth hormones; indole acetic acid and kinetin when applied in controlled conditions enhanced the growth of *Muco*r species and chitosan production with concurrent decrease in ethanol output (Safaei *et al.,* 2015). Fungi in the Zygomycota phylum can best be identified by molecular methods such as sequencing of the

internal transcribed spacer (ITS) region or the polymerase chain reaction-restriction length polymorphism molecular tools (Alvarez *et al*., 2009: Ziaee *et al*., 2016).

Chitosan has proven to promote plant growth, induce tolerance in various horticultural crops towards abiotic and biotic stress conditions as well as protecting the safety of edible products (Malerba *et al*., 2016). Chitosan has also been used as a biostimulator in the cultivation of potted freesia and okra (*Hibiscusasculentus* L) (Mondal *et al*., 2012; Salachina & Zawadzińska, 2014). Chitosan is documented to have improved the growth of the plantlets of *Vitis vinifera* l. to which protection against *Botrytis cinerea* was impacted (Barka *et al*., 2004). Given the ubiquitous nature of mucorales in their interaction with plants, it is probale that the presence of the species isolate in this study may have had an effect on the quality and vase life of the cut *Gladiolus* flowers.

Fungi of the genera *Madurella* and *Nigrospora*were also isolated from vase water of the cut *Gladiolu*s (**Plate 4.17**).The respective microscopic and macroscopic of an isolate belonging to the genus *Madurella*is as shown in **Plate 4.17** (**a,**, b., d.,e). This fungus was isolated from vase water of cut *Gladiolus* pulsed and wet cold stored for five days prior to vase study; showing colony morpology with brown pigmentation. Fungi in this genus haveyellow/brown to olive/brown glabrous colonies that may be raised to heaped, and sometimes they may be radially heaped (Kidd *et al*., 2016**).** These fungi possess septate hyphae and they may have black masses of sclerotia and chlamydospores (Ellis *et al*., 2007). *Madurella* species have been isolated from soil and *Acaci*a thorns (Kidd *et al*., 2016).

The two most common species of *Madurella: M. grisea* and *M. mycetomatis* have been associated with human infections. Since there is cross Kingdom jumps of microrganisms' pathogenicity (Van Barrlem *et al*., 2006) the isolate from the cut *Gladiolus* vase water could have influenced the postharvest quality of the flowers. The *Madurella* genus exhihits two types of conidia formation, In the first type, conidia are formed from flask like phialides. In the second type of conidiation, pyriform to globose spores are formed from conidiophores.

The *Nigrospora* species in this study was isolated vase water of cut *Gladiolus* that had been pulsed for 24 hours with 600 ppm 8-HQS plus 5% sucrose before study of postharvest qualities at ambient temperatures. **Plate 4.17** (**c**., **f**., **g**) refer to the respective microscopic and maroscopic appearances of *Nigrospra* species characterized by conidiophores reduced to conidiogenous cells that are monoblastic, discrete, solitary, determinate and hyaline (Mohammed *et al*., 2017). The fungus isolate was from vase water of cut *Gladiolus* that were pulsed with 600 ppm 8-HQS plus 5% sucrose prior to vase study.*Nigrospora* is a monophyletic genus belonging to the family *Apiosporaceae* (Wang *et al*., 2017).

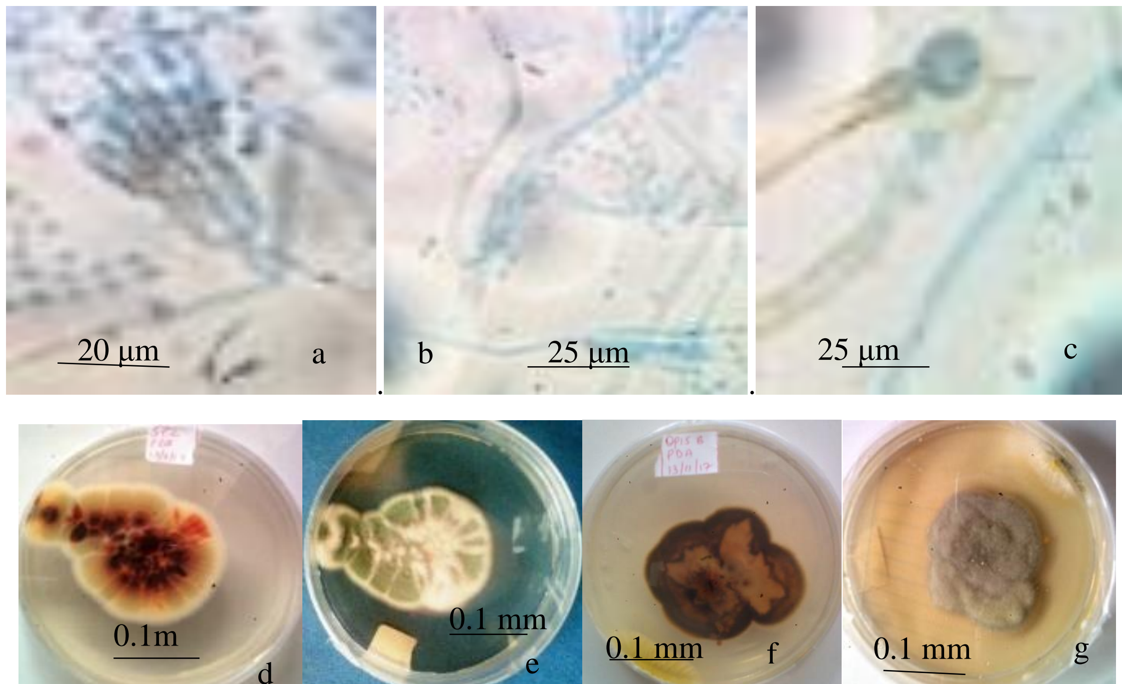


Plate 4.17: *Madurela* species with hyphae,flask-shaped phialides (a)., conidiophores and flask shaped phialides bearing conidiophores of *Madurella* species(b)hyphae, conidiogenous conidiophore and conidium of *Nigrospora* species (c)., the front (d) and reverse (e) surfaces of the fungus *Madurela* species., the front (f) and the reverse (g) surfaces of the fungus of *Nigrospora* species

Fungi in the genus *Nigrospora* are endophytes; though they are also plant and human pathogens. *Nigrospora* species are plant pathogens of many economic crops including fruits and ornamentals (Liu *et al.,*2016). One of the species; (*N. sphaerica*) causes leaf blight on *Camellia sinensis* (Liu *et al*., 2016).

Fungi in the genus *Nigrospora* produce secondary metabolites which have bioactivity against other fungi (Wang *et al*., 2017). A species of *Nigrospora* endophytic on the medicinal plant *Moringa oleifera* Lam which produced griseofulvin which had antifungal activity against eight plant pathogenic fungi (Zhao *et al*., 2012). Other studies indicated that *N. sphaerica* has bioactivity against sporangium and zoospore germination of *Phytophthora infestans* (Kim *et al*., 2001). *Nigrospora* species are known to produce novel metabolites (Wang *et al*., 2017). For instance, *Nigrosporasphaerica* produces a phytotoxin that is pathogenic on turfgrasses. On the other hand , plant growth hormones including indole acetic acid, salycylic acid and benzylaminopurine inhibited the growth of *N. oryzae* and *N. sphaerica* (Mohammed & Abbas, 2017). A collaborative study established that a victorivirus and two mitoviruses infected the plant pathogen *Nigrospora oryzae* (Liu *et al.,* 2019). Given that there is species diversity within *Nigrospora* (Liu *et al.,* 2016) with a general lack of host specificity, it is probable that the species

isolated from this study had an effect on the postharvest quality and vase life of the cut *Gladiolus*.

Fungi of the genera *Epidermophyton* and *Syncephalastrum* were also isolated from *Gladiolus* vase water **(Plate 4.18).** A **f**ungus of the Genus *Epidermophyton* was characterized for macroconidia that grow directly from the hyphae with a deep yellowish brown pigment (Kidd *et al*., 2016) (**Plate 4.18** (**a**)). The fungal isolate was from vase water of pulsed unstored *Gladiolus*. *Epidermophyton*species are characterizes by the presence of smooth thin-walled macroconidia growing directly from the hyphae. The colonies are greenish-brown on the front with a deep brown pigmentation. *Epidermophyton* species are anthrophilic dermatophytes with worldwide distribution which often cause tinea pedis, tinea cruris, tinea corporis and onchomycosis (Kidd *et al*., 2016). The presence of *Epidermorphyton* fungus may have affected the quality of the cut *Gladiolus* as a result of competion for the available nutrients. This may have have had an effect on the quality and vase life of the cut *Gladiolus.*

Sycephalastrum species (**Plate 4.18 (d**., **e**., **f**)) was isolated from vase water of cut *Gladiolus*that had been pulsed with 600 ppm 8- HQS plus 5% sucrose solution before isplay at ambient temperatures.The sporangial wall shrinks at maturity so that the spores appear in chains reminiscent of *Aspergillus* (Webster & Weber, 2007). Fungi in the genus *Syncephalastrum* have been isolated from the soil and dung (Kidd *et al*., 2016). The fungal diversity in a strawberry field included *Syncephalastrum* species among others (Biyik *et al*., 2018). Fungi in this genus are characterized by the presence of merosporangia and merospores (Ellis *et al.,* 2007). They are also considered as laboratory aerial contaminants Kidd *et al*., 2016). One of the species, *Syncephalatrum racemoseum* has been associated mucomycosis in human and host animals (Webster &Weber, 2007).

Otherwise, fungi of this genus are saprophytes that have been isolated from foodstuffs, cereal grains, other seeds and spicies (Kidd *et al.,* 2016). *Syncephalastrum* are fast growing fungi that are capable of thriving over a wide range of temperatures between 7 – 40 ºC (Kidd *et al*., 2016). These fungi form zygospores resembling other Mucorales (Webster& Weber, 2007). Methanol extracts of *Syncephalastrum racemosum* and *Trichodermalongibrachiatum* endophytes from the leaves of *Markhamia tomentosa* (Berth) K. Schum ex, Engl. *Bongnoniceae* had antifungal activity against pathogenic fungi and also antiproliferatic action on some cancer lines (Ibrahim *et al*., 2017). Fungi belonging to the genera *spergillus, Penicillium, Alternaria, Rhizopus* and *Syncephalastrum* have been isolated as contaminants in medicinal plants during storage (Siakrwar *et al*., 2014).

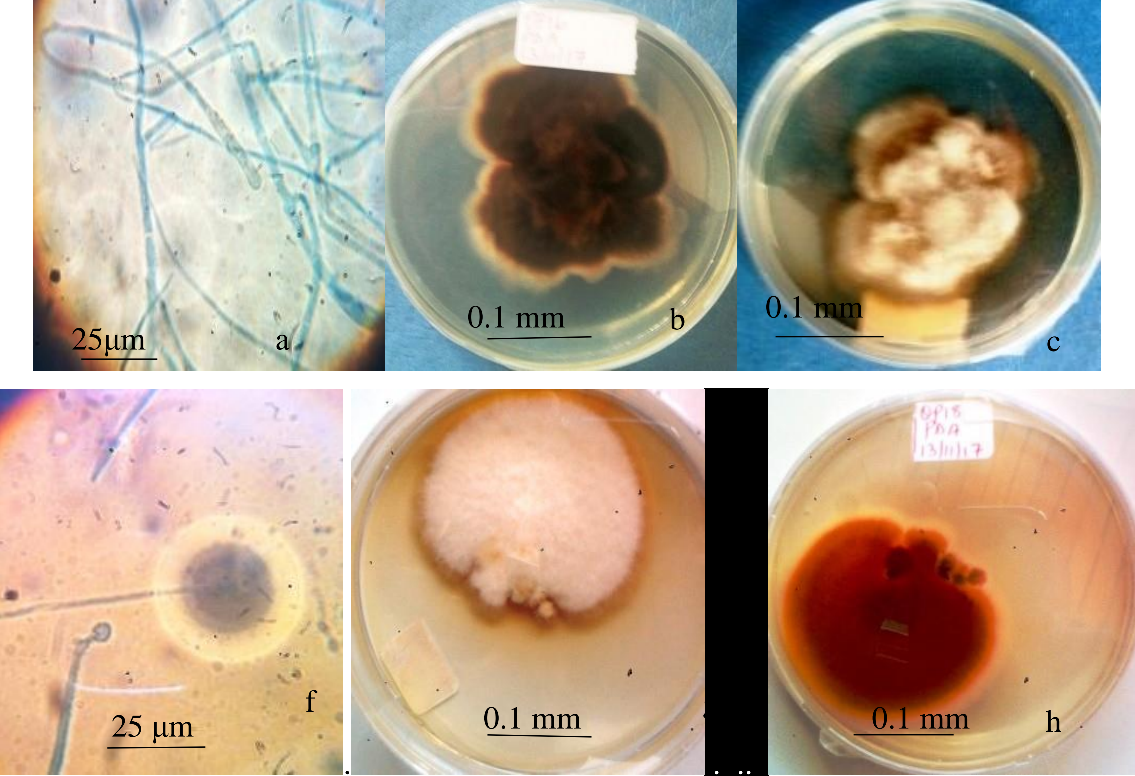


Plate 4.18: Microscopic appearance of *Epidermophyton* species (a)., The front (b) and the reverse (c) of the *Epidermophyton floccosum*., *Syncephalastrum Mucorale* from vase water of unstored pulsed *Gladiolus* spikes (d)., *Syncephalastrum* species showing cottony to fluffy white (e) colonies with red exudate (f)

Syncephalastrum species were among fungal isolates from selected crude herbal extracts (Toma *et al*., 2013). The presents of the *Syncephalastrum* species in the vase water of *Gladiolus* may have contributed to the quality and vase life of this cut flowers either as endophytic or contaminant. Fungi belonging to the genera *Histoplasma* and synnematous *Cephalotricum* (**Plate 4.19 (a., b)**) were alos isolated from the vase water of *Gladiolu*s cut flowers. The fungal isolate on **Plate 4.19 (a)** refer to the microscopic appearance of *Histoplasma*- like structure. Fungi in the genus *Histoplasma, Pythium* and *Sepedonium* are all characterized for possesing macroconidia (Kidd *et al*., 2016). *Sepedonium* differs from *Histoplasm*a by not being dimorphic, not forming microconidia and not reacting with specific *Histoplasma* probes. All the three genera above have worldwide distribution (Antinori, 2014;Teixeira *et al*., 2016).

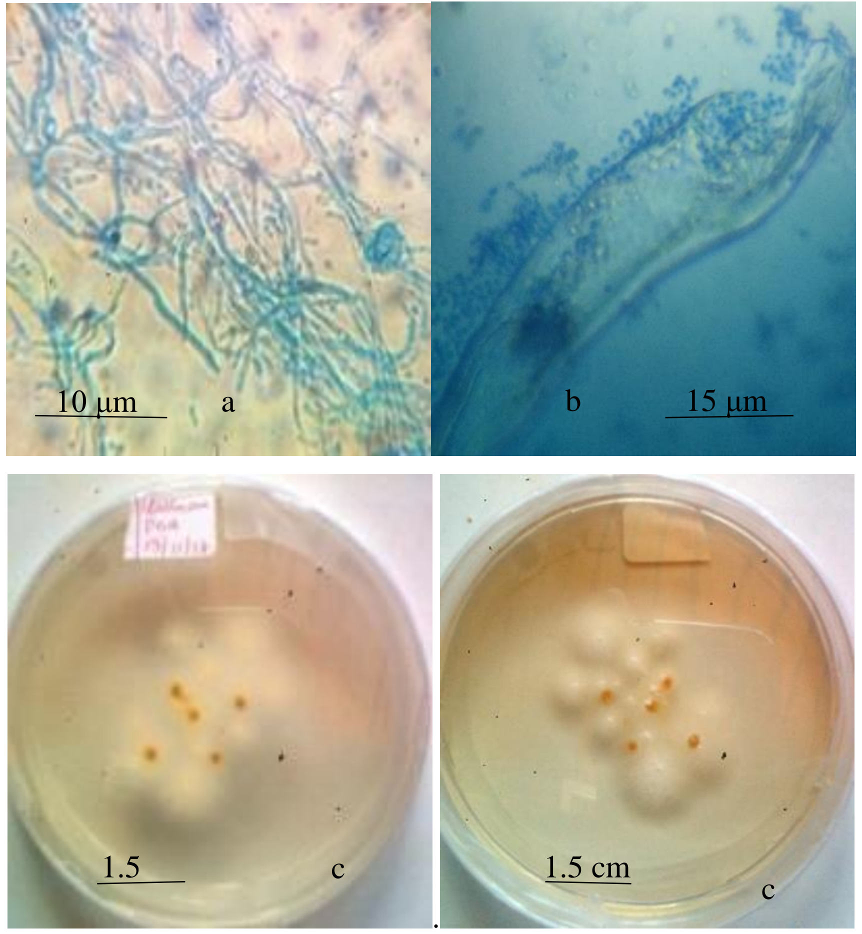


Plate 4.19: Histoplasma-like Fungus species with tuberculate macroconidia and globose microconidia (a)., synnematous microscopic appearance of the fungus in the genus *Cephalotricum* (b)., front (c) and reverse (d)surfaces of Histoplasma –like species after 14 days incubation for 5 days at 28 ±2 ºC on PDA

Sepedonium species was one of the fungal contaminants in smokeless tobacco products (Saleem *et al*., 2018). Fungi in the genus *Pythium* are morphologically polymorphic; found ubiquitous in soil and water, with diverse host ranges (Al-Sheikh, 2010). These fungi are the causal agents of damping – off, rots of seedlings, roots or basal stalks, decay of fruits, vegetables (during storage and transit) and generally causes sereous damage of various crops ((Al-Sheikh, 2010). *Pythium* has been implicated in the stem rot of Rose and *Chrysanthemum* (Tsukiboshi *et al*., 2007). A species of *Pythium, P. ultimum*, caused plant necrotrophy (Fawke *et al.,* 2015). The morphologically indistinguishable species of *Sepedonium* are credited for their role as biocontrol agents (Kiss, 2003). One of the species, *Sepedonium tulasneanum* produced tulasporins which had growth inhibitory activity against *Botrytis cinerea* and *Phytophthorainfestans* (Otto *et al*., 2016). In another study, the *Sepedonium* teleomorph *Hypomyles* was infective against basidiomycetus yeasts from the boletales fruiting bodies

(Yurkov *et al*., 2012). Another species of *Sepedonium*, *S. amplulosporum* produced an alkaloid (isoquinoline) which had inhibitory activity against the plant pathogen, *Cladosporium cucumerinum* (Quang *et al*., 2010). *Sepedonium* species are saprophytes but can be causative agents of fatal pneumonia in immunocompromised individuals (Areiano *et al*., 2017). They are filamentous fungi which inhabit the soil and plant materials (Kidd *et al*., 2016). From the cited literature, the fungus in **Plates 4.19 (a)** may have had an effect on the vase life and quality of the cut *Gladiolus* spikes. The use of molecular probes could help unravel the identity of this isolate.

The fungus in **Plate 4.19** (**b**) is a *Trichurus* species; a *Microascaceae* belonging to the synnematous genera of fungi (Sandowal-Denis *et al*., 2016). This fungus has a worldwide distribution , found inhabiting the soil and air (Manoharachany *et al*., 2005). Other synnematous fungi include *Cephalotricum*, *Doratomyces* and other related species such as *Gamsia*, *Wardomyces* and *Wardomycopsis* whose identification has been based on their morphology (Sandowal-Denis *et al*., 2016). A species of *Trichurus; T. spiralis* has been renamed as *Cephalotricum. gorgonifer,* which is the most abundant species isolated indoors (Woundenberg *et al*., 2017). An extract of this species of marine origin had antioxidant and free radical scavenging properties (Nehad *et al*., 2013). It is thus probable that the fungus isolate in **Plate 4.19** (**b**) affected the quality and vase life of the cut *Galdiolus* either by competing for nutrients or by by intermediary association to influence flower growth.

The relationship and interaction of the microbes in the vase water of the cut *Gladiolus* flowers needs to be established in this study. The pathogenicity / coorperativity of the microorganisms should be elucidated to ascertain the role each of the microbes may play in the postharvest life of the cut *Gladiolus grandiflorus* L. cv. ‘Fado’. Morphological profiling of the microbes should be coupled with phylogenetic and use of appropriate molecular specific probes to ascertain identification of the fungi. Quarum sensing studies can be undertaken to show the coorperation versus the competition interactions in the microbial populations in view of establinshing an effective biocide to control their populations. The interaction of pure colonies of the microbes profiled in this study upon cut *Gladiolusgrandiflorus* L. cv. ‘Fado’ can be done by assessing their pathogenic/ commensal relationship with this cut flower. Phytosanitary conditions should also be enhanced in view of ascertaining the epiphytic and endophytic populations versus the contaminants during the postharvest handling of the cut *Gladiolus grandiflorus* L. cv. ‘Fado’. However, this study established that the microbial flora in the cut *Gladiolus* vase water were significantly lowered in pulsed and wet cold spikes compared with

the non-pulsed spikes. The lowerd microbial load correlated with improved vase life and quality parameters in the cut pulsed cut *Gladiolus* flowers compared with the control.

CHAPTER FIVE
CONCLUSIONS AND RECOMMENDATIONS

5. 1 Conclusions

1. Effect of pulsing and wet cold storage durationon cut *Gladiolus* quality and vase life

The pulsing treatment coupled with cold storage had significant effect on the vase life and post-harvest quality of cut *Gladiolus grandiflorus* cv. Fado'. The quality parameters of fresh and dry weight, number of open florets per spike and water uptake were superior in *Gladiolus* spikes pulsed with 600 ppm 8-hydroxyquinoline sulphate coupled with 5% sucrose solution in comparison with the control. The pulsing treatment coupled with cold storage had significant effect on the vase life (4.75- 11.5 days; 142 % increase). The pulsing treatment elicited superior postharvest quality parameters: fresh weight on the third day in the vase (11.67 %); floral attributes i.e. opened florets (59.8 %); reduced unopened buds (61-28.88 %). There waselevated mean water uptake (23.15-31.98 milliliters, 38.14% increase) on the third day in the vase in pulsed wet cold stored cut gladioli compared with the control.It can be concluded that *Gladiolus* cut flowers can be wet stored at 3 ± 1°C up to a maximum of 4 days without any adverse effect on their subsequent vase life and quality. These results will assist stake holders who adopt the pulsing treatment of 600 ppm 8-HQS plus 5% sucrose with wet cold (3 ± 1°C) storage to minimize postharvest losses in cut*Gladiolus grandiflorus* cv. 'Fado'. Stake holders (mainly small holder farmers) may bulk and transport their produce within 4 days without compromising the vase life and quality parameters of the cut.*Gladiolus grandiflorus* cv. 'Fado' spikes.

2. Changes in the biomarkers: total starch, total soluble sugars, total free amino acids and totalphenols during senescence of pulsed wet cold stored cut gladioli flowers

This study establishedthat the biomarkers total starch, total soluble sugars, total free amino acids and total phenols trend could be used in assesement of vase life and quality parameters in cut *Galdiolus* (L. cv, 'Fado').The following trends could be correlated to the vase life and quality parameters of the cut spikes:(a) Maximum number of vase life (11.50 days) for pulsed and wet cold stored cut *Gladiolus* flowers was observed in spikes that attained highest levels of starch (10.33-42.29 μg/mg dw.; 309.39% increase) on the third day in the vase compared with the control; (b) The concentration of theTotal Soluble Sugars as a quality biomarker was significantly enhanced (24.01-48.30 μg/mg dw.; 101.16% increase., 33.83-45.67 μg/mg dw.; 11.84% increase) in pulsed wet cold stored cut *Gladiolus*on the third day in the vase and at senescence respectively, which also correlatedwith maximum vase life (11.50 days)compared

with the control; (c) Levels of Total free amino acids decreased(36.50-28.48µg/mg dw.; -21.97% reduction) on the third day in the vaseand at senescence (25.33-19.39 µg/mg dw.; 23.45% reduction) in pulsed wet cold stored cut *Gladiolus* which correlated with maximum vase life (11.50 days) and other quality parameters compared with the control; (d) Maximum accumulation of total phenols on the third day (24.37-49.50 µg/mg dw.; 104.14% increase) and at senescence (5.46-27.51 µg/mg dw); 441% increase) was registered in *Gladiolus* spikes wet cold stored for three days after pulsing with 600 ppm 8-HQS plus 5% sucrose; which also coincided with improved vase life (11.50 days) and other quality parameters compared with the control.

3. Effect of pulsing and wet cold storage on the anthocyanins in cut *Gladiolus*

The maximum levels of anthocyaninswere higher on the third in the vase (6.93-8.8 µg/mg dw;26.98% increase) and at senescence (6.25-7.52 µg/mg dw ; 20.32% increase) in *Gladiolu*s pulsed and wet cold for two days which attained superior post harvest parameters and attained a higher vase life (4.75-10 days; 122.22%); that was not significantly different from the best vase life attained in this study (11.50 days) compared with the control.

4. Effect of pulsing and wet cold storage on subsequent microbial flora in vase water of cut *Gladiolus*

The pulsing treatment of 600 ppm 8-HQS plus 5% sucrose reduced the meanmicrobial proliferation on the third day of the shelf life(3.288 $\log_{10}$ cfu ml^{-1}-3.261 $\log_{10}$ cfu ml^{-1};6.032% decrease) and at senescence 6.229$\log_{10}$ cfu ml^{-1}-5.92 $\log_{10}$cfuml^{-1}; 50.9 %) in the vase water of the cut *Gladiolus* flowers which translated into enhanced mean vase life (9.13-10.33 days; 13.14% and quality parameters in the pulsed wet cold stored spikes compared with the non – pulsed spikes. The probable microbial isolates from the control included the plant pathogens*Sepedonium, Pythium*, and *Nigrospora* while the bacteria belonged to*Pseudomonas putida, Shewanella putrefaciens* and *Staphylococcus warneri* species. Comparatively, the microbial profiles: *Pantoea agglomerans*, *Serratia marcescens*,*Bacillus* species, *Salmonella* species, *Emericela nidulans,Phytophthora* species and *Acremonium* isolates were from vase water of pulsed cut flowers wet cold stored for 0-5 days before vase study. It is possible that the microbial proliferation in and the species interaction may have had an effect on post harvest quality of the cut *Gladiolus*.

5.2 Recommendations

(i). Pulsing of*Gladiolus* (*Gladiolus grandiflorus* L. cv. Fado) with 600 ppm plus 5% sucrose for 24 hours followed by cold storage (3 ± 1°C) of up to 4 days treatments can be adopted as a postharvest stratege to enhance the vase life, floral attributes and quality parameters of this cut flower.

(ii). Pulsing cut *Gladiolus* (*Gladiolus grandiflorus* cv. Fado) flowers with. with 600 ppm 8-HQS plus 5% sucrose coupled with wet cold storage (3 ± 1°C) of upto 4 days should enhance the concentrations of total starch, total soluble sugars and total phenols while total free amino acids are reduced as biochemical markers during senescence of cut gladioli flowers. The cumulative effect of this trend of the total starch, total soluble sugars, total phenols, total soluble sugars and total free aminoacids correlates with improved vase life, floral attributes and other quality parameters of pulsed Gladiolus cold stored for up to 4 dayscold stored

(iii). Pulsing with 600 ppm 8-HQS plus 5% sucrose coupled with wet cold storage (3 ± 1°C) of upto 4 days should be adopted to enhances the anthocyanin content in the gladioli flowers at harvest, during storage and at senescence which corresponds with improved spike vase life, floral attributes and superior quality parameters.Since colour in cut flowers adds consumer satisfaction, the treatments in this study can be adjusted to accommodate colour enhancing agents in the pulsing treatment coupled with wet cold storage of upto 4 days.

(iv). The pulsing treatment of 600 ppm 8-HQS coupled with wet cold storage (3 ± 1°C) (of upto 4 days)should be used as a deterrent measure to lower proliferation of microbial flora and diversity in vase water of cut flowerswhose vase life, floral attributes qualities parameters get enhanced compared to the non-pulsed spikes. in cut *Gladiolus* (*Gladiolusgrandiflorus* cv. Fado).The use of holding solutions with biocidal activity during vase study of the cut *Gladiolus*flowers could improve the vase life and quality of cut *Gladiolus*. Identification of microbes by use of appropriate molecular specific tools could help unravel an effective control strategy in formulating the flower preservatives to be adopted and also type of mode of interactions between microbes and subsequent flower attributes.

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APPENDICES

Appendix A

Analysis of Variance (ANOVA)

Appendix A1: ANOVA data cut *Gladiolus* fresh weight after pulsing

Analysis of the Effect of pulsing with 600 ppm 8- HQS plus 5% sucrose solution and wet cold storage on the fresh weight of cut *Gladiolus grandiflorus* cv. 'Fado'

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	1	1	152.46151	10.0899	0.0031*
Storage duration	5	5	280.81561	3.7169	0.0082*
Treatment*Storage duration	5	5	201.55001	2.6677	0.0376*

Appendix A2: ANOVA data on *Gladiolus* fresh weight on third day in the vase

Analysis of the Effect of pulsing with 600 ppm 8- HQS plus 5% sucrose solution and wet cold storage on the fresh weight on the third day in the vase of cut *Gladiolus grandiflorus* cv. 'Fado'

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	1	1	36.2460	0.2067	0.2994
Storage duration	5	5	1745.8732	1.9912	0.1035
Treatment*Storage duration	5	5	593.1775	0.6765	0.6440

Appendix A3: ANOVA data on *Gladiolus* fresh weight just after storage

Analysis of the Effect of pulsing with 600 ppm 8- HQS plus 5% sucrose solution and wet cold storage on the weight after Storage of the vase of cut *Gladiolus grandiflorus* cv. 'Fado'

Effect Tests

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Treatment	1	1	0.03031	0.0015	0.9690
Storage duration	4	4	711.47745	9.0414	<.0001*

Treatment*Storage duration	4	4	546.45770	6.9443	0.0004*

Appendix A4: ANOVA data for cut *Gladiolu*s dry weight on third day in the vase

Analysis of the Effect of pulsing with 600 ppm 8- HQS plus5% sucrose solution and wet cold storage on the dry weight on the third day in the vase of cut *Gladiolus grandiflorus* cv. 'Fado'

Effect Tests

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Treatment	1	1	3.722431	6.0554	0.0188*
Storage duration	5	5	17.577163	5.7187	0.0006*
Treatment*Storage duration	5	5	4.074529	1.3256	0.2754

Appendix A5: ANOVA data on cut *Gladiolu*s fresh weight at senescence

Analysis of the Effect of pulsing with 600 ppm 8- HQS plus 5% sucrose solution and wet cold storage on the Fresh weight at Senescence of the cut *Gladiolus grandiflorus* cv. 'Fado'

Effect Tests

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Treatment	1	1	1.15972	0.0735	0.7879
Storage duration	5	5	807.66319	10.2348	<.0001*
Treatment*Storage duration	5	5	144.63270	1.8328	0.1311

Appendix A6: ANOVA data on cut *Gladiolu*s dry weight at senescence

Analysis of the Effect of pulsing with 600 ppm 8- HQS plus 5% sucrose solution and wet cold storage on the dry weight at Senescence of the cut *Gladiolus grandiflorus* cv. 'Fado'

Effect Tests

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Treatment	1	1	3.405871	5.3025	0.0272*
Storage duration	5	5	11.114547	3.4608	0.0118*
Treatment*Storage duration	5	5	15.717887	4.8941	0.0016*

Appendix A7: ANOVA data on cut *Gladiolus* number of open florets

Analysis of the Effect of pulsing with 600 ppm 8- HQS plus 5% sucrose solution and wet cold storage on the number of open florets of the cut *Gladiolus grandiflorus* cv. 'Fado'

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	1	1	2.475208	4.0674	0.0512
Storage duration	5	5	38.276042	12.5796	<.0001*
Treatment*Storage duration	5	5	10.976042	3.6073	0.0095*

Appendix A8: ANOVA data on cut *Gladiolus* number of unopened buds

Analysis of the Effect of pulsing with 600 ppm 8- HQS plus 5% sucrose solution and wet cold storage on the number of unopened buds of the cut *Gladiolus grandiflorus* cv. 'Fado'

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	1	1	5.901019	5.4222	0.0256*
Storage duration	5	5	27.866760	5.1211	0.0012*
Treatment*Storage duration	5	5	16.681344	3.0655	0.0209*

Appendix A9: ANOVA data on cut *Gladiolus* vase life

Analysis of the Effect of pulsing with 600 ppm 8- HQS plus 5% sucrose solution and wet cold storage on the vase life of the cut *Gladiolus grandiflorus* cv. 'Fado'

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	1	1	17.520833	16.4902	0.0003*
Storage duration	5	5	95.354167	17.9490	<.0001*
Treatment*Storage duration	5	5	22.354167	4.2078	0.0041*

Appendix A10:ANOVA data on cut *Gladiolus* water uptake after pulsing

Analysis of the Effect of pulsing with 600 ppm 8- HQS plus 5% sucrose solution and wet cold storage on the water uptake after pulsing treatment of the cut *Gladiolus grandiflorus* cv. 'Fado'

Effect Tests

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Treatment	1	1	928.54095	1505.802	<.0001*
Storage duration	5	5	159.81846	51.8351	<.0001*
Treatment*Storage duration	5	5	106.17953	34.4380	<.0001*

Appendix A11: ANOVA data on cut *Gladiolus* water uptake on the third day in thevase

Analysis of the Effect of pulsing with 600 ppm 8- HQS plus 5% sucrose solution and wet cold storage on the water uptake on the third day in the vase of the cut *Gladiolus grandiflorus* cv. 'Fado'

Effect Tests

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Treatment	1	1	937.2346	1042.218	<.0001*
Storage duration	5	5	1611.1054	358.3145	<.0001*
Treatment*Storage duration	5	5	398.7638	88.6862	<.0001*

Appendix A12:ANOVA data on cut *Gladiolus* water uptake at senescence

Analysis of the Effect of pulsing with 600 ppm 8- HQS plus 5% sucrose solution and wet cold storage on the water uptake at senescence of the cut *Gladiolus grandiflorus* cv. 'Fado'

Effect Tests

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Treatment	1	1	557.8283	343.2244	<.0001*
Storage duration	5	5	1720.2672	211.6915	<.0001*
Treatment*Storage duration	5	5	113.3869	13.9531	<.0001*

Appendix A13: ANOVA data on cut *Gladiolus* initial microbial population in vase water

Analysis of the Effect of pulsing with 600 ppm 8- HQS plus 5% sucrose solution and wet cold storage on the number of the initial microbial population in vase water of the cut *Gladiolus grandiflorus* cv. 'Fado'

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	1	1	0.0354253	28.5746	<.0001*
Storage duration	5	5	8.1403347	1313.222	<.0001*
Treatment*Storage duration	5	5	2.5921017	418.1652	<.0001*

Appendix A14: ANOVA data on cut *Gladiolus* vase water microbial population on the third day

Analysis of the Effect of pulsing with 600 ppm 8- HQS plus 5% sucrose solution and wet cold storage on the number of microbial population on the third day in the in vase water of the cut *Gladiolus grandiflorus* cv. 'Fado'

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	1	1	0.008480	0.3728	0.5453
Storage duration	5	5	15.480381	136.1013	<.0001*
Treatment*Storage duration	5	5	13.237629	116.3834	<.0001*

Appendix A15: ANOVA data on cut *Gladiolus* microbial population in vase water at senescence

Analysis of the Effect of pulsing with 600 ppm 8- HQS plus 5% sucrose solution and wet cold storage on the number of microbial population at senescence in the in vase water of the cut *Gladiolus grandiflorus* cv. 'Fado'

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	1	1	1.096563	5.8355	0.0209*
Storage duration	5	5	11.205810	11.9266	<.0001*
Treatment*Storage duration	5	5	13.812067	14.7005	<.0001*

Appendix A16: ANOVA data on cut *Gladiolu*s TSS on the third day in the vase

Analysis of the Effect of pulsing with 600 ppm 8- HQS plus 5% sucrose solution and wet cold storage on the total soluble sugars on the third day in the cut *Gladiolus grandiflorus* cv. 'Fado'

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	1	1	138.996	222.3874	<.0001*
Storage duration	5	5	12640.094	4044.731	<.0001*
Treatment*Storage duration	5	5	88.491	28.3165	<.0001*

Appendix A17:ANOVA data on cut *Gladiolu*s TSS at senescence

Analysis of the Effect of pulsing with 600 ppm 8- HQS plus 5% sucrose solution and wet cold storage on the total soluble sugars at senescence in the cut *Gladiolus grandiflorus* cv. 'Fado'

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	1	1	293.387	104.5747	<.0001*
Storage duration	5	5	19650.578	1400.849	<.0001*
Treatment*Storage duration	5	5	49.210	3.5081	<.0001*

Appendix A18:ANOVA data on cut *Gladiolu*s TS at the third day in the vase

Analysis of the Effect of pulsing with 600 ppm 8- HQS plus 5% sucrose solution and wet cold storage on the total starch on the third day in the in the cut *Gladiolus grandiflorus* cv. 'Fado'

Effect Tests

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Treatment	1	1	72.9221	167.6086	<.0001*
Storage duration	5	5	1821.4894	837.3244	<.0001*
Treatment*Storage duration	5	5	34.6691	15.9338	<.0001*

Appendix A19: ANOVA data on cut *Gladiolu*s TS at senescence

Analysis of the Effect of pulsing with 600 ppm 8- HQS plus 5% sucrose solution and wet cold storage on the total soluble starch at senescence in the in the cut *Gladiolus grandiflorus* cv. 'Fado'

Effect Tests

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Treatment	1	1	34.93206	33.0127	<.0001*
Storage duration	5	5	939.11402	177.5029	<.0001*
Treatment*Storage duration	5	5	36.04680	6.81232	<.0001*

Appendix A20: ANOVA data on cut *Gladiolu*s TFAA on the third day in the vase

Analysis of the Effect of pulsing with 600 ppm 8- HQS plus 5% sucrose solution and wet cold storage on the total free amino acids on the third day in the cut *Gladiolus grandiflorus* cv. 'Fado'

Effect Tests

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Treatment	1	1	63.0185	129.2838	<.0001*
Storage duration	5	5	4674.6280	1918.018	<.0001*

Treatment*Storage duration	5	5	1617.1830	663.5365	<.0001*

Appendix A21: ANOVA data on cut *Gladiolu*s phenols on the third day in the vase

Analysis of the Effect of pulsing with 600 ppm 8- HQS plus 5% sucrose solution and wet cold storage on the total phenols on the third day in the vase of the in the cut *Gladiolusgrandiflorus* cv. 'Fado'

Effect Tests

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Treatment	1	1	265.0659	6.2742	0.0169*
Storage duration	5	5	1287.7350	6.0962	0.0003*
Treatment*Storage duration	5	5	320.2618	1.5161	0.2092

Appendix A22:ANOVA data on cut *Gladiolu*s total phenols at senescence

Analysis of the Effect of pulsing with 600 ppm 8- HQS plus 5% sucrose solution and wet cold storage on the total phenols at senescence in the cut *Gladiolus grandiflorus* cv. 'Fado'

Effect Tests

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Treatment	1	1	958.0107	495.6320	<.0001*
Storage duration	5	5	1257.7142	130.1370	<.0001*
Treatment*Storage duration	5	5	1307.9704	135.3371	<.0001*

Appendix A23: ANOVA data on cut *Gladiolu*s anthocyanins on the third day in the vase

Analysis of the Effect of pulsing with 600 ppm 8- HQS plus 5% storage on the anthocyanins on the third day in the vase of the cut *Gladiolus grandiflorus* cv. 'Fado'

Effect Tests

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Treatment	1	1	0.00067500	1.7564	0.1934
Storage duration	5	5	0.00984942	5.1256	0.0012*
Treatment*Storage duration	5	5	0.01002175	5.2153	0.0011*

Appendix A24: ANOVA data on cut *Gladiolus* anthocyanins at senescence

Analysis of the Effect of pulsing with 600 ppm 8- HQS plus 5% sucrose solution and wet cold storage on the anthocyanins at senescence in the cut *Gladiolus grandiflorus* cv. 'Fado'

Effect Tests

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Treatment	1	1	0.00099919	3.5610	0.0672
Storage duration	5	5	0.01066185	7.5996	<.0001*
Treatment*Storage duration	5	5	0.03283619	23.4051	<.0001*

Appendix B

Peer Reviewed Journal Publications

Appendix B1:Peer reviewed paper in *Journal of Ornamental Plants*

Journal of Ornamental Plants
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Effect of Pulsing and Wet Cold Storage on the Quality and Vase Life of Cut Gladiolus (*Gladiolus grandiflorus* L.) 'Fado'

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Abstract

Floriculture is among Kenya's top foreign exchange earners. *Gladiolus* is one of the four famous cut flowers in the world whose popularity in Kenya is attributed to its heat tolerance, many spike forms and color combinations. However, the perishable nature of the flower renders it vulnerable to huge post-harvest losses. The study aimed to evaluate pulsing effect of cut *Gladiolus grandiflorus* 'Fado' with 600 ppm 8-hydroxyquinoline sulfate plus 5 % sucrose prior to wet cold storage duration (0–5 days) on quality and vase life against the control (distilled water). The *Gladiolus* were grown in the open field from corms at the Horticulture Research and Teaching Field, in the Department of Crops, Horticulture and Soils, Egerton University, Kenya, during two successive seasons. A two by six factorial experiment embedded in a completely randomized design with four replicates was adopted. Pro GLM model in two way ANOVA was used to determine differences using Tukey's test at 5 % level of significance. Pulsing treatment had significant effects on the *Gladiolus* quality parameters including: Fresh weight ($P = 0.0031$; 82.214 ± 0.7934 g) as compared to the control; dry weight ($P = 0.0272$); interactive effect of the pulsing and cold storage duration treatments ($P = 0.0004$); maximum vase life (11.5 ± 0.287 days) and opened florets (11 ± 0.15). The highest number of unopened buds (5.18 ± 0.212) were recorded in the control which also had least mean water uptake (23.87 ± 0.26 ml) as compared with the pulsed and cold stored spikes (31.98 ± 0.193 ml). Pulsed cold stored *Gladiolus* preserved quality up to 4 days. The knowledge gained from this study will augment existing technologies in improving quality and market value of this cut flower.

Keywords: Duration, Losses, Sucrose, 8-hydroxyquinoline sulfate.

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AppendixB2: Peer reviewed paper in*Journal of Progressive Horticulture*

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[Research Article]

Changes in total free amino acids as quality biomarkers in cut gladiolus spikes as affected by pulsing and wet cold storage

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ABSTRACT

This study aimed at evaluating and quantifying Total Free Amino acids (TFAA) as indicators of quality in cut *Gladiolus grandiflorus* L. cv. Fado as affected by pulsing with 600 ppm 8-hydroxyquinoline sulphate plus 5 % sucrose and subsequent wet-cold storage durations of 0 – 5 days. Spectrophotometric and thin layer chromatography of dried petal amino acid analyses of open field grown *Gladiolus* at Horticulture Research and Teaching Field at Egerton University, Kenya, were done. A two by six factorial experiment embedded in a completely randomized design with four replicates was adopted and data was analyzed using Proc GLM model in two way Anova. Means were separated by Tukey's test at 5 % level of confidence. Values of TFAA and the vase life were significantly ($P < 0.0001$) affected by the pulsing and wet-cold storage treatments with consequent enhanced mean vase life (10.33 ± 0.21 days) and lowered (28.006 ± 0.006 µg/mg dw) mean TFAA compared with that of the control (4.75 ± 0.21 days; 36.496 ± 0.006, respectively). Petals predominantly displayed proline, lysine, leucine, isoleucine, glutamine, glycine and threonine, which could be alternative respiratory substrates or physiological signals. The results from this study will augment existing technologies in improvement of postharvest handling of Gladiolus.

KEY WORDS: Gladiolus, pulsing, cold storage, vase life

Plant growth and development may be exposed to a number of abiotic stresses such as osmotic stress, salinity, excess or limitation of essential ions, heavy metals, high temperature, freezing, ozone, excess light and nutrient deficiency (Claeys *et al.*, 2014). Plants may respond to abiotic stresses in a variety of ways by accumulating a variety of compatible solutes and amino acids including proline (Szabados and Savoure, 2009), derivatives such as γ-aminobutyric acid (Signorelli *et al.*, 2015) and polyamines (Rakesh *et al.*, 2014). There are changes in the activities of enzymes involved in production/scavenging of reactive oxygen species and in expression of hundreds of genes (Carlson, 2014). Senescence is the final stage that marks the hallmark of programmed cell death (Shahri and Tahir, 2010). Protein degradation is an important process concomitant with the visible signs in petal senescence (Wagstaff *et al.*, 2002). The rise in some amino acids in plants during senescence or carbohydrate starvation predisposes them as alternative respiratory substrates (Hildebrandt *et al.*, 2015). Branched chain and aromatic amino acids increase during stress (Fernie and Stitt, 2012). The pool of free amino acids range in accordance with respective functions, protein biosynthesis, fine-tuned metabolic pathways' involvement and signal transduction processes (Hildebrandt *et al.*, 2015). The upsurge of proline relates to the oxidative stress a plant encounters (Aghdam *et al.*, 2017). Aromatic amino acids induce vegetative growth, improve chlorophyll a and b and promote early flowering in *Amaryllis* (*Hippeastrum vittatum*, Herb) and *Gerbera jamesonni* production (Geshnizjani and Khosh-Khui., 2016).

The sulphate and citrate salts of 8-hydroxyquinoline are the most commonly used biocidal compounds in flower handling (Loubaud and van Doorn, 2004). They act to influence enzyme activity by reducing the solution pH (Damunupola and Joyce, 2008). The solution of 8-HQS @ 600 ppm in combination with 5 % Sucrose at a pH of 2.78 used in pulsing *Gladiolus* may have inhibited microbial proliferation. 8-HQS promotes water uptake, stomata closure and exhibits chelating activity that renders it biologically active (Prachayasittikul *et al.*, 2014.;

Appendix B3: Peer reviewed paper in *African Crop Science Journal*

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CHANGES IN CARBOHYDRATES ASSOCIATED WITH SENESCENCE OF CUT GLADIOLUS SPIKES UNDER PULSING AND WET COLD STORAGE DURATIONS

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ABSTRACT

Several biochemical and metabolic changes are associated with senescence of cut gladioli, particularly in relation to quality characteristics, including dynamism of carbohydrates. The objective of this study was to evaluate the effect of pulsing and wet cold storage on the starch and sugar biomarkers on cut Gladiolus (*Gladiolus grandiflorus* L. cv. Fado) vase quality. Pulsing treatments of 600-ppm 8-hydroxyquinoline sulphate, plus 5% sucrose solution, *versus* distilled water, were administered prior to wet cold storage durations of 0 - 5 days, on the cut Gladiolus grown from corms. This was done in the open field at the Horticulture Research and Teaching Field, Egerton University in Kenya, during two successive seasons. There was a significant difference ($P < 0.01$) in total soluble sugars (TSS) and total starch of spikes pulsed with 600 ppm 8 - HQS + 5% sucrose, compared with the control, during the third day in the vase life of the cut flowers. Prolonged vase life of cut spikes was associated with a decrease in total soluble sugars and increase in total starch, as influenced by pulsing and wet storage duration up to 4 days.

Key Words: *Gladiolus grandiflorus,* soluble sugar, starch

Appendix B4: Kibabii conference attendance abstract paper

Effect of Pulsing and Wet Cold Storage on Post Harvest Quality and Vase Life of Cut Gladiolus (*Gladiolus Grandiflorus*) L. Cv. Fado

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Citation: KIBU International Conference (2018). Creativity and Innovation for Sustainable Development. Book of Abstracts of Kibabii University 3rd International Conference 12 - 14 June 2018. Kibabii University Main campus, Bungoma Kenya ISBN: 978-9966-59-011-5

Abstract
Floriculture is among Kenya's top foreign exchange earners..*Gladiolus* is one of the four famous cut flowers in the world whose popularity in Kenya is attributed to its heat tolerance, many spike forms and color combinations.. However, the perishable nature of the flower renders it vulnerable to huge post-harvest losses. The study aimed to evaluate pulsing effect of cut *Gladiolusgrandiflorus* L. CV, Fado with 600 ppm 8-hydroxyquinoline sulphate plus 5 % sucroseprior to wet cold storage duration (0 – 5 days) on quality and vase life against the control (distilled water). The *Gladiolus* were grown in the open field from corms at the Horticulture Research and Teaching Field, in the department of Crops, Horticulture and Soils, Egerton University, Kenya, during two successive seasons. .A two by six factorial experiment embedded in a completely randomized design with four replicates was adopted. Pro GLM model in two way Anova was to determine differences in pulsing and cold storage treatments on the flower quality and vase life. Differences in means were determined using Tukey's test at 5 % level of significance. Pulsing treatment had significant effects on the Gladiolus quality parameters including: fresh weight (P = 0.0031; 82.214 ± 0.7934 grams) as compared to the control; dry weight (P = 0.0272); interactive effect of the pulsing and cold storage duration treatments (P = 0.0004); maximum vase life (11.5 ± 0.287 days) and opened florets (11 ± 0.15). The highest number of unopened buds (5.18 ± 0.212) were recorded in the control which also had least mean water uptake (23.87± 0.26 mls) as compared with the pulsed and cold stored spikes ((31.98 ± 0.193 mls).The knowledge gained from this study will augment existing technologies in improving quality and market value of this cut flower.

Key Words: Pulsing; Cold Storage: Gladiolus, Quality

Appendix C

Application for exemption from obtaining ethical clearance

EGERTON UNIVERSITY

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P. O. BOX 536
EGERTON

DIVISION OF RESEARCH AND EXTENSION

EU/RE/DVC/009 **18th February, 2020**

Judith Kavulani Chore

Dear Judith,

RE: APPLICATION FOR EXEMPTION FROM OBTAINING ETHICAL CLEARANCE

Following your application for exemption from obtaining ethical clearance on your research project " *Biochemical Changes and Microbial Storage Profiles Affecting Quality and Vase of Cut Gladioli(Gladiolus grandiflorus)* ".

I wish to inform you that your research project mets criteria for exempt from ethical clearance. however we cannot give letter exemption retrospectively since your study commenced prior to your request for clearance.

Thank you.

EGERTON UNIVERSITY RESEARCH ETHICS Date. 18/2/2020

Prof. J. K. Kipkemboi.
CHAIRMAN, EGERTON UNIVERSITY RESEARCH ETHICS CTTEE

JKK/BK/

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Appendix D

Research licence

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Date of Issue: 10/March/2020

RESEARCH LICENSE

This is to Certify that Ms.. JUDITH KAVULANI CHORE of Egerton University, has been licensed to conduct research in Nakuru on the topic: BIOCHEMICAL CHANGES AND MICROBIAL PROFILES AFFECTING QUALITY AND VASE LIFE OF CUT GLADIOLI (Gladiolus grandiflorus L. FADO) FOLLOWING PULSING AND COLD WET STORAGE for the period ending : 10/March/2021.

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