PHYSIOLOGY OF SENESCENCE AND POSTHARVEST HANDLING IN SOME SELECT ORNAMENTAL FLOWERS OF CARYOPHYLLACEAE

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CERTIFICATE

Certified that ***Mr. Riyaz Ahmad Dar*** has worked under my supervision for his M. Phil. programme in Botany. The data contained in this dissertation is the bonafide work of the candidate. The dissertation entitled ***"Physiology of Senescence and Postharvest Handling in Some Select Ornamental Flowers of Caryophyllaceae"*** is forwarded herewith for evaluation.

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DEDICATED

TO MY

PARENTS

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LIST OF ABBREVIATIONS

ABA	Abscissic acid
ACC	1-aminocyclopropane-1-caboxylic acid
ACO	1-aminocyclopropane-1-caboxylic oxidase
ACS	1-aminocyclopropane-1-caboxylic synthase
AIF	Apoptosis inducing factor
AOA	Aminooxyacetic acid
APX	Ammonium persulphate
ATP	Adenosine triphosphate
AVG	Amino-ethoxyvinylglycine
BA	Benzyladenine
BAP	Benzyl amino purine
BSA	Bovine serum albumin
CAT	Catalase
cDNA	Complementary deoxyribonucleic acid
CHI	Cycloheximide
$CoCl_2$	Cobalt choloride
D	Day
DNA	Deoxyribonucleic acid
DW	Distilled water
EDTA	Ethylene diamene tetra acetate
ERS	Ethylene receptors sensor

GA_3	Gibberellic acid
GR	Glutathione reductase
8-HQS	Hydroxyquinoline sulphate
h	Hour
IAA	Indole acetic acid
JA	Jasmonic acid
LOX	Lipoxygenase
LSD	Least significant difference
mRNA	Messenger ribonucleic acid
NO	Nitrous oxide
PA	Polyamines
PAGE	Polyacrylamide gel electrophoresis
PCD	Programmed cell death
PCR	Polymerase chain reaction
PHDS	Postharvest dry storage
PHWS	Postharvest wet storage
PVP	Polyvinyl pyrrolidone
RH	Relative humidity
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RT	Room temperature
SA	Salicylic acid

SAG	Senescence associated genes
SAM	S-adenosyl methionine
MDA	malondialdehyde
SDS	sodium dodecyl sulphate
GST	glutathione S-transferase
SOD	Superoxide dismutase
SPD	Spermidine
SPM	Spermine
STS	Silver thiosulphate
Suc	Sucrose
TCA	Trichloroacetic acid
TEMED	Tetraethylmethylenediamine
Wm^{-1}	Watts per meter square

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CHAPTER-1

INTRODUCTION

Flowers are a part of the most important occasions in our lives. They are conspicuously presented on birthdays, funerals, graduation, weddings, etc. forming an integral part of our lives. Some flowers have religious significance too. It is not pertinent for any person to be completely unaware of meanings attributed to various flowers. For instance, *Dianthus* symbolizes pride and beauty. A red *Dianthus* symbolizes love, pride and admiration; a pink *Dianthus* symbolizes the love of a woman or a mother; a purple *Dianthus* symbolizes capriciousness; a yellow *Dianthus* symbolizes disdain, rejection or disappointment; while as a white *Dianthus* symbolizes innocence and pure love. A striped *Dianthus* conveys refusal. However, meanings are associated not only with roses and *Dianthus* but also with other flowers. Most people do not consider flower meanings before gifting flowers. Similarly, people who receive flowers may not know their meaning and hence miss the underlying message.

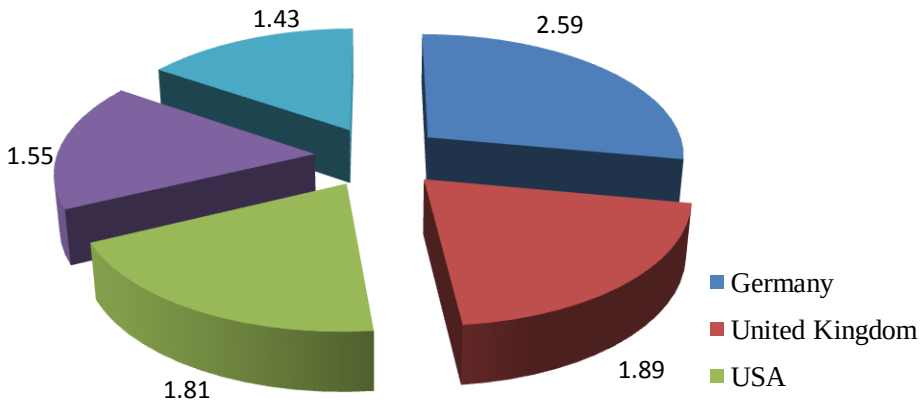
A cut flower can simply be defined as any flower that is cut from the plant, trimmed and used in a fresh flower arrangement. Cut Flowers are available at the florist or can be cut from the home garden. Most cut flowers are popular choices as gifts on special occasions, either as a single cut flower or as a bunch or a bouquet of cut flowers. Rose is the most popular cut flower. Dianthus, Gerberas, Chrysanthemums also enjoy a huge demand in the cut flower market. Gladioli, Lilies, Alstroemerias, Anthuriums etc., are also popular among flower lovers.

India ranked 23rd among the world exporters of floricultural products and its share in world exports was negligible at around 0.38%, 46 million US $ (Export-Import bank, India, 2007). In India the area under flowers has crossed one-lakh hectares concentrated mostly in Karnataka, Tamil Nadu, Andhra Pradesh, Maharashtra, and West Bengal (Patil *et al*., 2011). According to a report of the APEDA, the total area under flower crops in 2011-12 was estimated to be around 190, 000 ha. The productions of flowers are estimated to be 1.031 million loose flowers and 690.27 million cut flowers in 2011-12. The country has exported 3,09,26,023 MT of floriculture products to the world for the worth 73.06 million US $ in 2011-12. Growing at a compounded annual growth rate (CAGR) of about 30 per cent, India's

floricultural industry is likely to cross the 8 billion US $ mark by 2015. The floriculture industry in India is poised at about 3,70 billion US $ with just 0.61 per cent share in the global floriculture sector, which is likely to reach 0.89 per cent by 2015 (ASSOCHAM, 2013). Besides, the global floriculture industry is likely to cross 900 billion US $ mark by 2015 from about US$ 12 million now and is growing at a CAGR of 15 per cent. With a share of about 65 per cent rose flower industry in India accounts for over 2,40 billion US $ of the overall floriculture industry and rose accounts for 75 per cent of the global floriculture industry. Rising demand from Tier II and III cities apart from urban centres is likely to spur demand for roses this Valentine's Day (14^{th} feb. 2013), as price of export quality cut rose is likely to quadruple from its current average ruling price of about Rs 15 to Rs 20 per stem. The study survey carried by ASSOCHAM in Feb, 2013 of 250 rose merchants, including cultivators, exporters, wholesale flower dealers and florists in Bangalore, Chennai, Delhi, Mumbai and Pune to gauge the scenario vis-a-vis business of rose flower during Valentine's week, considering India also to be one of the most world's biggest rose grower. Demand for roses has spiralled upwards by over 25 per cent in domestic and about 30 per cent in international markets as Valentine's Day draws closer, claimed over 55 per cent of all respondents. Fall in the value of rupee against major currencies is the prime reason behind this upsurge in demand for roses in international markets of Australia, Germany, Greece, Italy, New Zealand, Netherlands, the US, UK and other countries of Europe and the Middle East. While, growing demand for roses from cities like Ahmedabad, Chandigarh, Hyderabad, Surat, Kanpur, Lucknow and Patna is driving the high demand for roses in the domestic circuit.

The world Cut Flower Trade is characterized by a high degree of concentration by sources. Exports from the Netherlands to Germany are a principal component of the world Cut Flower trade. The trade makes up a significant chunk of the intra EU trade, which accounts for a large part of the global trade. The global exports of floriculture products stood at US $ 17 billion in the year 2007. Fresh cut flowers and foliage accounted for around 49.1percent (US $ 8.31 billion), and live plants, bulbs and cuttings accounted for 50.9 percent (US $ 8.60 billion) of total floriculture products

exported in 2007. Developed countries in Europe, America and Asia account for more than 90 percent of the total world trade in floriculture products. With an annual average growth rate of 10.3 percent, world exports are expected to reach US $ 25 billion by 2012. The world imports of floriculture products in 2007, stood at US $ 16.7 billion. Germany (US $ 2.59 billion) was the largest importer, followed by the United Kingdom (US $ 1.89 billion), the USA (US $ 1.81 billion), the Netherlands (US $ 1.55 billion), and France (US $ 1.43 billion).



Trade (US$ billions)

In the Americas, Colombia is the major supplier to the United States. Japan receives its supplies from a more diversified base with Taiwan, New Zealand and Europe being the most important suppliers. The Netherlands has a good and functional trade system to facilitate the movement of Cut Flowers which form a majority of the flowers that are traded. Cut Flower growers from all over the world assemble at the famed flower auctions to find suitable buyers for their produce. Flowers are imported from various countries in order to create the largest possible assortment of flowers. Newly established players in the Cut Flower market include Kenya, Ecuador and Zimbabwe supplying to their newly emerging ambitious competitors in China, India, the Republic of Korea, Malaysia, Malawi, Mexico, Palestine, Peru, South Africa and Zambia.

Caryophyllaceae known as “Pink family” include the most beautiful flowers such as *Dianthus caryophyllus* (Pink) and *D. barbatus* (sweet william), *Gypsophila* (baby’s

breath), *Agrostemma* (corn cockle), *Saponaria* (soapwort), *Lychnis* (firepink), and *Silene* (campions). The family comprising of some 96 genera and 2415 species mainly distributed in all temperate parts of the world. Efforts to promote the export of cut flowers need a strong scientific base for developing postharvest handling and strong techniques. It is in this perspective that the present work was undertaken to study flower senescence with an ultimate aim to modulate it in some important members belonging to Caryophyllaceae. The study forms a part of the research work carried in our laboratory on various angiosperm families particularly having ornamental value in order to gain an insight into strategies of flower senescence and identify model systems for studying the important process of flower/petal senescence. Besides the study is aimed to identify the plants within the particular family which have ornamental or cut flower potential. The study is also aimed to develop adequate cost effective storage and handling techniques, besides postharvest protocols for the ornamental plants having cut flower importance in a particular family. During the present investigation experiment in this direction were conducted on *Dianthus caryophyllus, Dianthus barbatus* and *Silene armeria* which are considered to be important ornamentals within the family Caryophyllaceae.

A comprehensive review of literature has been written on "Physiology of flower senescence with special emphasis on Caryophyllaceae" in order to gain an insight into various strategies of flower senescence and important biochemical and molecular events associated with it.

CHAPTER-2

FLOWER SENESCENCE WITH SPECIAL EMPAHIS ON CARYOPHYLLACEAE

Flower senescence with special emphasis on Caryophyllaceae – A review

Senescence generally refers to the process of growing old and consists of those events that are part of genetically programmed events leading to cell death, often referred to as programmed cell death (PCD) in plants (Makrides and Goldthwaite,1981; Noh and Amasino, 1999; Buchanan-Wollaston and Morris, 2000; Rubinstein, 2000; Xu and Hanson, 2000; Mahagamaekera and David, 2001; Leverentz *et al*,. 2002; Wagstaff *et al*,. 2003; Jones *et al*., 2005; Rogers, 2006; Xu *et al*,. 2006; Hoeberichts *et al*,. 2007; Lerslerwong *et al*,. 2009; Yamada, 2009; Shahri and Tahir 2011., Shibuya, 2012). Senescence is an unavoidable process that can be seen in particular at the final stage of ontogenesis, during which irreversible changes are initiated leading to gradual cell disorganisation and death of the organism. This process leads to various physiological and biochemical modifications including loss of water from the senescing tissue, leakage of ions, transport of metabolites to different tissues, generation of reactive oxygen species (ROS), increase in membrane fluidity as also peroxidation, hydrolysis of proteins, nucleic acids, lipids and carbohydrates (Noodén *et al*., 1997; Delorme *et al*., 2000; Thomas *et al*., 2003; Tripathi and Tuteja 2007; van Doorn and Woltering, 2008; Fischer, 2012). Senescence can be referred to those changes that provide for endogenous regulation of death or the process that leads to the death of individuals and/or organs. While as PCD can be defined as the programme whereby a cell actively kills itself and whereby developmental or environmental stimuli activate a specific series of events that culminate in cell death. Thus it is the process by which cells promote their own death through the activation of self-destruction systems.

In the context of petal senescence it seems appropriate to use these words almost interchangeability (van Doorn and Woltering, 2003; Rogers 2006; van Doorn and Woltering, 2008). Additionally, as senescence generally leads to death, it should be viewed as a programmed cell death (PCD) process or apoptosis (Noodén *et al*., 1997; Lim *et al*., 2007a; Vicencio, 2008). The term apoptosis comes from Greek "apoptosis" that originally means the loss of petals or leaves (Narcin *et al*., 2005; Vicencio, 2008). Despite this, apoptosis is generally used in the context of animal cells and refers to morphological changes such as condensation, shrinkage, blebbing of the nucleus, decrease in cell size and changes at the cell surface that allow

phagocytosis (Jacobson *et al*., 1997; Orzăez and Granell, 1997; Xu and Hanson, 2000; Adrain & Martin, 2001; Yamada et al., 2001, 2003; Wagstaff et al., 2003, Hoeberichts, 2005; Elmore, 2007; Taylor et al., 2008; Wang and Youle, 2009; van Doorn, 2011). In addition animal apoptosis includes activation of caspases and DNA degradation. Though, there is no evidence for classical caspases in the *Arabidopsis* genome, caspase-like molecules do exist in plants and there is evidence for caspase substrates being cleaved during plant PCD (Woltering, 2004; Sanmartín *et al*., 2005). DNA is cleaved, often into nucleosomal ladders and several genes implicated in apoptosis seem to function during plant PCD (Danon *et al*., 2000). There are many striking similarities between apoptosis and this specific form of PCD in plant cell deaths, but at the same time differences also exist. The strict definition for apoptosis states that the cell eventually breaks up into apoptotic bodies that can then be engulfed by phagocytes. Therefore, we cannot define the morphology that we observe in plants as truly apoptotic. While there are recorded instances of plant cells breaking up, it is not a common feature of plant cell death (Mc Cabe& Pennell, 1996). Consequently it has been suggested that this morphologically defined PCD in plants should be termed apoptotic-like PCD (AL-PCD) (Danon *et al*., 2000). In plants, (AL-PCD) is a cell-death process that can be defined by the presence of a number of characteristic hallmark features. These defining characteristics include a recurring corpse morphology where the protoplast has shrunk from the cell wall and the degradation of DNA often but not always into DNA 'ladders'. It can be initiated as a part of several developmental processes in response to pathogen attack and following abiotic stress. The mechanism of execution of (AL-PCD) is often associated with the activation of caspase-like molecules and the release of mitochondrial proteins including cytochrome *c*. The death process is relatively quick, usually 6 h from initiation until the final destruction of the cell. This 6-h opportunity allows identification of an initiation phase, a later execution phase and a final degradation phase of the death process which aids in the identification of the components of the (AL-PCD) death cascade in plant cells. Use of this term concedes that there are similarities between (AL-PCD) and apoptosis, while recognizing that there are also discrete and possibly basic dissimilarities.

Flower senescence – current overview

Flowers provide the model systems for studying PCD in plants because they have a short, well-defined life span, the tissue is relatively homogenous, besides morphological and biochemical changes can easily be monitored without substantial wounding (Ashman and Schoen, 1994; Shykoff *et al* 1996; Ashman and Schoen, 1997; Xu and Hanson, 2000, Rubinstein, 2000, Eason *et al.*, 2002, Hoeberichts *et al* 2005., Shahri and Tahir, 2011). In addition, flower is a complex organ composed of different tissues that senesce at different rates, allowing studies on inter-organ communication (Borochov and Woodson, 1989, van Doorn, 2004; Shahri and Tahir, 2011). Petal senescence is a part of developmental continuum in the flower, preceded by tissue differentiation, maturation of petals, followed by growth and development of seeds coordinated by plant hormones (Eason, 2006). Floral senescence involves an ordered set of events coordinated at the plant, flower, organ and cellular level (Rogers, 2012). The process of flower senescence is a correlative one as it occurs at different rates in different parts of flower but the individual flower components are all connected to each other (van Staden, 1995). The onset of petal senescence is triggered by number of factors which initiate a series of physiological events orchestrated by plant growth regulators. Ethylene is a main regulator of petal senescence in some species such as *Dianthus, Ranunculus, Petunia* (Stead and van Doorn, 1994); while, in other species such as *Iris, Hemerocallis* and *Alstromeria* it appears to play a little or no part (Woltering and van Doorn, 1988; Wagstaff, *et al*., 2005; Islam, *et al*., 2011). The hormonal changes in various floral parts may activate a degeneration process that leads the flower to wilting or death. Senescence signals seem to be mediated by floral organs. For instance, pollination-induced ethylene in *Dianthus* is translocated from stigma to the petals via the style and ovary, where it upregulates ethylene biosynthetic genes and induces the production of ethylene (ten Have and Woltering, 1997; Tripathi and Tuteja, 2007). It is well known that phytohormones are involved in the regulation of flower senescence, but their specific activities can vary depending on the species (Halevy and Mayak, 1981; Saks *et al*., 1992; Hunter *et al*., 2004; Serek *et al*., 2006). Despite the complex nature of the flower, most studies of flower senescence have utilized intact or cut flowers (Thimann, 1978; Mayak and Halevey, 1980; Rogers,

2006). Isolated petals make an interesting system for examining the factors involved in flower senescence. Because of rapid and synchronous programmed cell death of petal cells, petals provide a model system to study the molecular aspects involving during the organ senescence. The death of petal cells is preceded by a loss of membrane permeability, due in part to increase in reactive oxygen species that is in turn related to up-regulation of oxidative enzymes and to a decrease in activity of certain protective enzymes (Mor and Reid, 1980; Desai *et al*., 2012; Rogers, 2012). In *Dianthus*, for example, the earliest morphological indication of advancing senescence is a striking inrolling of the petals, a phenomenon that has been termed sleepiness while as in *Ipomoea* flowers, inrolling of calyx and corolla takes place (Nichols 1968; Kende and Baumgarter, 1974; Jones *et al*. 2009). Evidence for an increase in membrane permeability during senescence of several flower species suggests that there occurs loss of membrane integrity which leads to intracellular decompartmentalization (Nichols, 1968; Hanson and Kende 1971, Mayak *et al*., 1977; Thomas *et al*., 2003). A strong correlation between membrane leakiness and phospholipid breakdown in senescing flowers has been reported in a number of flower systems like *Petunia*, *Mirabilis* and *Dianthus* (Nichols, 1968; Hanson and Kende 1971; Kende *et al*. 1976; Mayak *et al*., 1977; Suttle and Kende 1980; Tripathi and Tuteja, 2007). Peroxidation may occur in part by the action of lipoxygenase (LOX), which oxidizes fatty acids liberated from membranes (Siedow, 1991). Increase in LOX activity before senescence becomes obvious in carnation petals and in daylily, the specific activity of LOX increased before the flowers even open (Sylvestre *et al*., 1989; Panavas and Rubinstein, 1998). A common feature of PCD is the loss of protein during organ senescence (Smart, 1994; Callis, 1995). The breakdown products of these proteins are then transported from the senescing organ to other portions of the plant. In fact, after the daylily flower opens, it changes abruptly from a sink to a source for transported materials and carbohydrates as also amino acids continue to be exported many hours after the flower has wilted (Bieleski, 1995). Protein synthesis may decline after flower opening (Woodson and Handa, 1987), but the separation of petal proteins by PAGE reveals a decrease in higher molecular- weight proteins during senescence while lower molecular weight proteins increase (Woodson and Handa,

1995; Courtney *et al*., 1994). The decrease in protein levels in daylily petals appears to occur in both membrane-bound proteins and those in the soluble fraction (Stephenson and Rubinstein, 1998, 2000) and in the chloroplast of *Dianthus (* Rubinstein, 2000). During senescence of floral parts, the degradation of DNA and RNA is the most common feature (Thomas et al., 2003; Tripathi and Tuteja, 2007). Yamada *et al*. (2006) studied flower PCD in the petals of *Antirrhinum, Argyranthemum* and *Petunia* using DNA degradation and changes in nuclear morphology as parameters. The petals of all three flowers showed loss of turgor (wilting) and DNA degradation. Two distinct types of nuclear morphology were observed during PCD in these petals. One type was characterized by chromatin clumping into spherical bodies inside the nuclear membrane lacking nuclear fragmentation during PCD as in *Antirrhinum*. Nuclear fragmentation did not occur in *Antirrhinum*, whereas nuclear fragmentation was reported in *Argyranthemum* and *Petunia* (Tripathi and Tuteja, 2007). In conclusion the flower senescence is associated with several structural, physiological, biochemical and molecular changes in the senescent floral organs. These changes include loss of the membrane permeability, leakage of ions, upregulation of oxidative enzymes, generation of ROS, degradation of proteins, lipids, carbohydrates and nucleic acids, imbalance of plant hormones, polyamines, sugars, calcium and finally up and down regulation of several genes associated with the flower senescence. Several external factors such as light, injury, pollination, temperature and dehydartion also affect the petal senescence. Many components of signal transduction pathways including G-proteins, inositol phosphate, calcium, kinases and phosphatases are also known to play important roles in petal senescence. Petal senescence is usually associated with an increase in the expression of ethylene regulated genes. In ethylene-regulated PCD mechanism, the primary signal initiation and cascade is still not known and it needs to be elucidated. In ethylene-insensitive flowers, though the ABA plays a regulatory role but the exact mechanism is not well known. Several key regulatory genes are known to be involved in the degradation of lipids, proteins, nucleic acids and cell wall components. The roles of catalytic genes were reported in different species with spatial regulation, so it becomes important to remember that different species may have different signal

transduction system. Despite the many obvious advantages of studying petal senescence, the proper understanding of its regulation has been hampered by the lack of a good model species. This is due to several factors. Firstly two key features of petal senescence vary across species: sensitivity to ethylene and timing of abscission in relation to petal wilting.

Flower senescence in Caryophyllaceae- an introspection

Caryophyllaceae includes commercially important cut flower species like *Dianthus, Gypsophila, Silene, Saponaria, and Agrostemma*. As cut flowers constitute the major components of floriculture industry, their short life becomes the great hurdle in their marketability. As they senesce within a short span of time, extensive work has been carried to enhance their longevity and commercial availability. Consequently, understanding and manipulating flower senescence to create long-lived flowers should therefore result in both aesthetic and economic benefits to the floricultural industry. Considerable amount of work has been done on flower senescence of *Dianthus*, little on *Gypsophila* and no senescence related work has been done on *Silene, Saponaria, and Agrostemma.* Based on the ethylene sensitivity flower senescence can be divided into two groups (Woltering and van Doorn, 1988; Tripathi and Tuteja, 2007). One, with ethylene as the major regulator, i.e., ethylene-dependent pathway as in *Petunia, Dianthus*, *Ipomeoa*, rose, *Arabidopsis* etc., and the other ethylene-independent pathway as in *Iris, Freesia*, *Gladiolous, Sandersonia, Hemerocallis*, *Chrysanthemum and Alstroemeria* (Woltering and van Doorn, 1988; Serek *et al*., 1994; Zhong and Ciafre, 2011; Shibuya, 2012). Caryophyllaceae belong to that group where ethylene is regulator of senescence (Borochov and Woodson, 1989; Woodson *et al*., 1992; Chang, 2003; Shibuya, 2012). A large amount of ethylene is synthesized as flowers of Caryophyllaceae open during natural senescence and this increased production of ethylene accelerates inrolling of petals, resulting in wilting which is a characteristic feature of flowers from Caryophyllaceae (Nichols, 1977; Nichols *et al*., 1983; Manning, 1985; Peiser, 1986; Woodson *et al*., 1992; Larsen *et al*., 1995; ten Have and Woltering 1997, van Doorn, 2004). In addition to ethylene, plant hormones like abscissic acid, cytokinins, auxins, gibberellins, brassinosteroides, methyl jasmonates,

polyamines, sugars and reactive oxygen species also play important role in the regulation of flower senescence in various species belonging to the family Caryophyllaceae (Chang *et al*., 2003). In many species floral senescence is coordinated by endogenous ethylene production often induced by pollination. Researchers are in search of good models to elucidate the molecular mechanism involved in flower senescence. However, there are number of flowers which have been used as model systems in flower senescence. Among these *Dianthus, Petunia* and *Arabidopsis* are important. In these species processes of remobilization, characteristic of senescence leading to cell death are coordinated by the ethylene signal. Furthermore, treatment with exogenous ethylene accelerates senescence, while treatment with inhibitors of ethylene synthesis or ethylene signalling delay it (Stead and van Doorn, 1994).

Dianthus (family Caryophyllaceae) can be found in a wide range of colors and is a model system for studies of flower senescence. In addition, it is one of the most important flowers in the global floriculture industry (Tanase *et al*., 2012). Cut *Dianthus* flowers have been shown to be a convenient model system for the study of ethylene biosynthesis (Bufler *et al*., 1980, Broun and Mayak 1981, Mayak *et al*., 1981) and other physiological events (Paulin and Jamain, 1982) during flower senescence. The senescence pattern in *Dianthus* is characterized by the inrolling of petals, followed by wilting (Nichols, 1966; Maxie *et al*., 1973; van Doorn and Reid, 1992; Newman *et al*., 1998). The senescence of *Dianthus* is accompanied by a marked increase in the ethylene synthesis and a concomitant climacteric rise in respiration (Nichols, 1966). In *Dianthus* and other flowers, the time-course of ethylene production is triphasic: a low steady-state level is followed by a climacteric rise, which then declines (Smith *et al*., 1992). Visual symptoms of senescence become evident at the end of the second stage such as petal inrolling, wilting or colour change. These have generally been interpreted in terms of membrane changes and subsequent loss of compartmentalization (Kende and Baumgartner, 1974; Eze *et al.,* 1986; Smith *et al*., 1992). Senescence of *Dianthus (D. Caryophyllus* and *D. barbatus)* petals is associated with the climacteric-like increase in ethylene production during the final stages of flower development (Brandt and Woodson, 1992; van Doorn, 2004; Kazemi,

2011. Kazemi *et al*., 2012) have reported a strong correlation between membrane leakiness and phospholipid breakdown in senescing flowers. Ethylene accelerates the onset of membrane leakiness and phospholipid deterioration in petals of *Dianthus*. Paliyath and Thompson (1990) have demonstrated with freeze fracture electron microscopy and X-ray diffraction that phase changes occur in the membranes of ER and plasmalemma of *Dianthus* petals well before the visible signs of senescence set in. Lipid peroxidation and the activation of phospholipase were seen as important early events leading to membrane destabilisation taking place before visible senescence. Autocatalytic ethylene production from petals of Caryophyllaceae flowers (*Dianthus caryophyllus, Dianthus barbatus* and *Gypsophila*) in the natural senescence results in wilting of the petals. The whole mechanism of senescence of the flowers is far from being well understood. The gynoecium has been suggested to play a role in the induction of petal senescence, i.e., ethylene evolved from the gynoecium acts as a diffusible signal that is perceived by the petals and induces the onset of senescence in the petals of caryophyllaceous flowers (Nichols, 1977; Jones and Woodson, 1997; ten Have and Woltering, 1997; van Doorn, 2004; Satoh *et al*., 2005; Satoh, 2011). The expression of ACC synthase and ACC oxidase genes has been shown to increase in the gynoecium earlier than in petals, which is consistent with the observed ethylene production. Ethylene produced in the gynoecium has been proposed to be the signal that induces ethylene production in petals, as careful removal of the gynoecium prevents increases in petal-derived ethylene production and markedly prolongs flower life (Shibuya *et al*., 2000; Satoh, 2011). Ethylene produced in the gynoecium is also thought to be a primary signal for pollination-induced petal senescence in *Dianthus* (Jones and Woodson, 1997, 1999b). Ethylene from the gynoecium and/or endogenously induced ethylene in petals by other stimuli are perceived by ethylene receptors in petals and induce an autocatalytic increase in ethylene production, leading to petal senescence in ethylene-dependent flowers (*Dianthus caryophyllus* and *Dianthus barbatus*). In species with ethylene-sensitive floral senescence, pollination induces a burst of ethylene production associated with up-regulation of ethylene biosynthetic enzymes: S-adenosylmethionine (SAM) synthase, 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase (Jones 2004; Hoeberichts *et al*.,

2007). Ethylene production is associated with pollen tube growth in the style and later with fertilisation (Hoekstra and Weges, 1986; ten Have and Woltering 1997). This results in autocatalytic ethylene production in the petals (Woodson and Lawton 1988; Graham *et al*., 2012). Since last two decades researchers have isolated the genes for ethylene receptors and signal components in several ornamental plants such as *Dianthus, Alstromeria, Hemerocallis* and *Petunia* (Satoh, 2005; Rogers, 2012). In *Dianthus*, for example, three ethylene receptor genes, DcERS1, DcERST2, and DcETR1, were identified. DcERS2 and DcETR1 transcripts were present in considerable levels in petals at the full-opening stage (Shibuya *et al*., 2002). The levels of transcripts for DcERS2 and DcETR1 did not show clear changes in the petals during flower senescence, although DcERS2 transcript showed a decreasing trend when approaching the flower senescence stage. DcERS1 mRNA was not detected in petals at any stage in the flowers of *Dianthus caryophyllus* (Shibuya *et al*., 2002).

The role of cytokinins as anti-senescence agents in the senescence of *Dianthus* petals have been well documented (Halevy and Mayak, 1981). A correlation has been established between senescence and decrease in endogenous cytokinins in *Dianthus caryophyllus* (Staden and Dimaua, 1980). There was a senescence-associated fall in cytokinins and rise in ABA levels (Mayak and Halevy 1970; Borohov *et al*., 1976; van Staden *et al*., 1987; Chang *et al*., 2003). Both these changes seem to be connected to ethylene signalling. In *Dianthus caryophyllus* and *Dianthus barbatus* sensitivity to ethylene was reduced by treatment with cytokinin and inhibition of cytokinin metabolism delayed senescence (Taverner *et al.,* 2000). Furthermore treatment of *Dianthus caryophyllus* petals with cytokinins blocked the conversion of exogenously supplied ACC to ethylene (Mor *et al.*, 1983). In addition to ethylene and cytokinins abscisic acid (ABA) accumulation also accelerates the senescence of cut flowers as has been seen in number of flowers especially *Dianthus* (*Dianthus caryophyllus* and *Dianthus barbatus*) and is thus generally considered as a promoter of flower senescence (Mayak and Halevy, 1972; Mayak and Dilley, 1976; Eze *et al*., 1986; Le Page-Degivry *et al*., 1991; Panavas *et al*., 1998; Hunter *et al*., 2004). Ornamentals of Caryophyllaceae (*Dianthus caryophyllus, Dianthus barbatus* and *Gypsophila*) treated with exogenous ABA quickly lose their ornamental value as senescence of flowers is

accelerated (Ronen and Mayak 1981). The ABA appears to increase the sensitivity of flowers to ethylene, as the gene expression of some ethylene receptors increased after exogenous ABA treatment (Müller *et al*., 2000a). Therefore, ABA may accelerate senescence by increasing the sensitivity of the flower, acting at the level of ethylene receptors. In ethylene-sensitive flowers such as *Dianthus*, ABA-accelerated senescence appears to be mediated through induction of ethylene synthesis, since it is not seen in flowers that are pretreated with ethylene (Mayak and Dilley 1976; Ronen and Mayak 1981; Muller *et al*., 1999).

The effect of auxins and gibberellic acid is not well characterized in flower senescence. Applications of auxins to cut flowers of *Dianthus caryophyllus* stimulate senescence (Stead, 1992). Jones and Woodson (1999) reported that 2,4-D a synthetic auxin induced the expression of ACC synthetase genes in the styles, overies and petals. In *Dianthus caryophyllus* petals, a transient increase has been observed in the mRNA of an Aux/IAA gene following the application of auxins (Hoeberichts *et al*., 2007) Gibberellic acid is known to delay senescence in some cut flowers by acting as ethylene antagonist. Saks *et al*. (1992) showed that exogenous application of Gibberellic acid delayed the senescence of cut *Dianthus* flowers with reduced ethylene production. Jasmonic acid also shows stimulating effect on flower senescence. Jasmonic acid along with several other metabolites promotes senescence of orchid species, presumably by elevating ACC, thereby stimulating ethylene production. However, in orchid petals for 50 h after pollination induced senescence lipoxygenase activity or jasmonic acid content was found to remain unchanged.

In plants, polyamines (PAs) have been shown to have a well established role in the stimulation of cell division, growth and in the delay of senescence; hence called as 'juvenility' factors (Bagni and Serafini-Fracassini, 1973; Palavan and Galston, 1982; Tripathi and Tuteja, 2007). The major forms of PAs are putrescine (Put), spermine (Spm) and spermidine (Spd) which are found in all plant cells. Tassoni *et al.* (2005) reported that either the direct spray of spermidine on petals or continuous treatment in vase solution delay the senescence of *Dianthus caryophyllus* flowers. These results led them to hypothesize the presence of an interconversion pathway of spermidine to

putrescine through a spermidine acetylation process as previously reported in *Helianthus tuberosus* (Del Duca *et al*., 1995) or via a putative polyamine oxidase (PAO) as already hypothesized in *Zea mays* (De Agazio *et al*., 1995) and in *Arabidopsis thaliana* (Tassoni *et al*., 2000). However, spermidine treatment only seemed to delay *Dianthus caryophyllus* flower senescence (Tassoni *et al*., 2005). This process is in fact ethylene-dependent and once started, it seems to proceed irreversibly and independently of spermidine treatments. The mechanism with which polyamines could delay senescence in the flowers of Caryophyllaceae (*Dianthus caryophyllus* and *Dianthus barbatus*) could be mainly related to their capacity to stabilize and organize DNA and RNA structures. Spermine and spermidine can in fact affect DNA conformation by inducing the transition from B-DNA to Z-DNA and bind inside the narrow and the major groove of the helix thus stabilizing the double helix structure against thermal denaturation and promoting a better and faster transcription of DNA (Feuerstein *et al*., 1988; Tassoni *et al*., 2005). In addition, a role of polyamines in inhibiting DNase and RNase activities has been hypothesized in *Dianthus* (Speranza *et al*., 1984; Srivastava, 1987). Polyamines could also preserve membrane fluidity by means of their free radical scavenging action and their ability to inhibit lipoxygenase-1 activity (Maccarrone *et al*., 1998, Tassoni *et al*., 2005).

Climacteric rise in the ethylene production during the senescence of *Dianthus caryophyllus* and *Dianthus barbatus* results in the formation of reactive oxygen species (ROS) (Kazemi *et al*., 2011). ROS cause chlorophyll degradation and membrane lipid per oxidation and increase in malondialdehyde (MDA) product (Reezi *et al*., 2009). The production of O_2^- by microsomal membranes from *Dianthus* petals has been found to increase as the flower ages (Mayak *et al*., 1983; Droillard *et al*., 1989; Droillard and Paulin, 1990). Because the O_2^- radical or its derivatives have been found to induce membrane degradation (Kellog and Fridovich, 1975; Mayak *et al*., 1983; Droillard and Paulin, 1990), it has been suggested that O_2^- radicals play an important role in senescence of ornamental flowers of Caryophyllaceae. SOD is a group of metalloenzymes which catalyze the dismutation of O_2^- to O_2 and H_2O_2 and are considered to be the major enzymatic defense systems against O_2^- radicals (Halliwell, 1978). The protective function provided by SODs shows age-related

changes in plant tissues (Rabinowich and Fridovich, 1983; Droillard and Paulin, 1990). In *Dianthus* petals the total SOD activity has been shown to decrease during aging (Droillard *et al*., 1989; Droillard and Paulin, 1990). Plants utilize ROS scavenging systems to protect themselves from oxidative damage. These include enzymes (catalase, superoxide dismutase, ascorbate peroxidase) and antioxidant molecules such as ascorbate, glutathione and others (Apel and Hirt, 2004).

The role of sugars in delaying senescence of cut flowers has been reviewed by several authors (Halevy and Mayak, 1979; van Doorn, 1999; Butt, 2007; Muriithi and Ouma, 2011). However, very little coverage has been given to the role of sugars in the biosynthesis of ethylene or sensitivity to ethylene (Dilley & Carpenter, 1975; Mayak and Dilley, 1976; Monteiro *et al*., 2002; Pun and Ichimura, 2003; Verlinden and Garcia, 2004; van Doorn, 2004). Supplementation of exogenous sugars to cut flowers generally delays visible senescence in petals of many flowers such as *Gypsophila*, *Dianthus caryophyllus* and *Dianthus barbatus* (van Doorn, 2004). Sucrose treatment accelerates flower opening and delayed senescence but did not affect petal abscission and alteration of hormonal balance in several floral tissues (Arrom and Munne-Bosch, 2012). A large amount of soluble carbohydrates is required for flower bud opening as substrates for cell walls and respiration as well as for their osmotic properties. Since the carbon source of cut flowers is limited; sugars such as sucrose, fructose, trehalose, mannose and glucose added to vase water have been shown to be highly effective in promoting flower opening (Mayak *et al*., 1973; Farnham *et al*., 1978; Paulin and Jamain, 1982; Downs, 1988; Pun and Ichimura, 2003). The effect of sucrose on gene expression (with the exception of a few genes) was identical to that of STS in the cut *Dianthus* flowers. The delay of visible senescence after sucrose treatment is therefore associated with preventing the up-regulation of numerous genes. Since these genes are shown to be regulated by ethylene, the data indicate that sucrose regulates ethylene signalling. Sucrose treatment prevented the increase in transcript abundance of Dc-EIL3. It is tempting to speculate that endogenous levels of soluble sugars act as a regulator of flower senescence in *Dianthus caryophyllus* by influencing Dc-EIL3 gene expression. In addition, sucrose might promote EIL degradation in proteasomes as observed in *Arabidopsis* (Yanagisawa *et al*., 2003). Sucrose loading of a cell might

thus prevent the accumulation of one or more EIL proteins, because it promotes EIL degradation in proteasomes. The low level of EIL might then prevent the up-regulation of numerous genes including the gene(s) encoding EIL (Hoeberichts *et al*., 2007).

The senescence of *Dianthus caryophyllus* petals is associated with a series of physiological and biochemical changes such as increases in hydrolytic enzymes, degradation of macromolecules, increased respiratory activity and a loss of cellular compartmentalization (Lawton *et al*., 1989). *In vitro* translation of mRNAs isolated from petals of *Dianthus caryophyllus* at various stages of development has shown that senescence is accompanied by changes in mRNA populations (Woodson, 1987; ten Have and Woltering, 1997; Jones and Woodson, 1999; Verlinden, 2006). Exposure of pre-senescent petals to ethylene leads to premature petal senescence and induces changes in mRNA accumulation similar to those that occur during natural senescence (Woodson and Lawton, 1988). Several mRNAs that accumulate in senescing *Dianthus* petals have been cloned and identified (Woodson, 1994). Gene expression studies have revealed that *SR* genes in case of *Dianthus* could be grouped in two categories depending on their responsiveness to ethylene. The first group include the ethylene biosynthetic genes (ACC synthase and ACC oxidase) which show a clear delay in mRNA accumulation in response to ethylene. The second group of genes (*SR5, SR8, SR12* and *DCCP1*) show almost immediate mRNA accumulation in response to ethylene (Verlinden *et al*., 2002). Treatment of flowers with the ethylene biosynthesis inhibitor AOA or the ethylene perception inhibitor silver thiosulfate (STS) reduced the accumulation of *SR5* mRNA only slightly. This indicates that an important temporal factor may influence *SR5* mRNA accumulation (Lawton *et al*., 1989). On the contrary, these treatments have been shown to completely suppress the accumulation of *SR8* and *SR12* 38 transcripts (Lawton *et al*., 1989). A marker protein with the predicted peptide sequences of SR5 and SR12 revealed homologies greater than 50% with β-glucosidases and β-galactosidases respectively (Woodson, 1994). DCCP1 shares significant homology to several cysteine proteinases (Jones *et al*., 1995). *SR8* has been found to encode a glutathione S-transferase (GST1) in *Dianthus caryophyllus* (Meyer *et al*., 1991).

The genus *Gypsophila* belonging to family Caryophyllaceae is regarded to be one of the most important alternatives of product diversification in the cut flower sector. *Gypsophila paniculata* is used as fresh and dry cut flower and attracts attention as being one of the most indispensable elements of arrangement and bouquet in domestic market (Đnan, 2006; Korkmaz, 2007; Korkmaz and Hasan, 2011). However, very little is known about the senescence in cut *Gypsophila* flowers. *Gypsophila paniculata* is ethylene sensitive flower system. In case of *Ipomoea* (Winkenbach, 1970b), *Iris* (Bancher, 1941; van Doorn *et al*., 2003), *Hemerocallis* (Stead and van Doorn, 1994), *Alstromeria* (Wagstaff *et al*., 2003) reported that the petal mesophyll cells died considerably earlier than the epidermal cells. In contrast, no clear evidence for earlier death in the mesophyll cells, compared with the epidermis was found in *Gypsophila paniculata* (Hoeberichts *et al*., 2005). Senescence in *Gypsophila paniculata* is characterised by inrolling of petals, followed by wilting (Nichols 1966; Maxie *et al*., 1973; van Doorn and Reid 1992, Newman *et al*., 1998). Van Doorn and Reid (1992) reported that the wilting of petals in *Gypsophila paniculata* was preceded by a sharp rise in ethylene production. Panicles continuously held in ethylene (3µl/L) at 20ºC and 90 % RH had no effects on early flower senescence (almost half with respect to control). Relatively low concentrations of ethylene (0.3µl/L) 24h pulse did not result in senescence of half open flowers indicating lower sensitivity than in open flowers. Upon the prolonged exposure to ethylene; desiccation was observed in flower buds, half open flowers and in open flowers, as compared to panicles kept under controlled conditions. van Doorn and Reid (1992) observed that the flowers of *Gypsophila* became translucent, then showed inrolling, wilting and desiccation. The rise of ethylene production prior to visible symptoms suggest that petal senescence is regulated by ethylene (Van Doorn and Reid, 1992; Newman *et al*., 1998). Pulse treated panicles of *Gypsophila* with STS (4mM, 30min) delayed the onset of petal senescence in individual flowers irrespective of vase solution. The use of STS to overcome the problems associated with exposure to ethylene gas was demonstrated nearly 3 decades ago (Veen and van de Geijn, 1978). The aminoethoxyvinylglycine (AVG) analogs such as Florish inhibit ethylene biosynthesis in plant tissues (Yang and Hoffman, 1984). Newman *et al*. (1998) proposed that such AVG analogs do not

protect sensitive flowers against exogenous ethylene. Besides, 1-MCP showed relatively poor performance as compared to STS treated flowers (Newman *et al*., 1998). Although open flowers pretreated with 1-MCP were somewhat protected from ethylene action however, it was less effective than STS in extending vase life in control flowers or flowers that had been exposed to ethylene after opening in the vase. The *Gypsophila* inflorescence bears number of flowers that develop sequentially with young immature buds being present when other flowers are fully open (Downs, 1988). It appears, that 1-MCP treatment is able to protect fully open flowers but not buds that will later develop into flowers. Ethylene binding sites present at the time of 1-MCP treatment are inactivated but those that develop with the opening of young buds are not. STS protects newly opening flowers and this seems likely to be the reason for its superiority over 1-MCP in protecting *Gypsophila paniculata*. Possibly this stable complex is able to move within the inflorescence inactivating ethylene binding sites in developing buds.

Gypsophila paniculata petal senescence is a form of PCD with features of animal apoptosis suggesting that nuclear DNA degradation is an early regulatory event rather than a result of massive cell death in the final stages of senescence. To investigate whether petal senescence in *Gypsophila paniculata* flowers is accompanied by the degradation of genomic DNA, *in situ* DNA breakdown was studied in whole petals using TUNEL. TUNEL consists of the *in situ* labelling of DNA breaks in individual nuclei and relies on the specific binding of terminal deoxynucleotidyl transferase (TdT) to exposed 3´-OH ends of DNA followed by the synthesis of a labelled polydeoxynucleotide molecule. This method preferably labels double strand breaks thought to result from the action of endo-DNases involved in the cell death process (Gravieli *et al*., 1992). To demonstrate that petal cells of *Gypsophila paniculata* undergo apoptotic-like cell death (Hoeberichts. *et al.,* 2005), the flowers were treated with anticancer drug Camptothecin which brings about the inhibition of topoisomerase I that induces apoptosis in a variety of mammalian systems (Kaufmann, 1998; Hoeberichts *et al.,* 2005). During the study it was revealed that with the progression of senescence in flowers of *Gypsophila paniculata*, the TUNEL positive nuclei increase upto partially senescent stage. However, they were altogether absent in fully

senescent flowers of *Gypsophila*. The studies of Hoeberichts *et al*., (2005) revealed that senescence process starts well before it is visible to naked eye. DNA degradation occurs early in the senescence programme rather than being solely associated with the ethylene-induced massive cell death during the final stages of senescence. Cells with degraded DNA are no longer expected to actively participate in the senescence programme, which implicates that senescence has started already before the flower is fully open. Ethylene apparently only hastens the later stages of senescence when massive cell death and collapse of the epidermal layers occurs (Hoeberichts *et al*., 2005).

It is clear from the above discourse on flower senescence in caryophyllaceae that PCD is an important process during petal senescence in this family and is directly associated with postharvest quality. The information obtained through these studies may be applied to the objective of producing flowers with a longer vase life. Apart from preserving flower freshness during the transport or by designing new holding solutions, there is a lot of scope to modify the flowers genetically through the induction of useful target genes in these flower systems. Advanced techniques can be used to improve our knowledge on flower senescence in the members of caryophyllaceae. The use of microarray technology may give important clues about the genes involved in physiological or induced flower senescence providing us with an array of genes putatively involved in the complicated pathways leading to flower ageing that may be blocked or else modified to delay the progression of flower senescence in these ethylene sensitive systems.

CHAPTER-3

MATERIALS AND METHODS

Plant material

The present study deals with physiological processes and senescence of important genus *Dianthus* and *Silene* belonging to family Caryophyllaceae. Both the plant species belong to ethylene sensitive flower system. The physiological changes associated with flower development were carried out on *Dianthus caryophyllus*, *Dianthus barbatus* and *Silene armeria* under laboratory conditions.

Collection of plant material

The intact spikes/isolated flowers were collected from the plants growing in Kashmir University Botanic Garden (KUBG). The material was harvested at 800 h using sharp scalpel and immediately brought to the laboratory in beakers (100ml in case of isolated flowers) and immersed in distilled water. In order to study the changes during course of flower development and senescence the samples were divided into various stages of development (stage I-VI) in case of *Dianthus caryophyllus, Dianthus barbatus* and five (I-V) in case of *Silene armeria*). These stages were deciphered as tight bud stage, mature bud stage, brush stage, fully open stage, partially senescent stage and senescent stage. The isolated flowers were harvested at stage III (one day before anthesis) for different chemical treatments. The samples were transferred to the test solutions in the laboratory. Only the healthy plants were selected for the experimentation.

The spikes were harvested with their oldest bud at one day before anthesis for various temperature regimes. The spikes were recut under water to get a uniform length of 35 cm in case of *Dianthus caryophyllus* and *Dianthus barbatus*. These temperature regimes were tested for studying the optimal storage temperature.

Storage treatments

Various temperature regimes were tested for studying the optimal temperature required for transportation and increasing the vase life in spikes of *Dianthus caryophyllus, Dianthus barbatus* and *Silene armeria* under study. Two different storage treatments were practiced (dry and wet storage) at three different temperatures {5˚C, 10˚C and RT (20±2˚C)}. In postharvest dry storage (PHDS), the spikes were wrapped in moistened filter papers and kept at different temperatures for 72 h. In

postharvest wet storage (PHWS), the samples were kept in 1000 ml borosilicate glass beakers containing distilled water and kept at various temperatures for 72 h. After 72 h the spikes were transferred to the holding solutions for assessing their postharvest performance. Sucrose and distilled water were used as vase solutions. Sucrose solution was always freshly prepared at the start of the experiment.

The test solutions were kept in 100 ml Ehrlenmeyer borosilicate flasks containing 75 ml of vase solution. The flasks were thoroughly washed with detergent and rinsed with distilled water. The glassware was oven dried before experimentation. Each treatment was represented by 5 replicates (flasks) with each replicate containing 2 spikes. The day of transfer of samples to vase solutions after 72 h storage was designated as day zero (D0). A separate set of 5 flasks without spikes containing blank test solutions were kept along with each set of flasks with samples to monitor the volume of the vase solution evaporated on a particular day.

Visible effects

Visual changes occurring during the course of flower development and senescence were monitored regularly. Besides, the apparent changes that occurred in flowers and spikes during the course of experiments were recorded at regular intervals. These included the time and pattern of flower opening, color change and pattern of senescence.

Vase life

Vase life of spikes and flowers was measured in days. Vase life of spikes was measured as the number of days required by the last open flower on spike to senesce. Vase life was counted from the day zero (D0) i.e., the day of transfer of spikes to vase solutions. Vase life of isolated flowers was measured as the time taken by an open flower to get senesced. Vase life in isolated flowers was regarded as terminated when the visible signs of senescence like inrolling of petals and loss of turgidity will commence.

Number of blooms

Number of blooms was recorded regularly by counting the number of buds bloomed on each spike on each day and averaged by dividing the total number of buds bloomed on all the spikes in a particular treatment on a day by number of spikes receiving the particular treatment. The number of buds that failed to open and aborted was also recorded. Total number of buds on each spike was counted to express the data on percentage basis.

Solution uptake

The volume of holding solution absorbed by the spikes was recorded at regular intervals and was averaged for every two, four, six, seven, eight, sixteen and twenty days for different plants. The volume of holding solution absorbed was calculated by measuring the volume of solution on a particular day and subtracting it from the initial quantity of the vase solution kept in the flasks, taking into account the volume of solution evaporated by using blank flasks (containing particular vase solution without spikes) alongside the flasks with spikes.

Fresh and dry mass

Fresh mass of five to ten flowers from each treatment was recorded at regular intervals in each experiment. The flowers were then kept in paper bags and oven dried at 70^{o}C for 48h. The material was put in a dessicator for 24h before recording the dry mass.

Moisture content

Moisture content was computed as the difference between fresh and dry mass of flowers. Percent moisture content of the fresh tissue was also calculated.

Flower diameter

Flower diameter was measured as the mean of two perpendicular measurements across a flower.

Membrane permeability

Changes in membrane permeability were estimated by measuring ion leakage from the petal discs (5mm in diameter) incubated in the dark in 15ml of glass distilled water for 15h at 20^{o}C. The discs were floated on distilled water with their abaxial surface

downwards and were removed with a brush after 15h of incubation. Conductivity of leachates was measured by using CM- 180 ELICO Conductivity meter and was expressed in µS.

Fixation of plant material

1g of chopped petal tissue was fixed in hot 80% ethanol. The material was then macerated in a glass pestle and mortar and centrifuged at 3000xg for 20 minutes. The supernatants were pooled and made to a uniform final volume. A suitable aliquot from the supernatant was used for the determination of tissue constituents (sugars, α- amino acids and phenols). For the determination of soluble proteins and protease activity, fresh material was directly used. Each treatment was represented by three replicates.

Determination of soluble protein content

Proteins were extracted from 1g petal tissue, homogenized in 5ml of 5% sodium sulphite (w/v) and 0.1g of polyvinyl pyrrolidone (PVP) and centrifuged at 4000xg for 20 minutes in a refrigerated centrifuge. Proteins were precipitated from a suitable volume of supernatant with equal volume of 20% trichloroacetic acid (TCA), centrifuged at 2000xg for 15 minutes. The pellet was redissolved in 4ml of 0.1N NaOH and proteins were estimated from a suitable aliquot by the method of Lowry *et al*. (1951). The volume of the aliquot was made to 1ml with distilled water, to which 2ml of mixed alkaline copper reagent (copper sulphate solution: alkaline tartarate in the ratio of 1:100; GENEI protein estimation kit) was added. After 10 minutes 0.2ml of solution III (Folin-Ciocalteau reagent) was added and optical density was measured after 20 minutes at 700 nm in a Photochem-8 absorptiometer. A standard calibration curve was prepared using bovine serum albumin (BSA) as the standard.

Determination of Protease activity

1g of pre-chilled tepal tissue was homogenized in 15ml chilled 0.1M phosphate buffer (pH= 6.5) in a pre-cooled glass pestle and mortar. The contents were squeezed through pre-cooled four-fold muslin cloth and centrifuged for 15 minutes at 5000xg in

a (Remi K- 24) refrigerated centrifuge at -5^{o}C. The supernatant was used for the assay of protease activity by a modified method of Tayyab and Qamar (1992). 1ml of enzyme extract was mixed with 1ml of reaction mixture (0.1% BSA dissolved in 0.1 M phosphate buffer, pH= 6.5). The reaction was started by incubating the mixture at 37^{o}C for 2h and then stopped by adding 2 ml of 20% cold TCA. Blanks in which TCA was added prior to the addition of the enzyme extract were run along with each sample. The contents were centrifuged and supernatants collected. Free amino acids were estimated (as tyrosine equivalents) in a suitable aliquot of the supernatant by the method of Lowry *et al*. (1951) using tyrosine as the standard. The enzyme activity has been expressed as µg tyrosine equivalents liberated per mg protein.

Reducing sugars

Reducing sugars were estimated by method of Nelson (1944) using D-Glucose as the standard. A suitable aliquot from the supernatant (obtained after fixation) was made to 5ml with distilled water followed by the addition of 1ml of copper reagent, prepared by mixing copper reagent A and B in the ratio of 50:1. The mixture was heated at 100^{o}C for 20 minutes over a water bath. After cooling 1ml of arsenomolybdate reagent was added and the mixture was shaken gently. The final volume of the mixture was made to 25ml with distilled water. Absorbance was measured at 520 nm in a Photochem-8 absorptiometer.

Total sugars

Total sugars were estimated after the enzymatic conversion of non reducing sugars into reducing sugars by invertase. The volume of a suitable aliquot from the alcohol soluble fraction of fixed material was made to 4ml with distilled water, followed by the addition of 1ml of 0.2% invertase. A drop of toluene was layered on the top and the solution was incubated overnight at 25^{o}C. Total sugars were then estimated by the method of Nelson (1944). D-Glucose was used as the standard for the preparation of calibration curve.

Non-reducing sugars

Non-reducing sugars were calculated as the difference between total sugars and reducing sugars.

Determination of α-amino acid content:

α-amino acids were estimated by the method of Rosen (1957). Calibration curve was prepared by using glycine as the standard. A suitable aliquot from the alcohol soluble fraction of tissue extract was made to 1ml with distilled water and 0.5ml of cyanide acetate buffer (pH=5.4) was added followed by 0.5ml of 3% ninhydrin (freshly prepared in ethylene glycol monomethyl ether; methyl cellusolve). The contents were then heated at 100^{o}C for 15 minutes, over a boiling water bath. After removing the tubes from the water bath 5ml of diluent (isopropyl alcohol: water; 1:1v/v) was immediately added and the contents were shaken vigorously. The tubes were allowed to cool at room temperature. Absorbance was measured at 570nm in a Photochem-8 absorptiometer.

Determination of total phenolic content

Total phenols were estimated by the method of Swain and Hillis (1959). A suitable aliquot from alcohol soluble fraction of tissue extract was diluted to 7ml with distilled water followed by the addition of 0.5ml of Folin- Dennis reagent. After 3 minutes 1ml of saturated solution of sodium carbonate was added and the total volume was made to 10ml with distilled water. Absorbance was measured after 30 minutes at 725nm in a Photochem-8 absorptiometer. Gallic acid was used as the standard for the preparation of calibration curve.

Procedure for SDS-PAGE

1g of petal tissue was homogenized in 1ml of 0.1M phosphate buffer (pH= 7.2- 7.4), adding 0.1g of PVP. The mixture was centrifuged at 5000xg at 5^{o}C in a refrigerated centrifuge (Remi K-24) for 15 minutes. The supernatant was collected in eppendorf tubes and used for SDS-PAGE. The extracted protein mixture was denatured by mixing equal volumes of protein mixture and 2X sample loading buffer (0.5 M Tris pH 6.8, 10% SDS, 10% glycerol, 5% β- mercaptoethanol, 0.1% bromophenol blue). The mixture was incubated in boiling water for 5- 7 minutes. The concentration of the

proteins was determined in both the original extracts and the TCA precipitated samples by the method of Lowry *et al* (1951) using BSA as the standard.

One dimensional vertical gel electrophoresis was carried out according to the method as described by Ausubel *et al* (1989). Slab gels 0.7mm thick contained 12% resolving gel {(Acrylamide + bisacrylamide), (1.5M Tris pH 8.8), 10% SDS, TEMED, 10% Ammonium persulphate (APX)} and 3% stacking gel {(Acrylamide + bisacrylamide), (0.5M Tris pH 6.8), 10% SDS, TEMED and 10% APX}. 80µl of the SDS- denatured protein extract was loaded into each lane. Electrophoresis was carried out at room temperature with a constant voltage of 50V during stacking and 150V during running. GENEI molecular weight standards were used for determining approximate molecular weights {Myosin, Rabbit muscle 205,000; phosphorylase b 97,400; Bovine serum albumin 66,000; Ovalbumin 43,000; Carbonic anhydrase 29,000; Aprotinin 6,500; Insulin (α and β chains) 3,000}. Following electrophoresis the gels were stained overnight using 0.25% coomassie brilliant blue in 45% methanol: 10% acetic acid. Gels were destained in 45% methanol: 10% acetic acid, then in 7% methanol: 5% acetic acid.

Statistical analysis of the data

The values given in the tables represent the mean of several independent replicates. Standard deviation has been computed as $\delta = \sqrt{\frac{\sum x^2}{N-1}}$. Difference between various treatments has been evaluated by simple analysis of variance and least significant difference (LSD) computed at $P_{0.05}$ using MINITAB (v15.1.2-EQUINOX_Softddl.net) software.

CHAPTER-4

FLOWER SENESCENCE AND CHEMICAL REGULATION OF VASE LIFE IN DIANTHUS CARYOPHYLLUS

***Dianthus caryophyllus* L.**

Botanical name:	*Dianthus caryophyllus* L.
English name	Carnation, Clove pink
Family:	Caryophyllaceae
Flowering period:	May- July
Plant height:	30-50 cm
Calyx:	5 sepals which are connate
Corolla:	5 petals generally clawed or serrated and vary from white to pink or purple in color.
Androecium:	Stamens 10 in number in two whorls.
Gynoecium:	Bicarpellary syncarpous, ovary superior.
Fruit:	Capsule
Flower longevity:	5 days
Symptoms and pattern of senescence:	The flower senescence in *Dianthus caryophyllus* is marked by the loss of petal turgidity followed by inward rolling of the petals. The coloured petals turn yellowish during senescence.

EXPERIMENT- 4.1

Physiological and biochemical changes associated with flower development and senescence of *Dianthus caryophyllus* L.

Experimental

Flowers of *Dianthus caryophyllus* growing in Kashmir University Botanic Garden (KUBG) were selected for the present study. Flower development and senescence was divided into six stages (stage I-VI). These stages were deciphered as I-tight bud stage, II-mature bud stage, III-brush stage, IV-fully opens stage, V-partially senescent stage and VI-senescent stage (Plate 4.1.1). Visible changes were recorded throughout flower development and senescence. Floral diameter, fresh mass, dry mass and water content was determined at each stage.

For the estimation of tissue constituents from petal tissues, 1g chopped material was fixed in hot 80% ethanol at each stage of flower development and senescence. The material was macerated and centrifuged three times. The supernatants were pooled and used for the estimation of soluble proteins, α-amino acids, total phenols, reducing sugars and total sugars from the suitable aliquots. Non- reducing sugars were calculated as the difference between total and reducing sugars. Soluble proteins were extracted from 1g of petal tissue drawn separately from five different flowers at each of the five stages. Protease activity was expressed as µg tyrosine equivalents liberated per 100mg fresh mass, besides specific protease activity has been computed as µg tyrosine equivalents liberated per mg protein. At each stage the homogenates from the petal tissues were used for studying electrophoretic profiles of proteins by SDS-PAGE. The tissue was homogenized in 0.1M phosphate buffer (pH=7.2). Suitable amount of aliquot was loaded in each lane. Each value represented in the tables corresponds to the mean of five to ten independent replicates. The data has been analyzed statistically by computing standard deviation.

Results

Visible changes

The flower senescence in *Dianthus caryophyllus* was marked by the loss of petal turgidity followed by inrolling of petal tips. The greenish buds opened into pinkish white. The pinkish white petals turn yellowish brown during senescence (Plate 4.1.1). The average life span of an individual flower after it opened fully was about 5 days.

Fresh mass, dry mass and water content of flowers

Fresh and dry mass of individual flowers increased with flower development upto stage IV and declined thereafter as the flower senescence progressed. Water content showed a continuous increase towards the flower opening and thereafter declined as the senescence progressed through stages V and VI (Table.4.1.1; Fig.4.1.1-4.1.3).

Floral diameter

Floral diameter increased as the flower development progressed upto Stage IV and thereafter showed a constant decrease as the senescence progressed towards stage VI (Table 4.1.1; Fig.4.1.4).

Soluble proteins

The concentration of soluble proteins from petal tissues increased upto stage IV and thereafter showed a decline through stages V to VI. Same trend was observed the when data was expressed on per flower basis (Table 4.1.2; Fig.4.1.5).

Specific protease activity

The specific protease activity (expressed as µg tyrosine equivalents per mg protein) from petal tissues generally decreased during flower development from stages I to IV and increased sharply thereafter as the senescence progressed through stages V and VI (Table 4.1.2; Fig.4.1).

α-amino acids

The α-amino acid content of petal tissues increased up to stage IV and thereafter a considerable decrease was registered as senescence progressed through stages V and VI. Almost same trend was observed when the data was expressed on per flower basis (Table 4.1.2, Fig.4.1.7).

Total phenols

The concentration of total phenols in petal tissues increased from stage I to stage II, showed a decrease at stage III which remained more or less consistent upto stage V, and registered a sharp increase at stage VI. However, when the data was expressed on per flower basis, the total phenolic content increased as the flower development progressed through stages I to V and then showed a sharp decrease as the senescence progressed from stage V to VI (Table 4.1.2; Fig.4.1.8).

Total sugars

The total sugar content of petal tissues showed an increase as the flower development progressed from stage I to IV and decreased as flower senescence progressed through stages V and VI. The trend became more apparent when the data was expressed on per flower basis (Table 4.1.3, Fig.4.1.9).

Reducing Sugars

The reducing sugar content of petal tissues increased as the flower development progressed through stages I to IV and thereafter decreased as the senescence progressed through stages V and VI. The same trend became apparent when the data was expressed on per flower basis (Table 4.1.3; fig.4.1.10)

Non-reducing sugars

The tissue content of non-reducing sugars increased from stage I to IV and decreased as the senescence progressed through stages V and VI. Same trend was observed when the data was expressed on per flower basis (Table 4.1.3; Fig.4.1.11).

Table 4.1.1: Table showing fresh mass, dry mass, water content and floral diameter during flower development and senescence in flowers of *Dianthus caryophyllus.*

Stages of flower development	Fresh mass (g)	Dry mass (g)	Water content (g)	Floral diameter (cm)
I (tight bud stage)	0.046±0.009	0.012±0.001	0.033±0.002	0.5±0.04
II (loose bud stage)	0.049±0.002	0.014±0.001	0.035±0.01	0.9±0.06
III (brush stage)	0.153±0.01	0.117±0.01	0.036±0.003	2.8±0.16
IV (open bud stage)	0.169±0.02	0.127±0.02	0.042±0.0	4.9±0.5
V (partially senescent stage)	0.122±0.011	0.014±0.001	0.008±0.001	2.3±0.09
VI (senescent stage)	0.017±0.002	0.012±0.003	0.004±0.001	1.8±0.12

Each value is the mean of 6 independent replicates ±SD

Table 4.1.2: Table showing soluble proteins, specific protease activity (expressed as µg tyrosine equivalents librated per mg protein) α-amino acids and phenols (expressed as mg g^{-1} fresh mass) during flower development and senescence in flowers of *Dianthus caryophyllus.*

Stages of flower development	Soluble proteins	Protease activity	α-amino acids	Total phenols
I (tight bud stage)	4.64±0.30 (0.213)	0.32±0.04	3.77±0.05 (0.173)	2.81±0.02 (0.129)
II (loose bud stage)	5.36±0.06 (0.262)	0.21±0.02	5.52±0.06 (0.270)	4.82±0.03 (0.236)
III (brush stage)	6.32±0.04 (0.966)	0.18±0.2	6.42±0.12 (0.982)	3.17±0.0 (0.485)
IV (open bud stage)	7.2±0.06 (1.216)	0.14±0.05	8.23±0.22 (1.390)	3.05±0.01 (0.515)
V (partially senescent stage)	6.32±0.09 (0.771)	0.31±0.1	4.23±0.01 (0.516)	3.36±0.07 (0.405)
VI (senescent stage)	4.88±0.3 (0.082)	0.63±0.07	2.21±0.0 (0.037)	7.15±0.02 (0.121)

Each value is the mean of 6 independent replicates ±SD
The values in parenthesis represent the values on per flower basis.

Table 4.1.3: Table showing total, reducing and non-reducing sugars during flower development and senescence in flowers of *Dianthus caryophyllus.*

Stages of development	Total sugars	Reducing sugars	Non reducing sugars
I (tight bud stage)	10.80±0.4 (0.496)	7.85±0.2 (0.361)	2.95±0.09 (0.315)
II (loose bud stage)	23.85±0.51 (1.168)	16.98±0.3 (0.832)	6.87±0.07 (0.336)
III (brush stage)	33.86±0.04 (5.180)	21.83±0.32 (3.34)	12.03±0.1 (1.840)
IV (open bud stage)	40.85±0.0 (6.903)	27.02±0.02 (4.566)	13.83±0.3 (2.337)
V (partially senescent stage)	34.82±0.023 (4.248)	23.19±0.11 (2.83)	11.63±0.02 (1.418)
VI (senescent stage)	27.62±0.11 (0.469)	19.6±0.32 (0.333)	8.02±0.7 (0.136)

Each value is the mean of 6 independent replicates ±SD.
The values in parentheses represent the values on per flower basis.

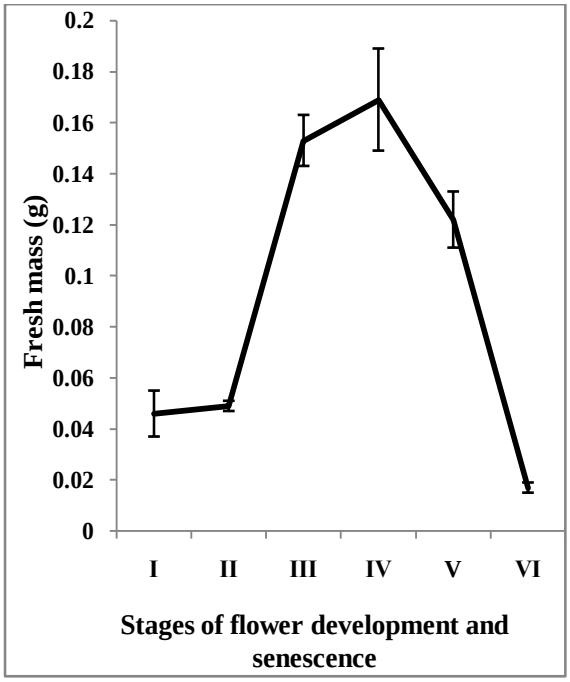


Fig.4.1.1

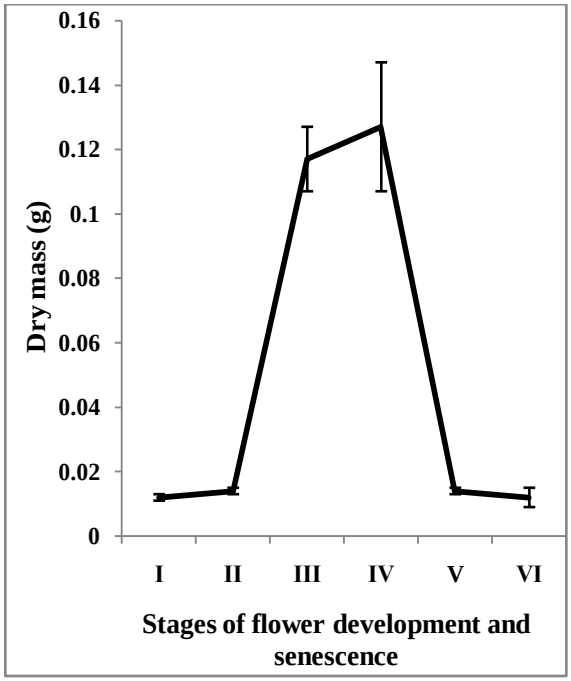


Fig.4.1.2

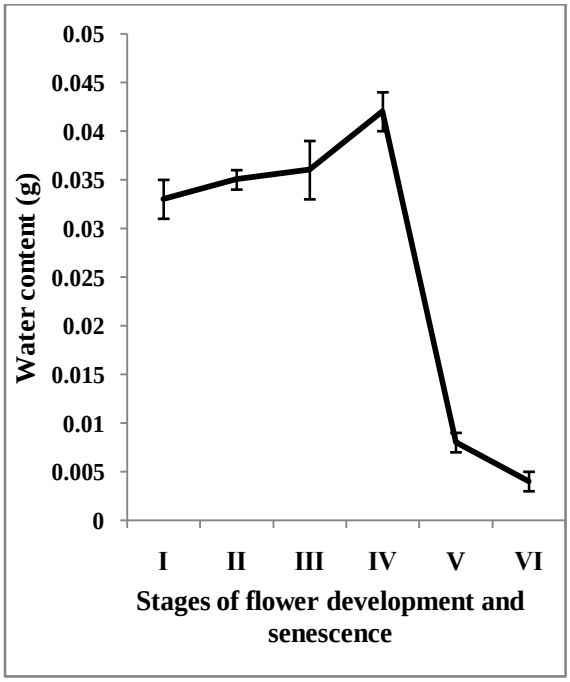


Fig.4.1.3

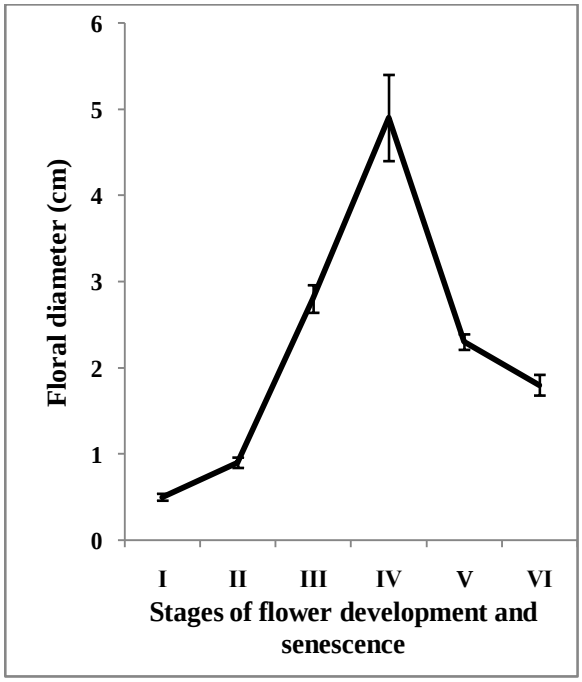


Fig.4.1.4

Fig.4.1.1-4.1.4: Changes in fresh mass, dry mass, water content of flowers and floral diameter during various stages of flower development and senescence in *Dianthus caryophyllus.*

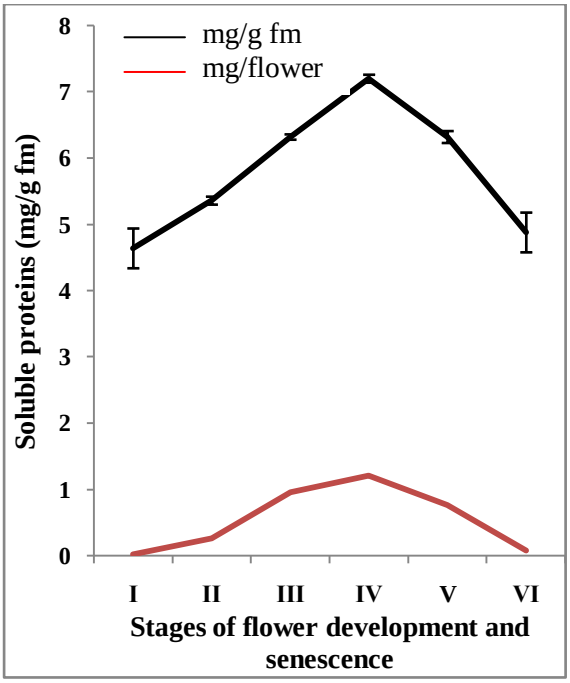


Fig.4.1.5

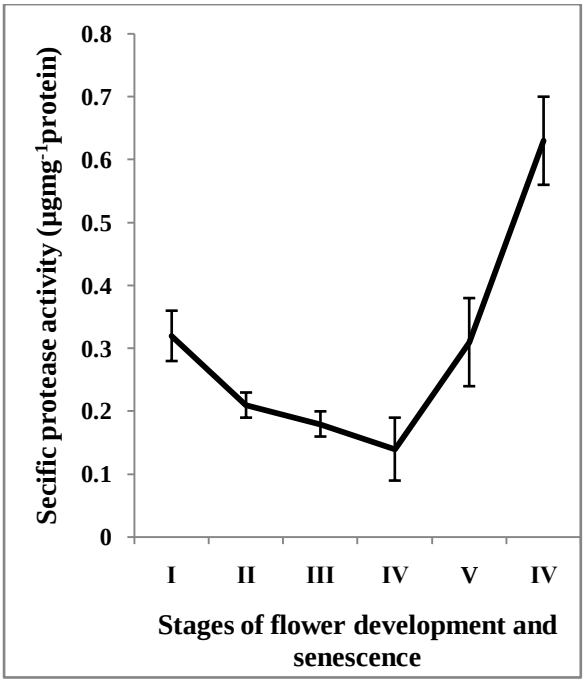


Fig.4.1.6

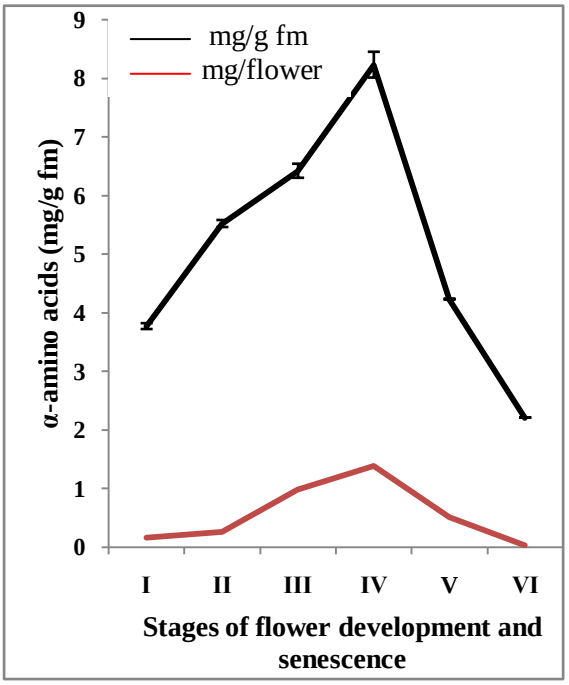


Fig.4.1.7

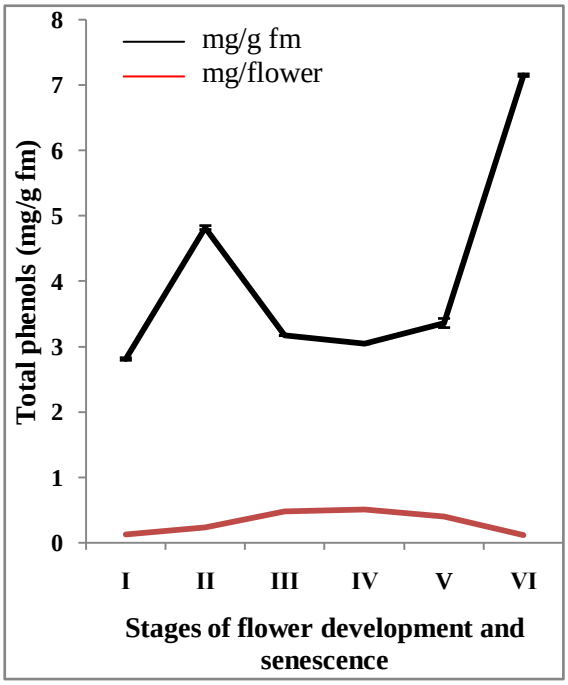


Fig.4.1.8

Fig.4.1.5-4.1.8: Changes in soluble proteins, specific protease activity, α-amino acids and total phenols during various stages of flower development and senescence in *Dianthus caryophyllus.*

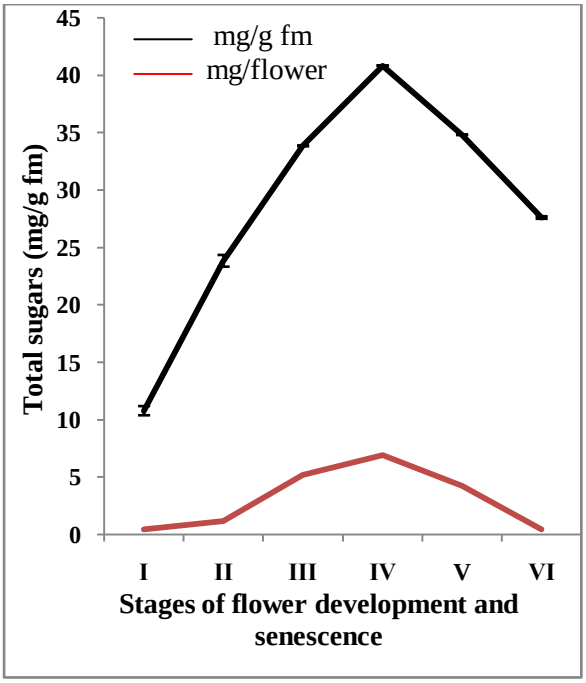


Fig.4.1.9

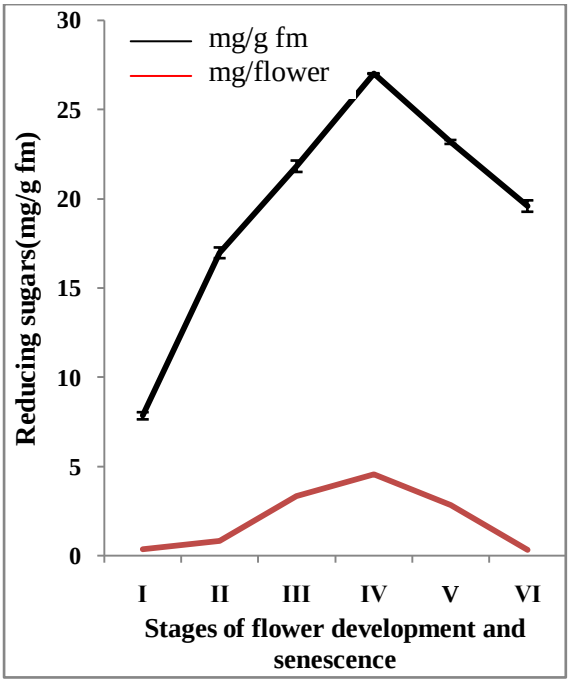


Fig. 4.1.10

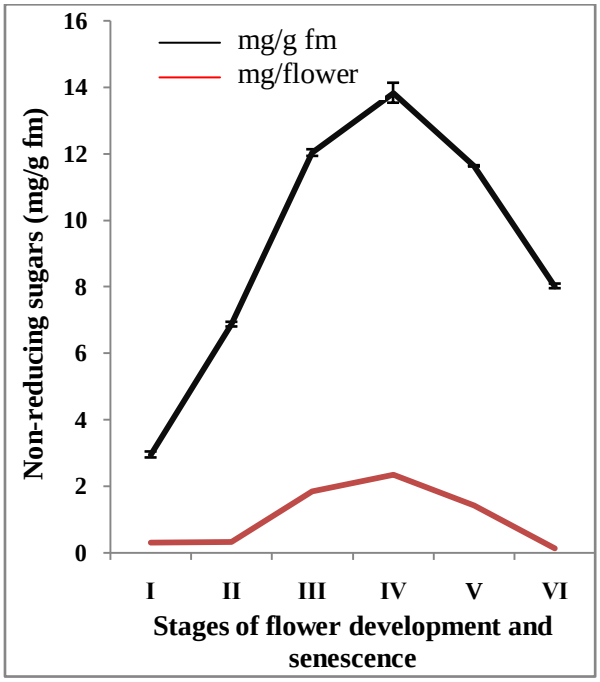


Fig.4.1.11

Fig.4.1.9-4.1.11: Changes in total, reducing and non reducing sugars during various stages of flower development and senescence in *Dianthus caryophyllus.*

Plate 4.1.1

Stages of flower development and senescence in *Dianthus caryophyllus*.

Fig. a: From left to right are arranged six stages of flower development and senescence in *Dianthus caryophyllus.* Stages I (Tight bud stage), II (loose bud stage), III (pencil stage), IV (open stage), V (partially senescent stage) and VI (senescent stage).

Fig. b: SDS-PAGE of equal amounts of extractable protein at various stage (I-VI) of flower development and senescence from petal tissue of Dianthus caryophyllus. The gel was stained with coomassie blue. Numbers above lanes correspond to developmental stages. Molecular weight standards are indicated on the left (kDa) and the ca molecular weights of major polypeptides to the right of the gel (kDa).

Fig. c: Scanning electron microscopic analysis of the petal tissues from the different stages of flower development and senescence in *Dianthus caryophyllus* L. From left to right are arranged the scanning electron micrographs of the stages I to VI.

I II III IV V VI

Fig. a: Stages of flower development and senescnce in *Dianthus caryophyllus.*

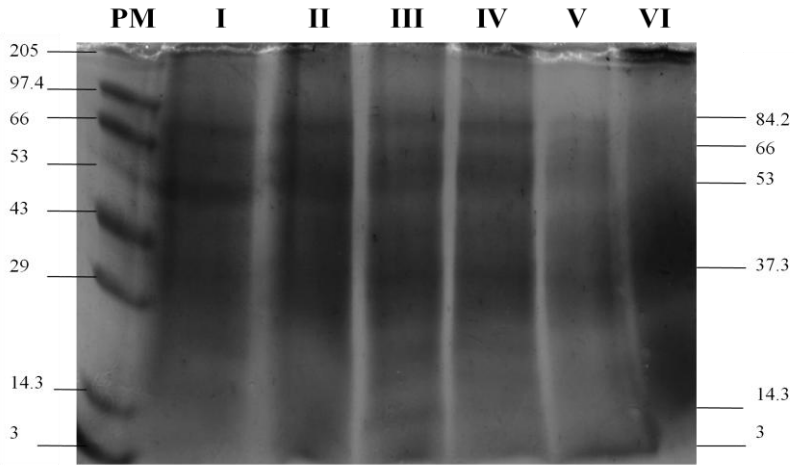


Fig. b: Electophoretogram of the stages of flower development and senescence of *Dianthus caryophyllus.*

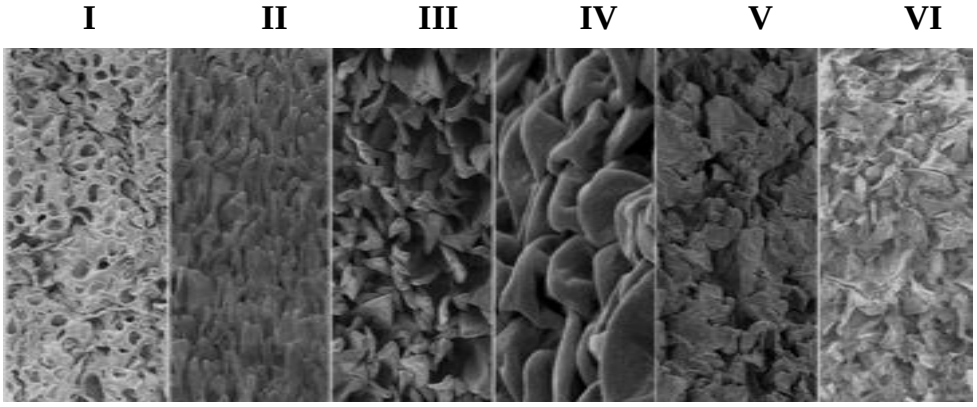


Fig. c: Scanning electron micrographs of different stage of flower development in *Dianthus caryophyllus*.

Plate 4.1.1

EXPERIMENT 4.2

Effect of postharvest wet storage (PHWS) at three different temperature regimes RT, 10ºC and 5ºC) for 72 h and subsequent transfer to vase solutions {distilled water (DW) and sucrose (SUC)} on postharvest performance of cut spikes of *Dianthus caryophyllus* L.

Experimental

Uniform and healthy spikes of *Dianthus caryophyllus* growing in the Kashmir University Botanic Garden (KUBG) were used for this study. The buds were harvested at 800 h with their oldest bud at 1 day before anthesis (i.e. brush stage). The harvested spikes were brought to the laboratory, cut to a uniform length of 27 cm, divided into three sets with each set containing equal number of spikes and held in DW in 500ml borosilicate beakers. One set was kept at 5°C and second at 10°C. The third set was kept at room temperature (20±2°C). After 72 h the spikes were kept at room temperature after transferring them to 250 ml Ehrlenmeyer flasks containing 200 ml of vase solution {Distilled water (DW) and Sucrose (SUC) 0.05 M}. Each treatment was represented by 5 replicates (flasks) with each flask containing two spikes. The day of transfer of spikes to vase solutions (DW and SUC) was designated as D0. The average vase life of spikes was counted from the day of transfer (D0) to holding solution and was regarded to be terminated when the last flower lost its display value (marked by color change and loss of petal turgidity). Visual changes were observed at periodic intervals. Floral diameter, number of blooms and volume of holding solution absorbed were estimated on Day 2, 8 and 15. Changes in tissue constituents including soluble proteins, α- amino acids, total phenolics and sugar fractions were estimated on day 3 and day 8 of transfer of spikes to vase solution (DW and SUC). The results have been analyzed statistically and LSD computed at $P_{0.05}$ using MINITAB (v15.1.2-EQUINOX_Softddl.net) software.

Results

Visible effects

The spikes previously wet stored for 72 h at 5°C maintained their premature status during storage, but the spikes maintained at 10°C opened up during storage. However,

the oldest flower buds on spikes wet stored at room temperature (20±2°C) opened during the storage (Plate 4.2.1, Fig.4.2.b). All the buds at 1 day before anthesis (i.e. brush stage) on the spikes previously stored at 5°C opened up on day 1 after the transfer to vase solution. Flower senescence was characterized by the changing of color from pinkish white to yellowish and inrolling of petals. Flower senescence was observed to be same as was the case under field conditions, but flowers remained persistent even after complete senescence.

Vase life

The average vase life of spikes previously wet stored at various temperature regimes i.e. RT (20±2°C), 10 and 5°C before transfer to DW was approximately 6, 8 and 15 days respectively; whereas the vase life of the corresponding spikes wet stored at RT (20±2°C), 10 and 5°C before transfer to SUC was approximately 9, 11 and 18 days respectively. The maximum vase life of 18 days was recorded in spikes wet stored at 5°C before transferred to SUC (Table 4.2.1; Fig.4.2.1).

Floral diameter

The floral diameter of the spikes previously wet stored at 10°C was higher at day 2 as compared to the flowers from the corresponding spikes held at RT (20±2°C) and 5°C irrespective of the holding solution. Floral diameter showed an increase with the progression in time from day 2 to day 8 of transfer of spikes to vase solution (DW and SUC). Floral diameter was maximum in the flowers from the spikes previously wet stored at 5°C for 72 h and transferred to SUC on day 15 (Table 4.2.1;Fig.4.2.2).

Number of blooms per spike

The number of blooms was appreciably higher in spikes previously wet stored at 5°C and transferred to SUC. However, the number of blooms increased with the progression in time from day 2 to day 15 of the transfer of spikes to various holding solutions irrespective of the particular temperature regime and holding solution (Table 4.2.1; Fig 4.2.3.).

Volume of holding solution absorbed

The volume of holding solution absorbed increased with progression in time from day 2 to day 15 of the transfer of spikes to various vase solutions irrespective of the

particular temperature regime and holding solution. The solution uptake was significantly higher in spikes previously wet stored for 72h at 10 and 5°C as compared to the corresponding spikes held at RT irrespective of the holding solution. A higher solution uptake was recorded in the spikes held in DW as compared to SUC irrespective of the various temperature regimes. The maximum solution uptake was noticed in the spikes previously wet stored at 5°C for 72h and transferred to DW on day 15 (Table 4.2.2;Fig.4.2.4).

Soluble proteins

The soluble protein content petal tissue samples from spikes transferred to sucrose was higher as compared to corresponding samples from spikes transferred to DW, irrespective of temperature regime. However, soluble protein content registered a significant increase with the progression in time from day 3 to day 8. Maximum protein content was found in the samples from the spikes previously wet stored at RT for 72h and transferred to SUC on day 8 (Table 4.2.2;Fig.4.2.5)

Specific protease activity

Specific protease activity (expressed as µg tyrosine equivalents released per mg protein) of the petal tissue samples from the spikes previously wet stored at 10°C for 72h was significantly higher as compared to the samples from corresponding spikes held at RT and 5°C irrespective of the holding solution used. The specific protease activity decreased with the progression in time from day 3 to day 8 irrespective of the holding solution. Minimum specific protease activity was observed in the samples from spikes previously wet stored for 72h at 10°C transferred to sucrose (Table 4.2.2; Fig.4.2.6)

α- amino acids

The α-amino acid content of the petal tissue samples from spikes transferred to SUC was much higher than the samples from spikes transferred to DW irrespective of the various temperature regimes. The α- amino acid content of samples from spikes wet stored for 72 h at RT decreased with the progression in time from day 3 to day 8 of transfer irrespective of vase solution used. Whereas, the α- amino acid content from the samples of spikes wet stored at 10°C and 5°C increased with the progression in

time from day 3 to day 8 of transfer. Maximum α- amino acid content was recorded in the samples from spikes previously wet stored for 72 h at RT (20±2ºC) and transferred to SUC on day 3 (Table 4.2.2;Fig.4.2.7).

Total phenolics

The total phenolic content of petal tissue samples from the spikes transferred to DW and SUC showed an increase with the progression in time from day 3 to day 8.The samples from the spikes previously wet stored for 72 h and transferred to SUC at different temperature regimes showed a higher total phenolic content compared to the corresponding spikes transferred to DW. The maximum total phenolic content was recorded in the spikes previously wet stored for 72 h at 5ºC and transferred to SUC on day 8 (Table 4.2.3;Fig.4.2.8)

Total sugars

The total sugar content of the tissue samples of the spikes previously wet stored for 72 h at various temperature regimes and then transferred to SUC was significantly higher than the corresponding spikes transferred to DW. The total sugar content generally decreased sharply with the progression in time from day 3 to day 8 of the transfer of the spikes to their respective vase solutions. Maximum total sugar content was maintained in the spikes previously stored at RT or 10ºC and transferred to SUC on day 3 and day 8 respectively (Table 4.2.3; Fig.4.2.9).

Reducing sugars

The reducing sugar content of the samples from the spikes previously wet stored for 72 h at different temperature regimes was found to be significantly higher in the samples held in SUC as compared to the samples from corresponding spikes held in DW. The reducing sugar content decreased substantially with the progression in time from day 3 to day 8 irrespective of temperature treatment and vase solutions. However, the decrease was more pronounced in spikes held in SUC as compared to DW. Maximum reducing sugar content was observed in the samples from the spikes previously wet stored at 5°C for 72h and transferred to SUC on day 3 (Table 4.2.3;Fig.4.2.10).

Non-reducing sugars

The maximum non reducing sugar content was recorded from the samples of spikes previously wet stored at 10°C for 72 h and transferred to SUC at day 8. However, the non reducing sugar content showed a significant decreases with progression in time from day 3 to day 8 of transfer in spikes transferred to DW irrespective of temperature regime, while as the non reducing sugar content of the spikes previously wet stored for 72 h and transferred to SUC irrespective of the temperature regimes showed a substantial increase with the progression in time from day 3 to day 8 (Table 4.2.3; Fig.4.2.11).

TABLE 4.2.1: Effect of postharvest wet storage (PHWS) at different temperatures (RT, 10°C and 5°C) for 72h and subsequent transfer to different holding solution (DW and SUC) on vase life, floral diameter and no. of blooms per spike of cut spikes on day 2, 8 and day 15 of transfer in *Dianthus caryophyllus* L.

Treatments	**Vase life (days)**	**Days of transfer**					
		Floral diameter (cm)			**No. of blooms per spike**		
		D2	**D8**	**D15**	**D2**	**D8**	**D15**
SET A (DW)							
RT	6.33	3.22	-	-	2.66	4.34	5.0
10 °C	8.20	3.92	4.06	-	3.0	5.66	5.99
5 °C	15.0	2.685	3.093	3.0	2.0	5.0	6.33
SET B (SUC)							
RT	8.87	3.75	4.20	-	2.92	4.5	6.33
10 °C	11.2.	4.02	4.26		4.21	6.32	7.04
5 °C	18.43	3.86	3.96	4.19	4.33	7.12	8.33
LSD at $P_{0.05}$	0.213	0.098	0.052	--	0.021	0.112	0.217

Each value is mean of 6 independent replicates.
Room temperature (RT= 20±2ºC)

Table 4.2.2: Effect of postharvest wet storage (PHWS) at different temperatures (RT,10 °C and 5 °C) for 72h and subsequent transfer to different holding solution (DW and SUC) on volume of holding solution absorbed per spike, soluble proteins, protease activity and α-amino acids of cut spikes on day 3 and day 8 of transfer in *Dianthus caryophyllus* L.

Treatments	**Days of transfer**								
	Volume of holding solution absorbed per spike (ml)			**Soluble proteins (mg/g fm)**		**Protease activity (µg/100mg protein)**		**α-amino acids (mg/g fm)**	
	D2	**D8**	**D15**	**D3**	**D8**	**D3**	**D8**	**D3**	**D8**
Set A (DW)									
RT	1.16	7.33	9.5	4.08	--	0.22	--	4.36	--
10 °C	1.00	8.83	12.0	3.12	7.04	0.56	0.50	2.73	2.95
5 °C	0.66	11.0	13.16	3.28	4.48	0.60	0.31	2.10	2.84
Set B (SUC)									
RT	0.66	3.66	4.00	6.32	8.16	0.44	0.29	5.98	4.73
10 °C	1.00	4.83	4.5	3.26	7.04	0.66	0.64	4.28	5.84
5 °C	0.33	10.8	7.33	5.52	6.88	0.64	0.18	3.88	5.10
LSD at $P_{0.05}$	0.03	0.05	0.011	0.04	0.05	0.012	0.002	0.02	0.048

Each value is mean of 6 independent replicates.
Room temperature (RT= 20±2ºC)

Table 4.2.3: Effect of postharvest wet storage (PHWS) at different temperatures (RT,10 °C and 5 °C) for 72h and subsequent transfer to different holding solution(DW and SUC on total phenols, total, reducing and non-reducing sugars of cut spikes on day 3 and day 8 of transfer in *Dianthus caryophyllus* L.

Treatments	**Days of transfer**							
	Total phenols (mg/g fm)		**Total sugars (mg/g fm)**		**Reducing sugars (mg/g fm)**		**Non-reducing sugars (mg/g fm)**	
	D3	**D8**	**D3**	**D8**	**D3**	**D8**	**D3**	**D8**
Set A (DW)								
RT	1.8	--	8.83	--	6.42	--	2.41	--
10 °C	0.80	2.80	14.46	4.41	6.42	3.21	8.04	1.2
5 °C	1.60	1.80	17.51	4.01	11.25	3.21	6.26	0.8
Set B (SUC)								
RT	2.65	2.15	32.94	11.25	28.13	3.21	4.82	8.04
10 °C	1.15	3.05	28.76	32.94	26.51	14.46	2.25	18.48
5 °C	2.05	3.20	28.76	17.67	30.53	10.80	1.77	16.87
LSD at $P_{0.05}$	0.120	0.132	0.991	0.546	0.659	0.432	0.591	0.509

Each value is mean of 6 independent replicates.
Room temperature (RT= 20±2ºC)

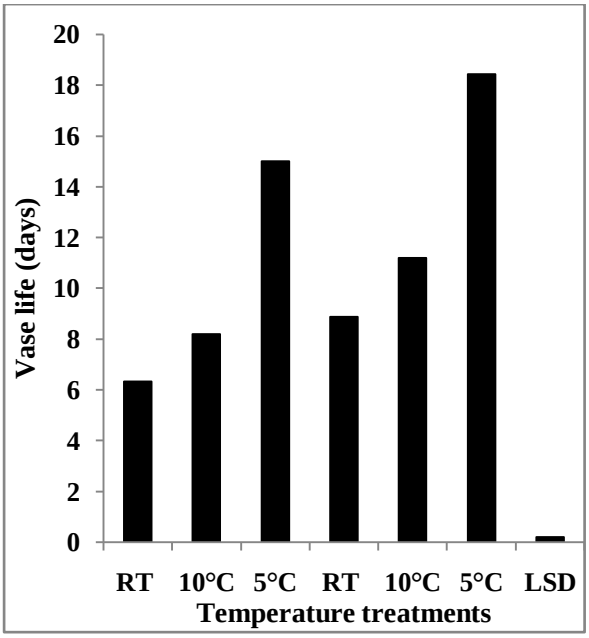
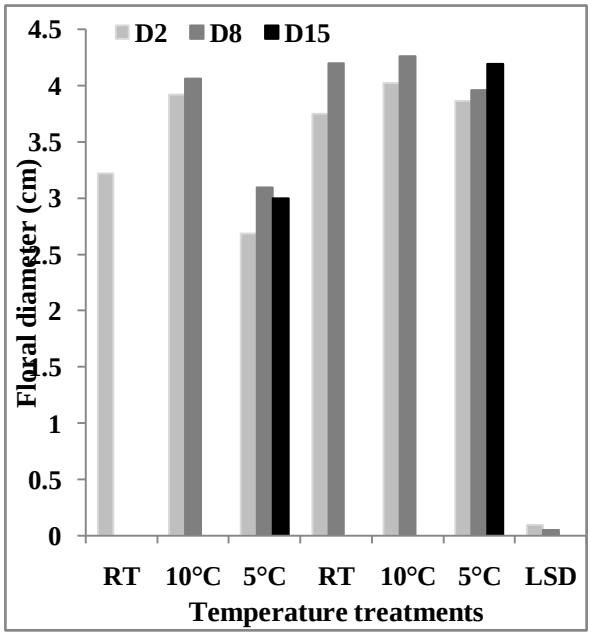


Fig. 4.2.1



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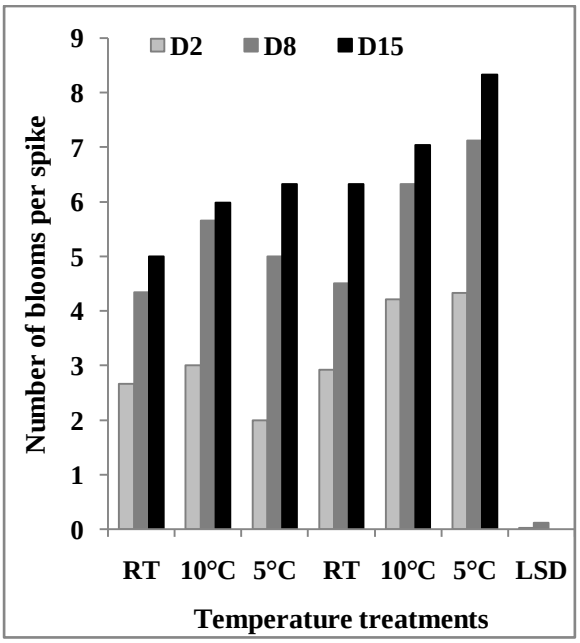

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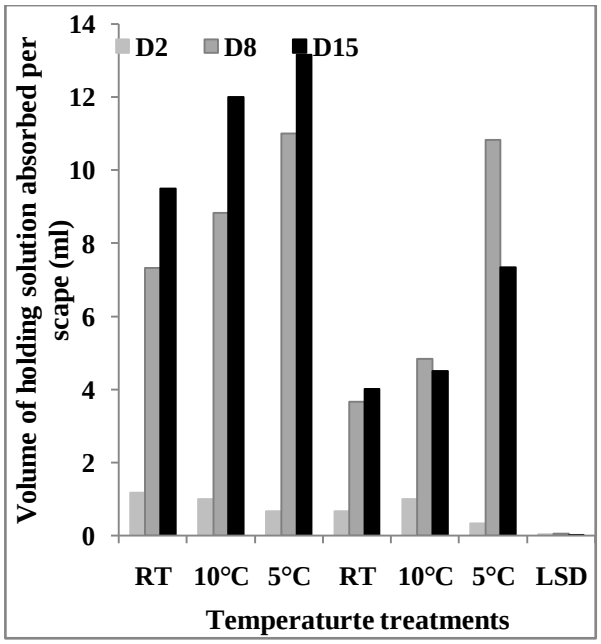


Fig. 4.2.4

Fig. 4.2.1-4.2.4: Effect of postharvest wet storage (PHWS) at different temperatures (RT,10 °C and 5 °C) for 72 h and subsequent transfer to different holding solution (DW and SUC) on vase life, floral diameter, no. of blooms per spike and volume of holding solution absorbed per spike on D2, D8 and D15 of cut spikes of *Dianthus caryophyllus.*

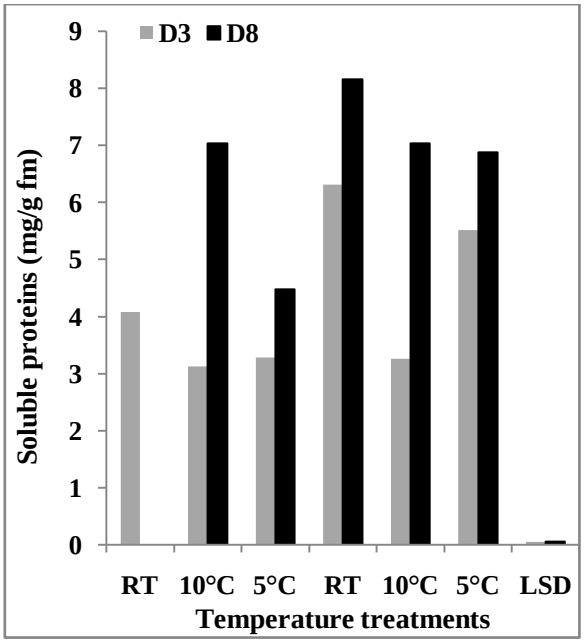
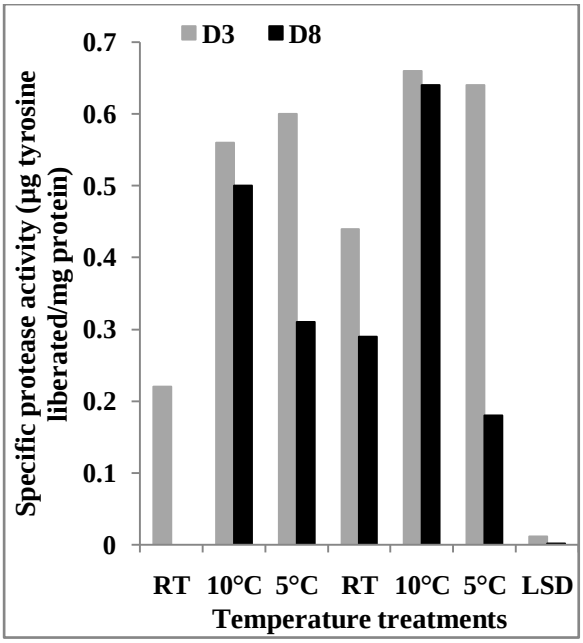


Fig. 4.2.5



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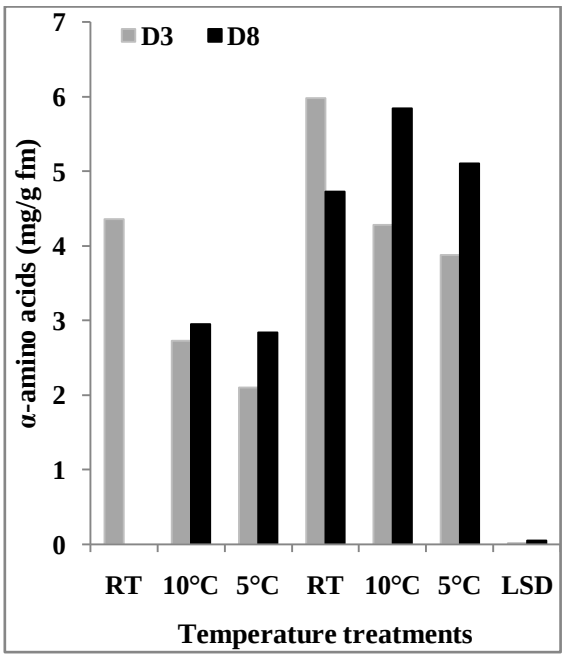

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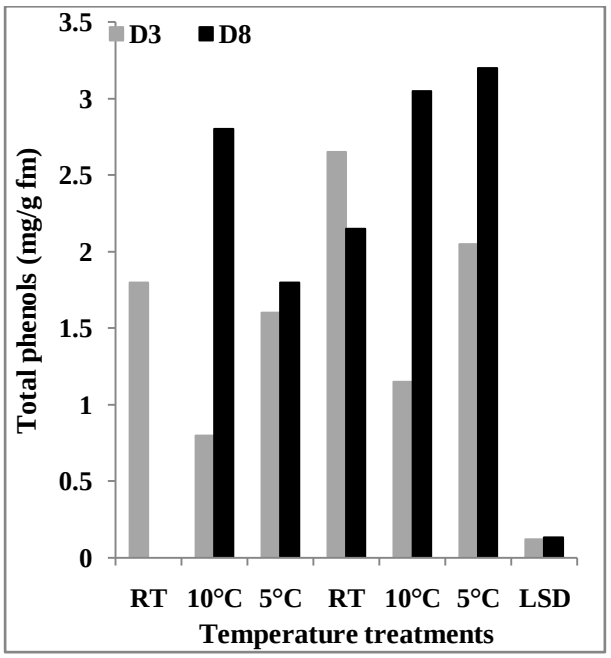


Fig. 4.2.8

Fig.4.2.5-4.2.8;: Effect of postharvest wet storage (PHWS) at different temperatures (RT,10 °C and 5 °C) for 72h and subsequent transfer to different holding solution (DW and SUC) on soluble proteins, specific protease activity, α-amino acids and total phenols of cut spikes on day 3 and day 8 of transfer in *Dianthus caryophyllus.*

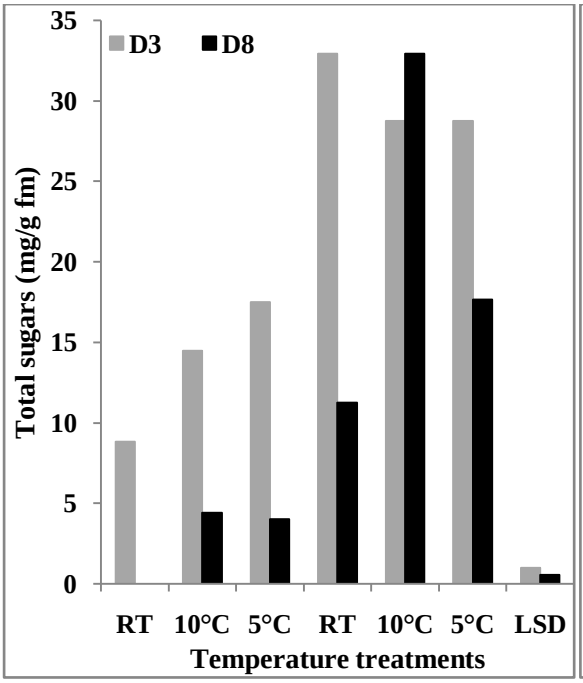


Fig. 4.2.9

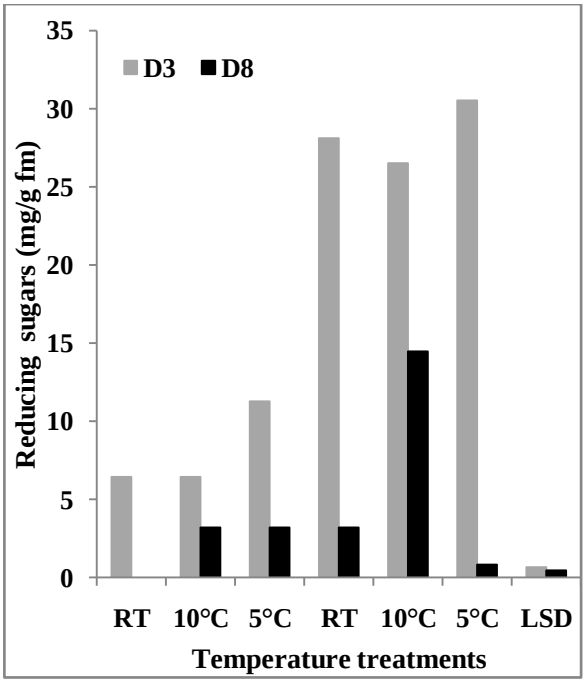


Fig. 4.2.10

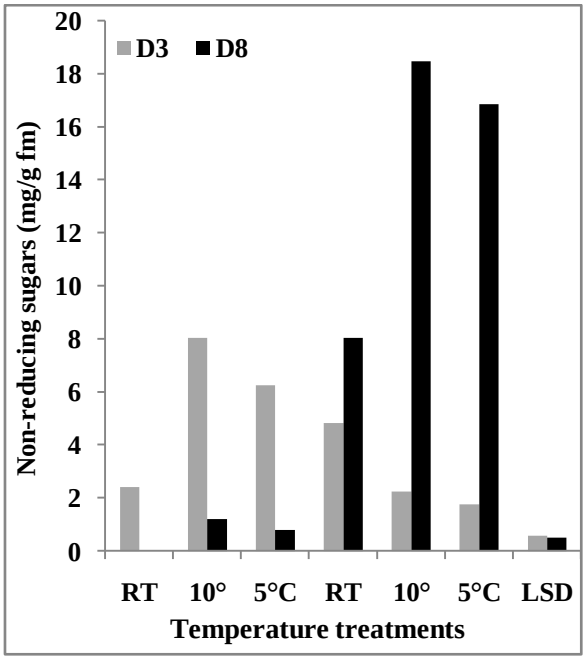


Fig. 4.2.11

Fig.4.2.9-4.2.11: Effect of postharvest wet storage (PHWS) at different temperatures (RT, 10°C and 5°C) for 72h and subsequent transfer to different holding solution (DW and SUC) on reducing, total and non-reducing sugars of cut spikes on day 3 and day 8 of transfer in *Dianthus caryophyllus.*

Plate 4.2.1

Effect of postharvest wet storage (PHWS) for 72 h at room temperature (RT), 10°C and 5°C before transfer to DW and SUC (0.05M) in cut spikes of *Dianthus caryophyllus*

Fig. (a): From left to right are arranged spikes before wet storage for 72 h.

Fig. (b): From left to right are arranged spikes after wet storage for 72 h.

Spikes of *Dianthus caryophyllus* held in distilled water (DW) and sucrose (SUC) after 72 h wet storage at day 8 (Fig. c) and day 18 of transfer (Fig. d). From left to right are arranged flasks containing spikes which were previously held at different temperatures {(RT, 22±2˚C), 10˚C and 5˚C} and in different vase solutions (DW and SUC).

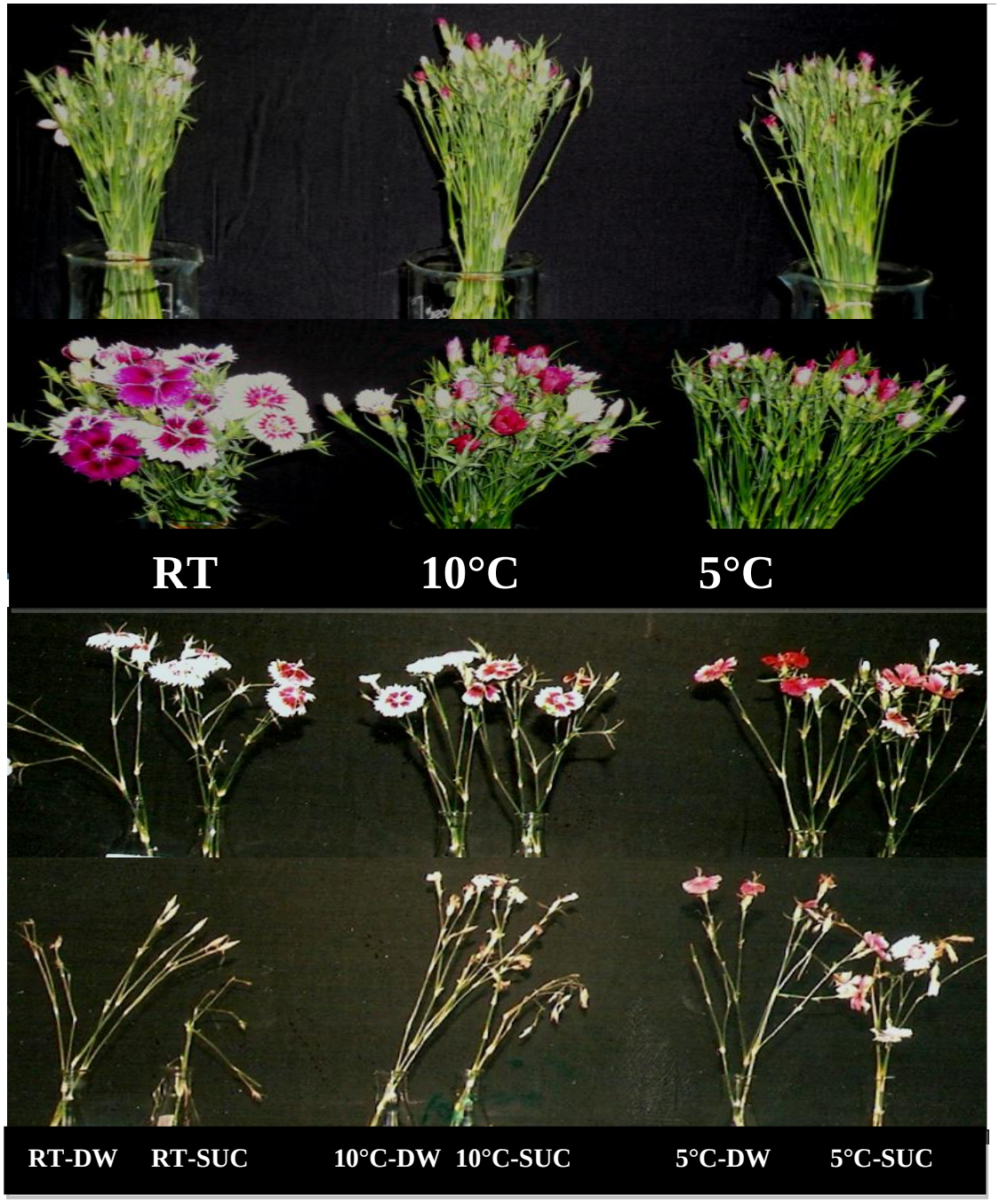


PLATE 4.2.1

EXPERIMENT 4.3

Effect of postharvest dry storage (PHDS) at different temperatures {5, 10 and RT (20±2°C)} for 72 h and subsequent transfer to vase solutions {distilled water (DW) and sucrose (SUC)} on postharvest performance of cut spikes of *Dianthus caryophyllus* L.

Experimental

Uniform and healthy spikes of *Dianthus caryophyllus* growing in the Kashmir University Botanic garden (KUBG) were used for this study. The buds were harvested at 800 h with their oldest bud at 1 day before anthesis (i.e. brush stage). The harvested spikes were immediately brought to the laboratory, cut to a uniform length of 27 cm, wrapped in moistened filter papers, packed in perforated polyethylene flower sleeves (50 cm long and 15 cm wide top) and kept at 5 and 10°C. A separate set of spikes was kept at room temperature (20±2°C). After 72 h the spikes were kept at room temperature after transferring them to 250 ml Ehrlenmeyer flasks containing 200 ml of vase solution {Distilled water (DW) and Sucrose (SUC) 0.05 M}. Each treatment was represented by 5 replicates (flasks) with each flask containing two spikes. The day of transfer of spikes to vase solutions (DW and SUC) was designated as day zero (D0). The average vase life of spikes was counted from the day of transfer to holding solution (D0) and was regarded to be terminated when the last flower had lost its display value (marked by petal inrolling). Visual changes were observed at periodic intervals. Floral diameter, volume of holding solution absorbed and number of blooms per spike was estimated on day 2, 8 and 15. Changes in tissue constituents including soluble proteins, α-amino acids, phenolics and sugar fractions were estimated at day 6 and 14 of transfer to vase solution (DW and SUC). The results have been analyzed statistically and LSD computed at $P_{0.05}$ using MINITAB (v15.1.2-EQUINOX_Softddl.net) software.

Results

Visible effects

The spikes dry stored for 72 h at 5 and 10°C maintained their premature status during storage. But the spikes maintained at 5°C were fresh as compared to spikes kept at 10°C. However, the oldest flower buds on spikes dry stored at room temperature (20±2°C) have lost their turgidity and were closed. All the buds at 1 day before anthesis (i.e. brush stage) on the spikes previously dry stored at 5 and 10°C opened up on D1 after the transfer to vase solution. Senescence of the oldest buds on the spikes previously kept at RT (20±2°C) occurred on day 4 as the flaccid buds regained turgidity once transferred to vase solution dry stored at RT (20±2°C) . Flower senescence was characterized by the loss of petal turgidity followed by initiation of petal inrolling and change from pinkish white petals to pale yellow. Flower senescence was observed to be same as was the case under the field conditions.

Vase life

The average vase life of spikes previously dry stored at various temperatures i.e. RT (20±2°C), 10 and 5°C before transfer to DW was approximately 4, 7 and 11 days respectively; whereas the vase life of the corresponding spikes dry stored at RT (20±2°C), 10 and 5°C before transfer to SUC was approximately 6, 10 and 15 days respectively. The maximum vase life of 15 days was recorded in spikes previously dry stored for 72 h at 5°C and subsequently transferred to SUC (Table 4.3.1; Fig.4.3.1).

Fresh and dry mass and water content

The maximum fresh mass, dry mass and water content was recorded from the tissue samples of spikes dry stored for 72 h at 5°C and transferred to SUC followed by samples of spikes dry stored at the same temperature and transferred to DW. The lowest fresh & dry mass and water content was recorded from samples of the spikes dry stored at RT irrespective of vase solution used. However, the fresh and dry mass decreased significantly with progression in time from day 2 to day 8 (Table 4.3.1; Fig.4.3.2-4.3.4).

Floral diameter

A higher floral diameter was recorded in the flowers previously dry stored at 5°C as compared to the flowers from the corresponding spikes held at 10 and RT (20±2°C)

and transferred to sucrose. Floral diameter showed increase with the progression in time from day 2 to day 8 but thereafter showed a decrease from day 8 to day 15. Maximum floral diameter was recorded on day 8 in the flowers of the spikes previously dry stored at 5°C for 72h and transferred to sucrose (Table 4.3.2; Fig.4.3.5).

Volume of holding solution absorbed

The volume of holding solution absorbed increased with progression in time from day 2 to day 15 of the transfer of spikes to various vase solutions irrespective of the particular temperature regime and holding solution. The solution uptake was significantly higher in spikes previously dry stored for 72h at 10 and 5°C as compared to the corresponding spikes held at RT irrespective of the holding solution. The solution uptake was significantly higher in the spikes previously dry stored for 72h and transferred to DW than the corresponding spikes transferred to SUC irrespective of the temperature treatments. The maximum solution uptake was noticed on day 15 in the spikes previously dry stored at 5°C for 72h and transferred to DW (Table 4.3.2;Fig.4.3.6).

Number of blooms per spike

The number of blooms per spike as also the percent blooming increased with the progression from day 2 to day 15 of the transfer of spikes to various holding solutions irrespective of the particular temperature regime and holding solution. However the rate of blooming was markedly higher in spikes dry stored at 5 and 10°C than spikes dry stored at RT. Spikes previously dry stored at 10 and 5°C for 72 h and transferred to SUC exhibited 71% and 90% blooming by day 15. Number of blooms as also the percent blooms was marginally higher in the spikes held in SUC than the corresponding spikes held in DW (Table 4.3.2; Fig.4.3.7).

Soluble Proteins

The soluble protein content of petal tissue samples from spikes increased with the progression in time from day 6 to day 11 of transfer irrespective of vase solution and temperature treatment in spikes dry stored for 72 h at 5˚C. Maximum soluble protein

content was recorded in the samples from spikes previously stored for 72 h at 10˚C and transferred to SUC on day 6 (Table 4.3.3;Fig.4.3.8).

α- amino acids

The α- amino acid content of the samples from the spikes previously dry stored at 10 and 5°C for 72h mostly registered a decrease as compared to the samples from the corresponding spikes held at RT irrespective of the holding solution. The α-amino content increased with the progression in time from day 6 to day 11 of the transfer of spikes dry stored for 72 h at 5˚C irrespective of nature of vase solution. Highest α-amino acid content was recorded on day 6 in the samples of the spikes previously dry stored at RT (20±2˚C)and transferred to SUC (Table 4.3.3;Fig.4.3.9) .

Total Phenolics

A significantly lower phenolic content was usually maintained in the tissue samples from the spikes previously dry stored at 10 and 5°C for 72h as compared to the samples from the corresponding spikes held at RT. The total phenolic content generally increased with the progression in time from day 6 to day 11 of transfer in case of spikes dry stored at 5˚C irrespective of vase solution. Lowest phenolic content was observed in the samples from the spikes previously stored at 5˚C and transferred to DW on day 6 (Table 4.3.3; Fig.4.3.10).

Total Sugars

The total sugar content of the samples from the spikes previously dry stored for 72h at 10˚C and was much higher as compared to the samples from corresponding spikes held at RT irrespective of the transfer to various vase solutions. The total sugar content decreased sharply with the progression in time from day 6 to day 11 in samples from the spikes dry stored for 72 h at 5˚C held irrespective of vase solution. Maximum total sugar content was recorded in the samples from the spikes previously dry stored for 72 h at 5°C and transferred to SUC on D6 (Table 4.3.4; Fig.4.3.11).

Reducing Sugars

The reducing sugar content of the samples from the spikes previously dry stored for 72h at 10 and 5°C registered a significant increase as compared to the samples from

corresponding spikes held at RT irrespective of the transfer to various vase solutions. However, at each of the temperature regimes the reducing sugar content was found to be higher in samples from the spikes held in SUC as compared to the samples of the spikes held in DW. Reducing sugar content decreased sharply with the progression in time from day 6 to day 11 of the transfer irrespective of the treatment. A higher tissue content of reducing sugars was maintained in the samples from spikes previously dry stored at 5°C for 72h and transferred to SUC (Table 4.3.4;Fig.4.3.12)

Non-reducing Sugars

The non-reducing sugar content increased with the progression in time from day 6 to day 11 in spikes dry stored for 72 h at 5˚C and transferred to SUC, while a decrease was registered in same spikes transferred to DW. However, at each of the temperature regimes the non reducing sugar content was found to be higher in samples from the spikes held in SUC as compared to the samples from the corresponding spikes held in DW. Maximum non reducing sugar content was noticed on day 6 in the samples from the spikes previously dry stored at 5°C for 72h and transferred to SUC (Table 4.3.4; Fig.4.3.13).

Table 4.3.1: Effect of postharvest dry storage (PHDS) at various temperatures (RT, 10 °C and 5 °C) for 72h and subsequent transfer to different holding solution (DW and SUC) on fresh mass, dry mass and water content on day 2 and day 8 of transfer in *Dianthus caryophyllus.*

Treatments	**Days of transfer**						
	Vase life (days)	**Fresh mass (g)**		**Dry mass (g)**		**Water content (g)**	
		D2	**D8**	**D2**	**D8**	**D2**	**D8**
Set A (DW)							
RT	4.078	0.066	--	0.054	--	0.012	--
10 °C	7.32	0.253	--	0.053	--	0.2	--
5 °C	11.0	0.273	0.212	0.064	0.049	0.209	0.163
Set B (SUC)							
RT	6.20	0.088	--	0.066	--	0.022	--
10 °C	10.43	0.363	0.247	0.064	0.055	0.299	0.192
5 °C	15.66	0.394	0.377	0.068	0.060	0.326	0.317
LSD at $P_{0.05}$	0.321	0.009	0.007	0.005	0.007	0.006	0.008

Each value is mean of 6 replicates.
Room temperature RT= (20±2ºC)

Table 4.3.2: Effect of postharvest dry storage (PHDS) at various temperatures (RT, 10°C and 5°C) for 72h and subsequent transfer to different holding solution (DW and SUC) on floral diameter, volume of holding solution absorbed per spike and no. of blooms per spike on day 2, 8 and day 15 of transfer in *Dianthus caryophyllus.*

Treatments	**Days of transfer**								
	Floral diameter (cm)			**Volume of holding solution absorbed per spike (ml)**			**Number of blooms per spike**		
	D2	**D8**	**D15**	**D2**	**D8**	**D15**	**D2**	**D8**	**D15**
Set A (DW)									
RT	3.226	--	--	2.3	6.3	17.5	2.5 (16.6)	6.06 (40.4)	6.06 (40.4)
10 °C	3.412	--	--	5.33	11.0	23.0	3.33 (22.2)	6.33 (42.2)	6.33 (42.2)
5 °C	3.776	4.17	--	6.0	15.0	26.25	3.5 (27.33)	8.62 (57.46)	11.14 (74.21)
Set B (SUC)									
RT	3.92	--	--	2.15	5.4	14.0	2.66 (17.73)	7.12 (47.46)	7.12 (47.4)
10 °C	3.665	4.30	--	3.66	9.0	17.5	3.76 (25.06)	8.1 (54.66)	10.65 (71)
5 °C	3.969	4.31	3.9	3.66	14.4	23.4	4.17 (27.8)	8.8 (58.66)	13.54 (90.2)
LSD at $P_{0.05}$	0.1	0.07	--	0.36	0.55	1.07	0.06	0.09	0.56

Each value is mean of 6 replicates.
Values in parentheses represent percent blooms per spike.
Room temperature (RT= 20±2 ºC)

Table 4.3.3: Effect of postharvest dry storage (PHDS) at various temperatures (RT, 10 °C and 5 °C) for 72h and subsequent transfer to different holding solution (DW and SUC) on soluble proteins, α–amino acids and total phenols from samples of cut spikes on day 6 and day 11 of transfer in *Dianthus caryophyllus.*

Treatments	**Days of transfer**					
	Soluble proteins (mg/g fm)		**α–amino acids (mg/g fm)**		**Total phenols (mg/g fm)**	
	D6	**D11**	**D6**	**D11**	**D6**	**D11**
Set A (DW)						
RT	1.92	--	2.95	--	3.44	--
10°C	10.24	--	1.88	--	2.0	--
5 °C	9.12	9.92	1.02	1.68	1.96	2.11
Set B (SUC)						
RT	10.08	--	4.35	--	4.55	--
10°C	12.96	--	1.39	--	3.66	--
5 °C	8.0	9.76	0.96	1.80	2.02	2.77
LSD at $P_{0.05}$	1.05	--	0.009	--	0.08	--

Each value is the mean of 6 replicates
Room temperature = (20±2 ºC)

Table 4.3.4: Effect of postharvest dry storage (PHDS) at various temperatures (RT,10 °C and 5 °C) for 72h and subsequent transfer to different holding solution (DW and SUC) on total, reducing and non reducing sugars of cut spikes on day 6 and day 11 of transfer in *Dianthus caryophyllus.*

Treatments	**Days of transfer**					
	Total sugars (mg/g fm)		**Reducing sugars (mg/g fm)**		**Non reducing sugars (mg/g fm)**	
	D6	**D11**	**D6**	**D11**	**D6**	**D11**
Set A (DW)						
RT	16.07	--	12.5	--	3.57	--
10 °C	25.89	--	23.21	--	2.68	--
5 °C	57.32	25.89	43.75	9.82	13.57	6.07
Set B (SUC)						
RT	23.5	--	14.10	--	9.40	--
10°C	48.75	--	32.67	--	16.08	--
5 °C	62.88	44.64	44.28	21.42	18.6	23.22
LSD at $P_{0.05}$	1.098	--	1.021	--	0.410	--

Each value is the mean of 6 replicates

Room temperature = (20±2 °C)

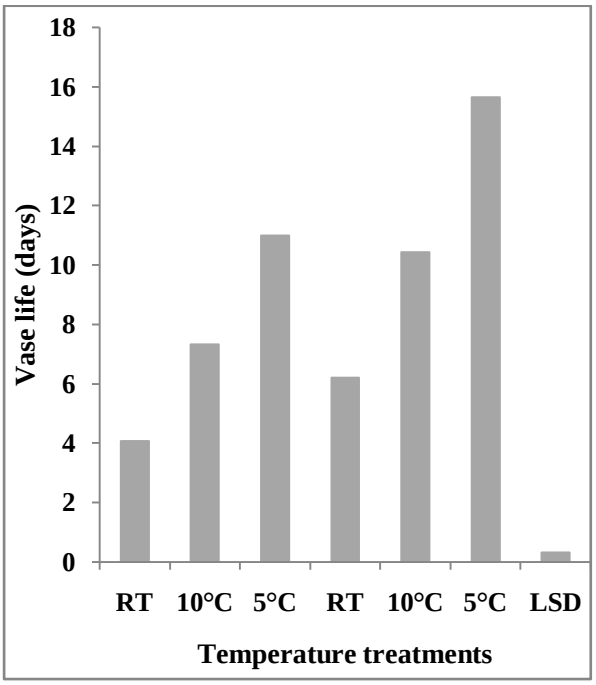


Fig 4.3.1

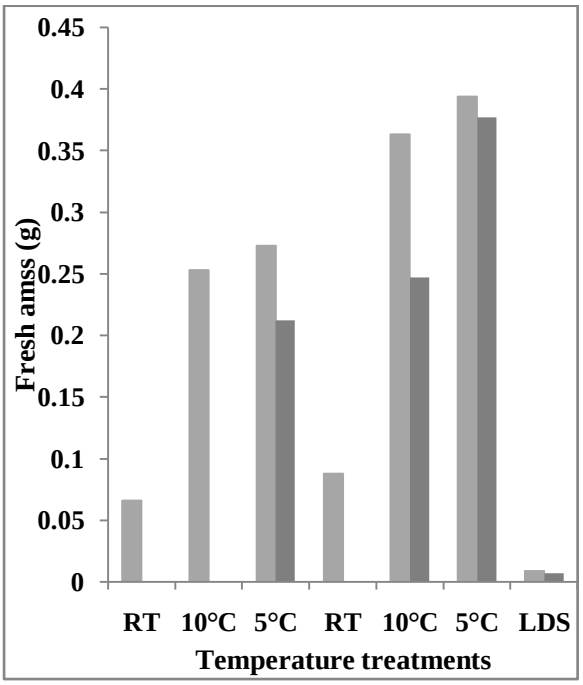


Fig 4.3.2

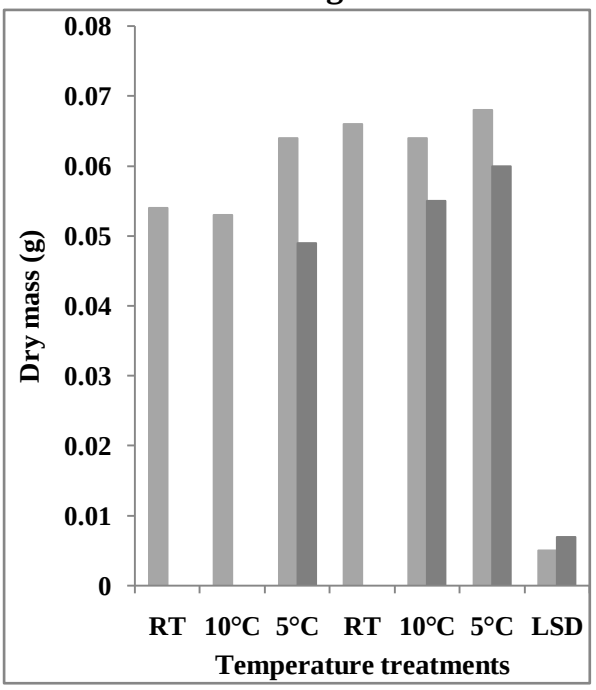


Fig 4.3.3

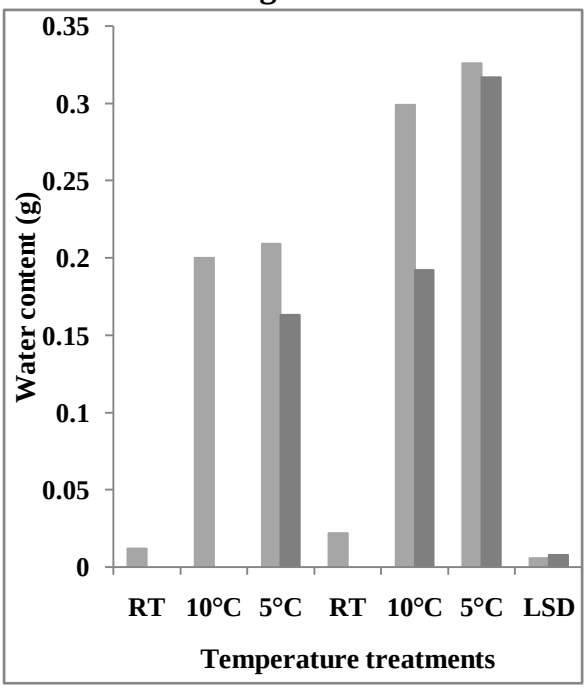


Fig 4.3.4

Fig.4.3.1-4.3.4: Effect of postharvest dry storage (PHDS) at various temperatures (RT, 10°C and 5°C) for 72 h and subsequent transfer to different holding solutions

(DW and SUC) on vase life, fresh and dry mass, and water content in cut spikes of *Dianthus caryophyllus* L.

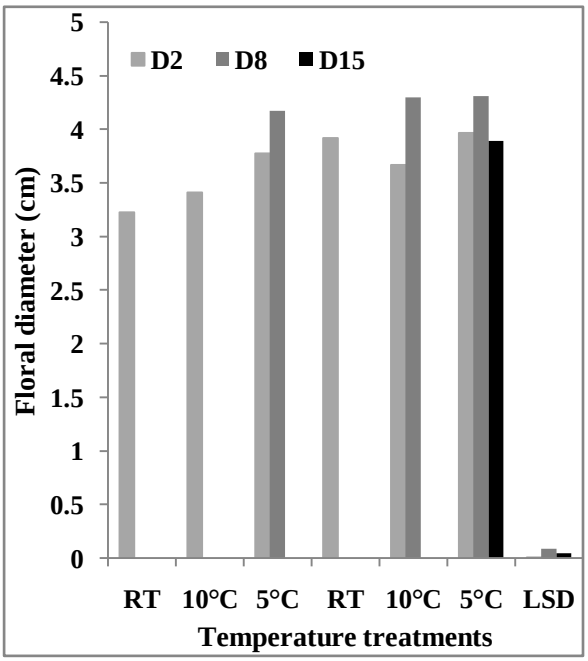


Fig 4.3.5

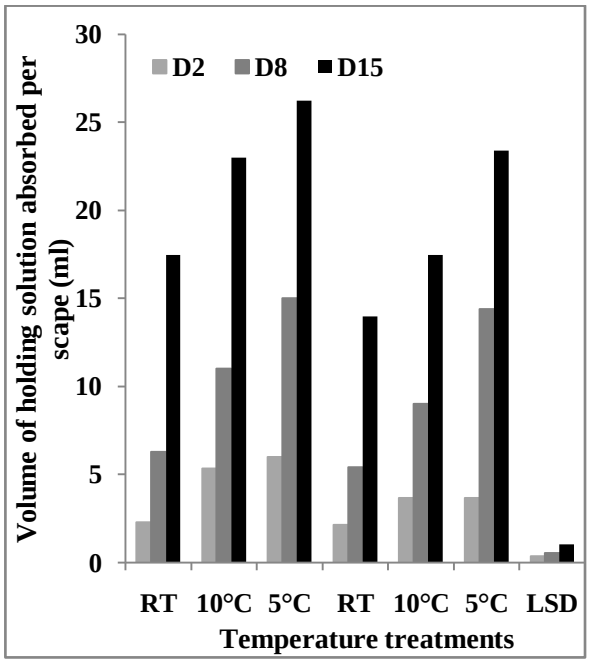
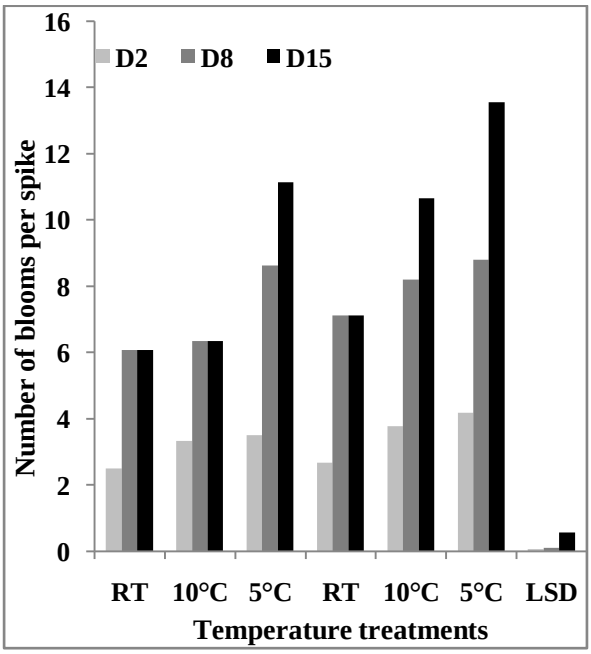


Fig 4.3.6



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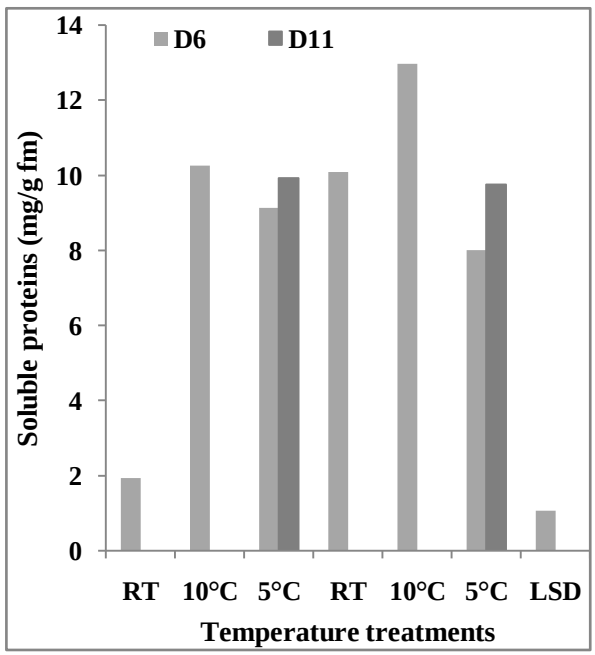


Fig 4.3.8

Fig.4.3.5-4.3.8: Effect of postharvest dry storage (PHDS) at various temperatures (RT, 10°C and 5°C) for 72 h and subsequent transfer to different holding solutions

(DW and SUC) on floral diameter, volume of holding solution absorbed, no. of blooms per spike and soluble proteins in cut spikes of *Dianthus caryophyllus* L.

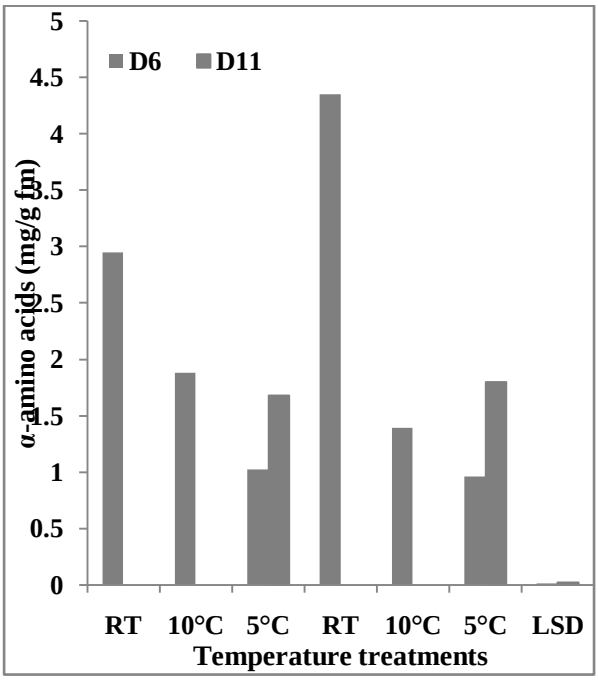


Fig 4.3.9

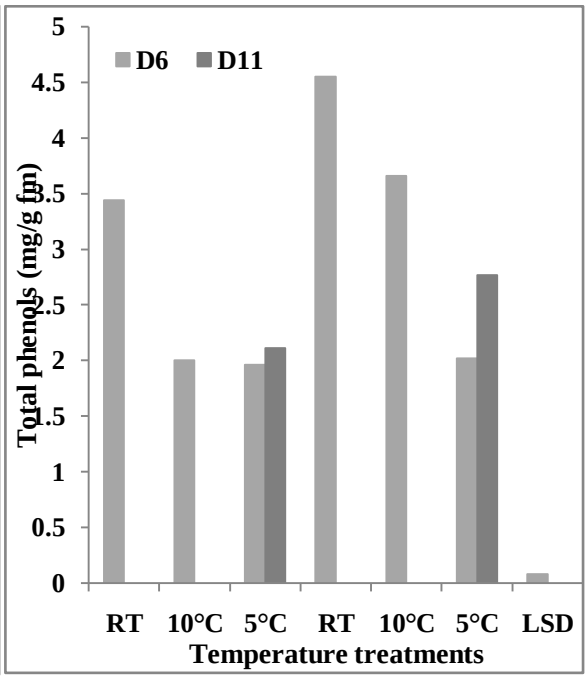
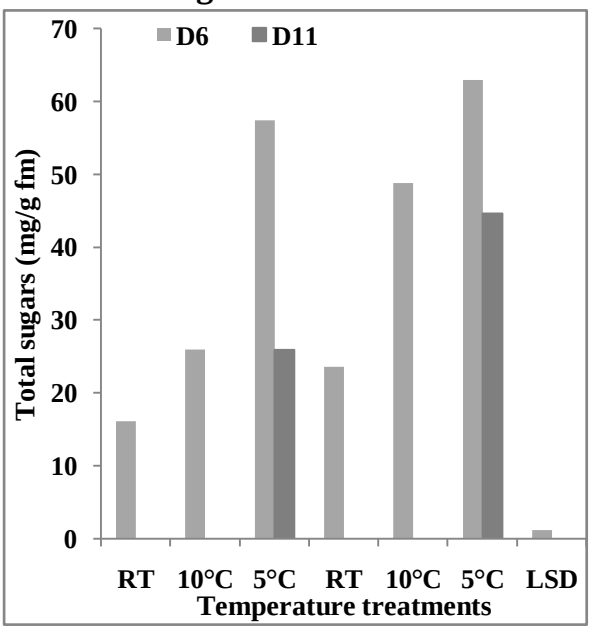


Fig 4.3.10



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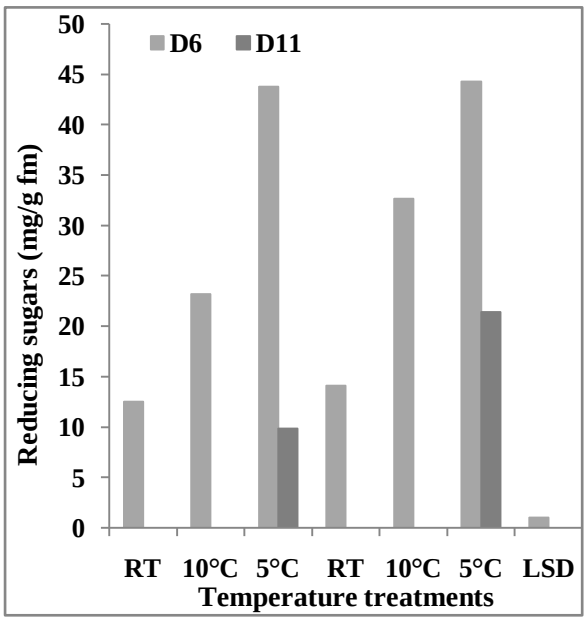


Fig 4.3.12

Fig.4.3.9-4.3.12: Effect of postharvest dry storage (PHDS) at various temperatures (RT, 10°C and 5°C) for 72 h and subsequent transfer to different holding solutions (DW and SUC) on α-amino acids, total phenols, total, and reducing sugars in cut spikes of *Dianthus caryophyllus* L.

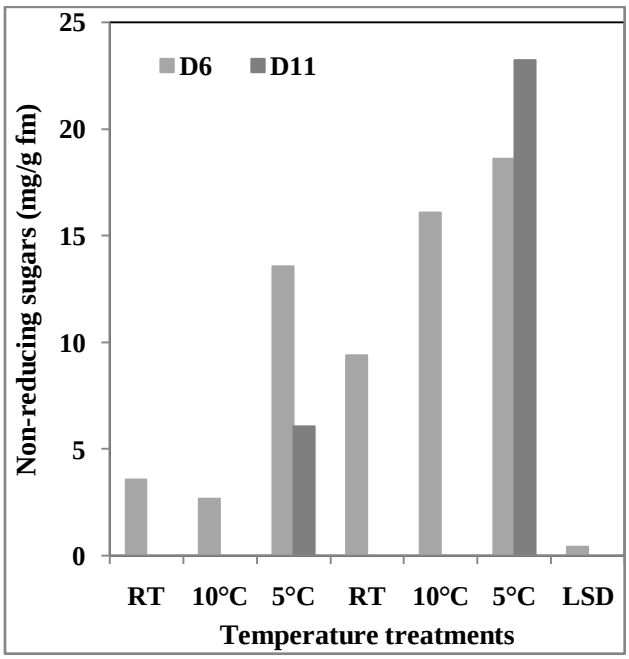


Fig.4.3.13

Fig.4.3.13: Effect of postharvest dry storage (PHDS) at various temperatures (RT, 10°C and 5°C) for 72 h and subsequent transfer to different holding solutions (DW and SUC) on non reducing sugars in cut spikes of *Dianthus caryophyllus* L.

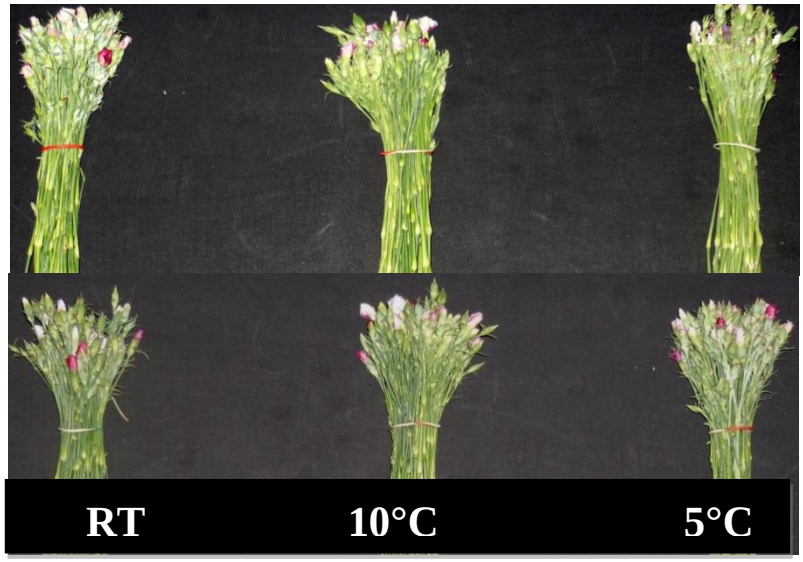
Plate 4.3.1

Effect of postharvest dry storage (PHDS) for 72 h at room temperature (RT), 10°C and 5°C before transfer to DW and SUC (0.05M) in cut spikes of *Dianthus caryophyllus*

Fig. (a): From left to right are arranged spikes before dry storage for 72 h

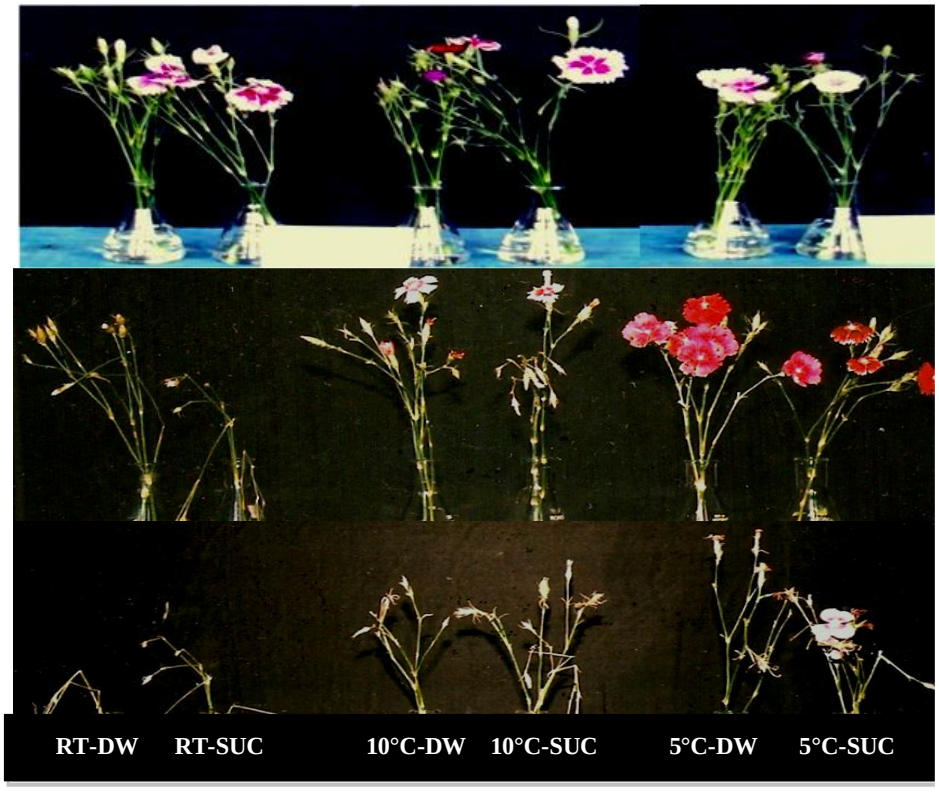
Fig. (b): From left to right are arranged spikes after dry storage for 72 h.

Spikes of *Dianthus caryophyllus* held in distilled water (DW) or sucrose 0.05 M (SUC) after 72 h dry storage at day 2 (Fig. c), day 8 (Fig. d) and day 15 (Fig. e) of transfer to respective vase solutions.



(a)

(b)



(c)

(d)

(e)

Plate 4.3.1

EXPERIMENT 4.4

Effect of protein synthesis inhibitor cycloheximide (CHI) on the cut spikes of *Dianthus caryophyllus* L. before, during and after 72 h wet storage at 5ºC.

Experimental

Mature and uniform spikes growing in the Kashmir University Botanic Garden (KUBG) were used for the present study. The buds were harvested at 800 h with their oldest bud at brush stage. The harvested spikes were immediately brought to the laboratory, cut to a uniform length of 27cm and divided into three sets containing equal number of spikes. One set of spikes was pulse treated with 0.1mM CHI for 1 h before 72 h wet storage at 5ºC, another set of spikes was pulse treated with 0.1mM CHI for 1 h during 5ºC wet storage. The third set of spikes was pulse treated with 0.1mM CHI for 1 h after storing it at 5ºC for 72 h. A separate set of spikes was kept unpulsed and stored at 5ºC for 72 h. This set of spikes represented the control. After 72 h the spikes were kept at RT (20±2 ºC) before transferring them to 250 ml Ehrlenmeyer flasks containing 200 ml of vase solution {Distilled water (DW) and Sucrose (SUC) 0.05 M}. Each treatment was represented by 5 replicates (flasks) with each flask containing two spikes. The day of transfer of spikes to vase solutions (DW and SUC) was designated as day zero (D0). The average vase life of spikes was counted from the day of transfer of the spikes to respective holding solutions (D0) and was regarded to be terminated when the last flower had lost its display value (marked by color change and loss of petal turgidity). Visual changes were observed at periodic intervals. Floral diameter, volume of holding solution absorbed per spike and number of blooms was estimated on Day 2, 8 and 16. Fresh mass, dry mass and water content was estimated on day 4 and day 12. Changes in tissue constituents (soluble proteins, α- amino acids, phenolics and sugar fractions) were estimated on day 4 and 12 of transfer of spikes to vase solution (DW and SUC). The results have been analyzed statistically and LSD computed at $P_{0.05}$ using MINITAB (v15.1.2-EQUINOX_Softddl.net) software.

Results

Visible effects

The spikes pulse treated with 0.1 mM CHI for 1 h before; during and after 72 h wet storage maintained their premature status during storage. All the buds at brush stage on the spikes opened up on D1 after the transfer to vase solutions. The spikes pulsed with 0.1mM CHI before 72h wet storage resulted in spike bending and the opening of oldest bud was arrested. Flower senescence was characterized by loss of petal turgidity, change of color from pinkish white to pale yellow and petal inrolling. Flower senescence was observed to be same as was the case under field conditions.

Vase life

The vase life of approximately 21 days was recorded in spikes treated with 0.1Mm CHI after storage and transferred to SUC as compared to control where it was 18 days. The vase life of the spikes transferred to SUC was considerably more than the respective spikes transferred to DW irrespective of the CHI treatment (Table 4.4.1; Fig.4.4.1).

Fresh & dry mass

The fresh and dry mass of spikes treated with 0.1mM CHI for 1 h before, during and after 72 h wet storage was comparable irrespective of CHI treatment. However, the fresh and dry mass showed a decrease with the progression in time from day 4 to day 12 of transfer of spikes either to DW or SUC (Table 4.4.1; Fig.4.4.2, 4.4.3).

Floral diameter

Treatment of spikes with 0.1mM CHI during and after 72 h wet storage resulted in increased floral diameter as compared to unpulsed spikes. However, the increase was more pronounced in case of spikes treated with CHI. The floral diameter almost remained constant on various days of transfer to different vase solutions irrespective of the nature of holding solution and CHI treatment (Table 4.4.2; Fig.4.4.4).

Volume of holding solution absorbed

Volume of holding solution absorbed per spike increased significantly from day 2 to day 16 in all spikes irrespective of various treatments. The spikes pulse treated with 0.1 mM CHI before and during 72 h wet storage showed considerably more water

absorption as compared to control (unpulsed) and spikes treated with CHI after 72 h wet storage (Table 4.4.2;Fig.4.4.5).

Number of blooms per spike

The number of blooms as also the percent blooming increased with the progression in time from day 2 to day 16 of the transfer of spikes to various holding solutions irrespective of CHI treatment. Maximum numbers of blooms were recorded in the spikes pulse treated with CHI after 72 h wet storage and transferred to SUC on day 16 (Table 4.4.3; Fig.4.4.6).

Soluble proteins

The soluble protein content of the samples from spikes previously pulse treated with 0.1mM CHI for 1 h before, during and after 72 h wet storage showed significantly lower protein content as compared to the respective controls irrespective of the nature of the holding solution. The samples from spikes held in sucrose showed lower soluble protein content than the corresponding samples from spikes held in DW irrespective of the nature of CHI treatment (Table 4.4.3; Fig.4.4.7).

α- amino acids

The α- amino acids content of the samples from the spikes pulse treated with 0.1mM CHI for 72 h before, during and after 72 h wet storage was higher as compared to the samples from unpulsed spikes irrespective of the holding solution. Higher α- amino acids content was recorded in the samples of the spikes pulse treated with 0.1mM CHI for 1 h and transferred to SUC as compared to the corresponding samples from spikes transferred to DW irrespective of the nature of CHI treatment (Table 4.4.3; Fig.4.4.8).

Total Phenolics

The total phenolic content was almost maintained in all the treatments (before, during & after 72 h wet storage) including the controls. The samples from spikes held in SUC maintained marginally a higher phenolic content irrespective of the nature of 0.1mM pulse treatments as compared to the corresponding samples from spikes held in DW. Maximum phenolic content was recorded in the samples from the spikes pulse treated with 0.1mM CHI before storage and transferred to DW (Table 4.4.3; Fig.4.4.9).

Total Sugars

A higher content of total sugars was maintained in the samples from the spikes pulse treated with 0.1mM CHI for 1 h before, during and after 72 h wet storage as compared to unpulsed (controls) spikes irrespective of nature of the vase solution. Higher total sugar content was recorded in the samples from the spikes previously pulse treated with 0.1mM CHI for 1 h and transferred to SUC as compared to the samples from corresponding spikes held in DW. Maximum total sugar content was recorded in the samples from the spikes previously pulse treated with 0.1mM CHI for 1 h after 72 h wet storage and transferred to SUC (Table 4.4.4; Fig.4.4.10).

Reducing sugars

A higher tissue content of reducing sugars was maintained in samples from spikes pulse treated with 0.1mM CHI for 1 h before, during and after storage as compared to control. Higher reducing sugar content was recorded in the samples from the spikes pulse treated with 0.1mM CHI for 1 h and transferred to SUC irrespective of the nature of CHI treatment as compared to the samples from corresponding spikes held in DW. Maximum reducing sugar content was maintained in the samples from the spikes pulse treated with 0.1mM CHI for 1 h after 72 h wet storage and transferred to SUC (Table 4.4.4; Fig.4.4.11).

Non-reducing sugars

A higher tissue content of non-reducing sugars was maintained in samples from spikes treated with 0.1mM CHI before, during and after storage as compared to the control. The non-reducing sugar content was comparatively higher in samples pulse treated with 0.1mM CHI for 1 h and transferred to SUC as compared to the samples from corresponding spikes held in DW. Maximum non-reducing sugar content was maintained in the samples of the spikes transferred to SUC irrespective of treatments. However the non-reducing sugar content of the samples from spikes pulse treated with 0.1mM CHI for 1 h after 72 h wet storage and transferred to SUC was found to be higher (Table 4.4.4; Fig.4.4.12).

Table 4.4.1: Effect of protein synthesis inhibitor cycloheximide (CHI) on vase life, fresh and dry mass and water content of cut spikes of *Dianthus caryophyllus.*

Treatments	**Vase life**	**Days of transfer**

	(days)	Fresh mass (g)		Dry mass (g)	
		D4	D12	D4	D12
Set A (DW)					
1. Control	14.33	0.228	0.212	0.042	0.032
2. (CHI) before storage	15.33	0.228	0.215	0.045	0.034
3. (CHI) during storage	16.65	0.235	0.229	0.056	0.039
4. (CHI) after storage.	18.33	0.298	0.290	0.061	0.053
Set B (SUC)					
1. Control	18.00	0.232	0.228	0.045	0.041
2. (CHI) before storage	15.42	0.226	0.224	0.040	0.038
3. (CHI) during storage	17.04	0.237	0.234	0.043	0.038
4. CHI) after storage	20.83	0.301	0.301	0.063	0.059
LSD at $P_{0.05}$	0.091	0.05	0.054	0.02	0.01

Each value is the mean of 6 independent replicates.

Table 4.4.2: Effect of protein synthesis inhibitor cycloheximide (CHI) on volume of holding solution absorbed and no. of blooms per spike of cut spikes of *Dianthus caryophyllus.*

Treatments	**Days of transfer**					
	Floral diameter (cm)			**Volume of holding solution absorbed per spike (ml)**		
	D2	**D8**	**D16**	**D2**	**D8**	**D16**
Set A (DW)						
Control	2.685	2.693	-	0.66	11	17.66
(CHI) before storage	3..83	3.875	-	6.78	14.54	26.33
(CHI) during storage	4.10	4.11	3.00	6.98	12.89	25.33
(CHI) after storage	4.20	3.231	3.09	5.45	9.66	19.65
Set B (SUC)						
Control	3.86	3.865	2.939	0.33	10.831	17.33
(CHI) before storage	4.06	4..070	-	5.54	12.98	25.0
(CHI) during storage	4.16	4..16	3.00	5.67	11.49	23.21
(CHI) after storage	4.5	4.52	3.9	4.93	11.04	20.09
LSD at $P_{0.05}$	0.41	0.17	0.06	0.51	0.42	0.73

Each value is the mean of 6 independent replicates.

Table 4.4.3: Effect of protein synthesis inhibitor cycloheximide (CHI) on soluble proteins, α-amino acids, total phenols, total sugars, reducing sugars and non-reducing sugars (expressed as mg/g fresh mass) of cut spikes of *Dianthus caryophyllus.*

Treatments	Days of transfer						
	No. of blooms per spike			Soluble proteins (mg/g fm)		α-amino acids (mg/g fm)	
	D2	D8	D16	D4	D12	D4	D12
Set A (DW)							
Control	2.06(17)	4.23(42)	4.7(52)	2.08	1.96	2.10	2.18
CHI) before storage	2.18(18.3)	4.73(42.4)	5.0(52.5)	1.86	1.82	3.451	3.54
(CHI) during storage	2.18(18.3)	4.62(43)	5.2(54.5)	1.36	1.29	4.107	4.97
(CHI) after storage	3.03(21.4)	5.42(49.4)	6.5(64.5)	1.04	0.98	5.751	6.32
Set B (SUC)							
Control	2.24 (17)	5.14(42.5)	6.01 (63)	2.12	2.21	5.10	6.43
CHI) before storage	2.14(19.02)	-	-	1.2	1.27	8.544	9.04
(CHI) during storage	2.34(19.01)	4.62(48.9)	5.33(64.2)	0.78	0.98	5.422	6.03
(CHI) after storage	3.33 (22.0)	5.92(57.9)	7.04 (72)	1.02	1.24	6.572	7.36
LSD at $P_{0.05}$	0.081	0.072	0.028	0.04	0.02	0.10	0.25

Each value is the mean of 6 independent replicates.

Table 4.4.3: Effect of protein synthesis inhibitor cycloheximide (CHI) on soluble proteins, α-amino acids, total phenols, total sugars, reducing sugars and non-reducing sugars (expressed as mg/g fresh mass) of cut spikes of *Dianthus caryophyllus.*

Treatments	Days of transfer							
	Total phenols (mg/g fm)		Reducing sugars (mg/g fm)		Non reducing sugars (mg/g fm)		Total sugars (mg/g fm)	
	D4	D12	D4	D12	D4	D12	D4	D12
Set A (DW)								
Control	1.60	2.09	11.25	9.87	6.26	5.39	17.71	14.65
(CHI) before storage	1.44	1.975	20.53	18.09	7.15	6.76	27.68	24.85
(CHI) during storage	1.22	2.03	22.32	15.04	13.39	16.03	35.71	31.07
(CHI) after storage	1.39	1.98	31.40	27.54	15.63	26.5	47.03	42.13
Set B (SUC)								
Control	1.60	2.13	30.23	28.09	6.49	3.34	36.72	31.43
(CHI) before storage	1.61	2.10	27.68	24.43	12.17	26.77	39.85	35.98
(CHI) during storage	1.61	2.43	34.02	29.76	13.48	11.91	47.5	41.67
(CHI) after storage	1.44	2.38	40.53	37.89	17.65	13.51	57.65	51.4
LSD at $P_{0.05}$	0.10	0.11	0.32	0.42	0.54	0.32	0.41	0.65

Each value is the mean of 6 independent replicates.

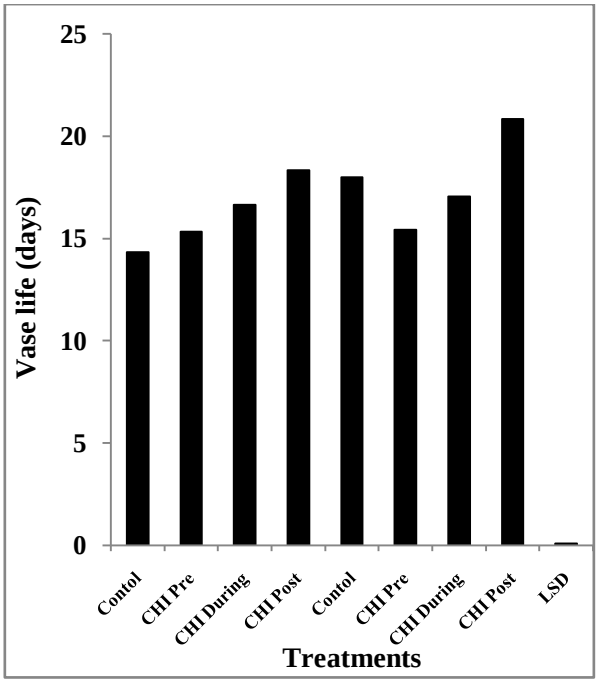
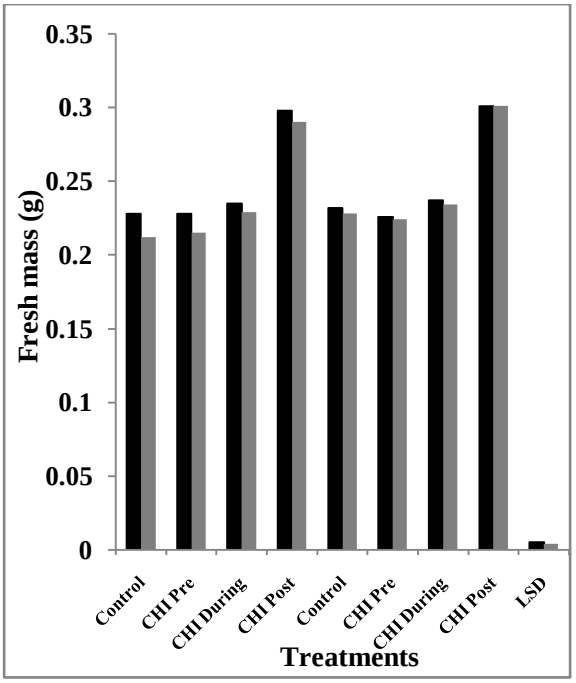


Fig. 4.4.1



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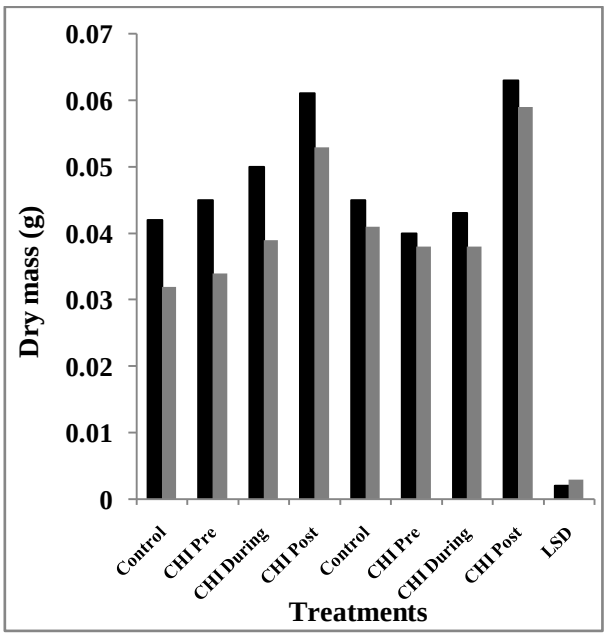


Fig. 4.1.3

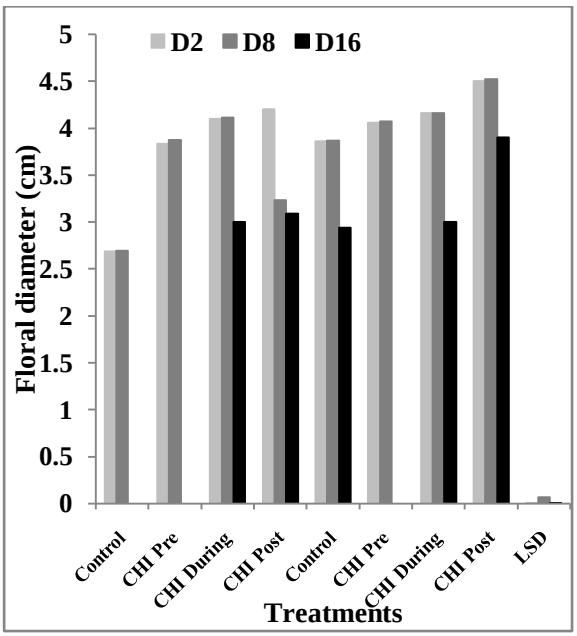


Fig. 4.1.4

Fig. 4.4.1-4.4.4: Effect of 1 hour pulse treatment with 0.1mM CHI (before, during and after 72h wet storage) on the vase life, fresh mass, dry mass and floral diameter of cut spikes of *Dianthus caryophyllus L.*

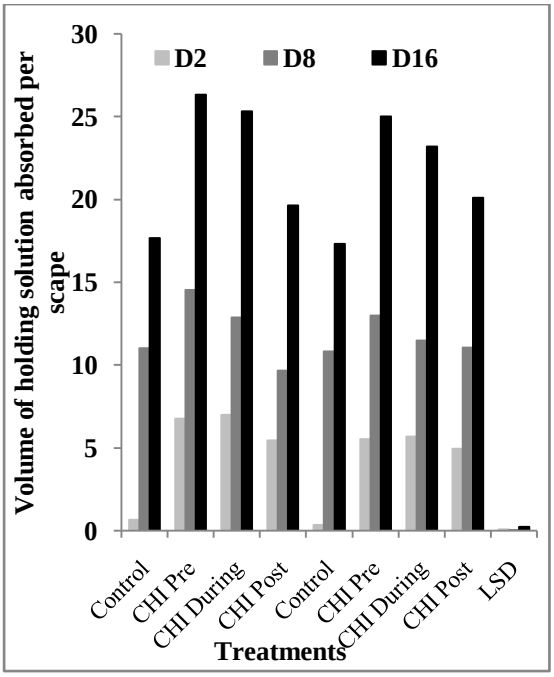


Fig. 4.4.5

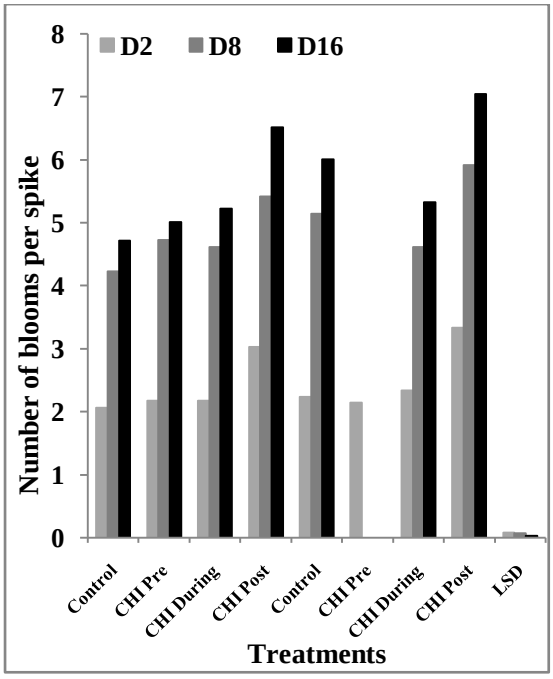


Fig. 4.4.6

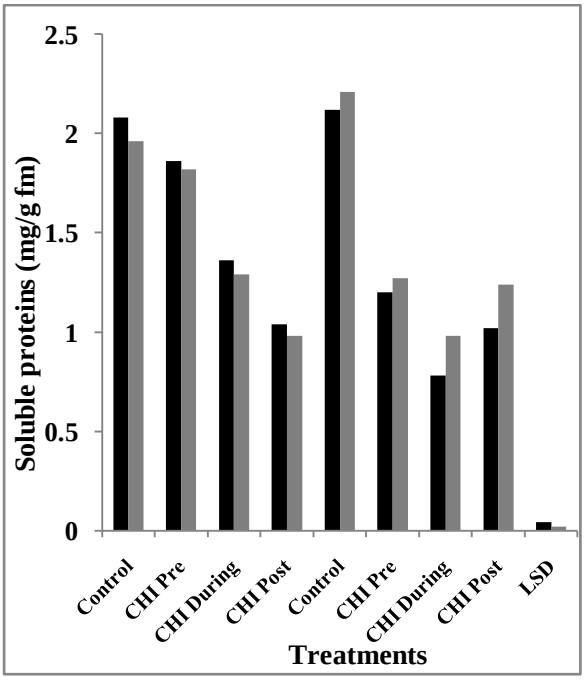


Fig. 4.4.7

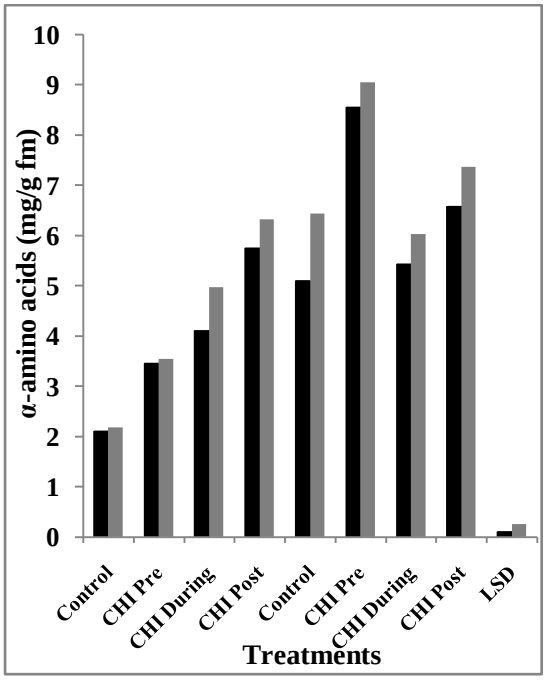


Fig. 4.4.8

Fig.4.4.6-4.4.8: Effect of 1 hour pulse treatment with 0.1mM CHI (before, during and after 72h wet storage) on volume of holding solution absorbed per spike, number of blooms per spike, soluble proteins and α-amino acids of cut spikes of *Dianthus caryophyllus.*

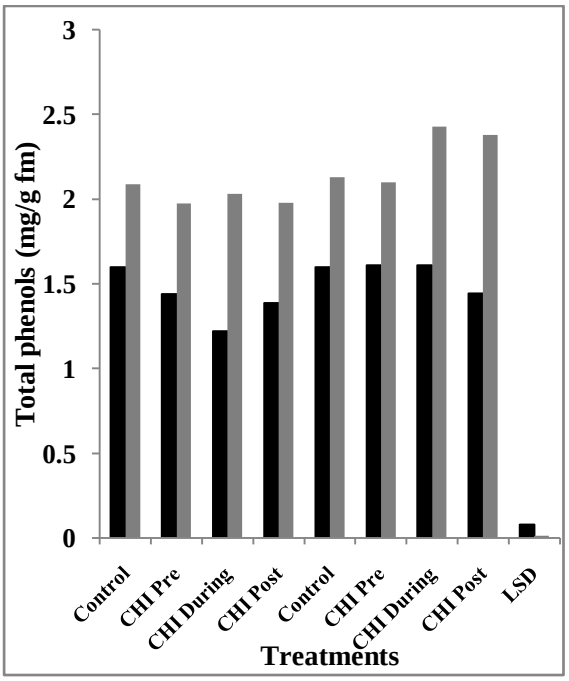


Fig. 4.4.9

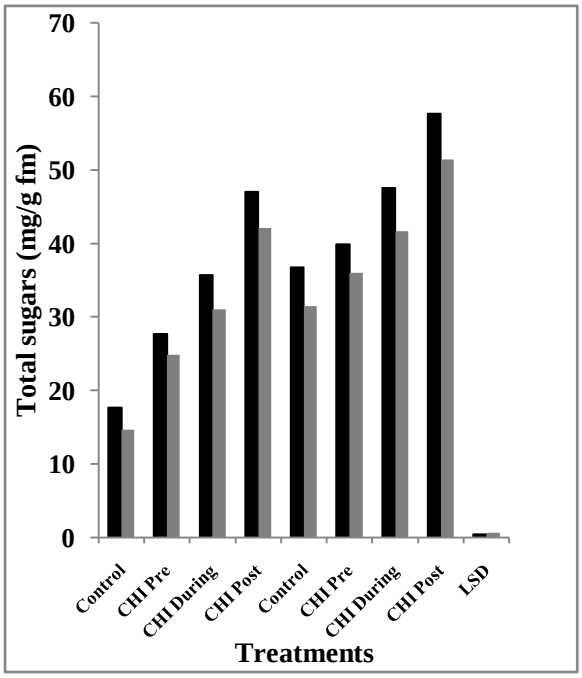


Fig. 4.4.10

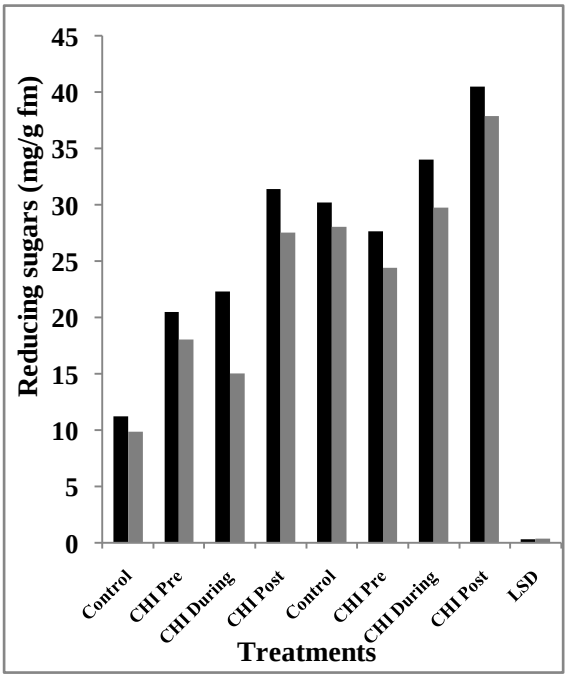


Fig. 4.4.11

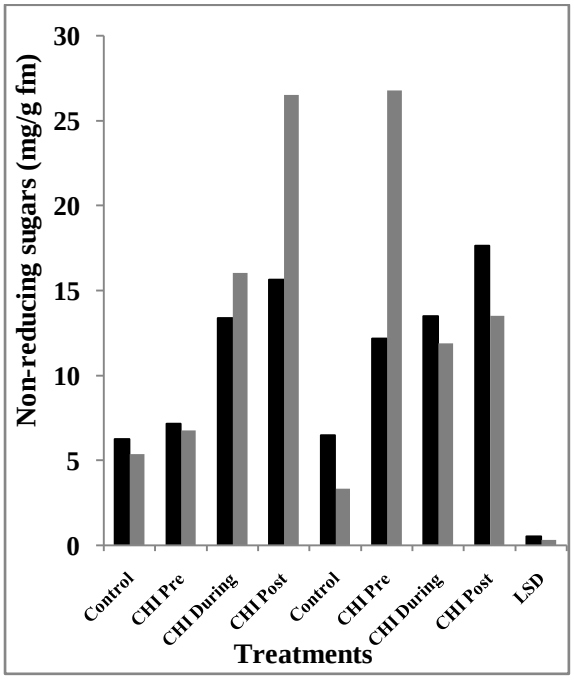


Fig. 4.4.12

Fig4.4.9-4.4.12: Effect of 1 hour pulse treatment with 0.1mM CHI (before, during and after 72h wet storage) on the total phenols, total, reducing and non-reducing sugars of cut spikes of *Dianthus caryophyllus.*

Plate 4.4.1

Effect of pretreatment with cycloheximide (0.1mM CHI, 1 h pulse) before, during and after 72 h postharvest wet storage before transferred to DW and SUC (0.05M) on postharvest performance of *Dianthus caryophyllus L*.

Fig. (a): From left to right are arranged spikes before storage.

Fig. (b): From left to right are arranged spikes after storage.

Effect of pretreatment with cycloheximide (0.1mM CHI, 1 h pulse) before, during and after 72 h postharvest wet storage (PHWS) at 5ºCbefore transferred to DW and SUC on day 2 (Fig. c), day 8 (Fig. d) and day 20 (Fig. e) of transfer to the respective vase solutions.

I, III & V represent spike transferred to DW and II, IV & VI represent spikes transferred to sucrose.

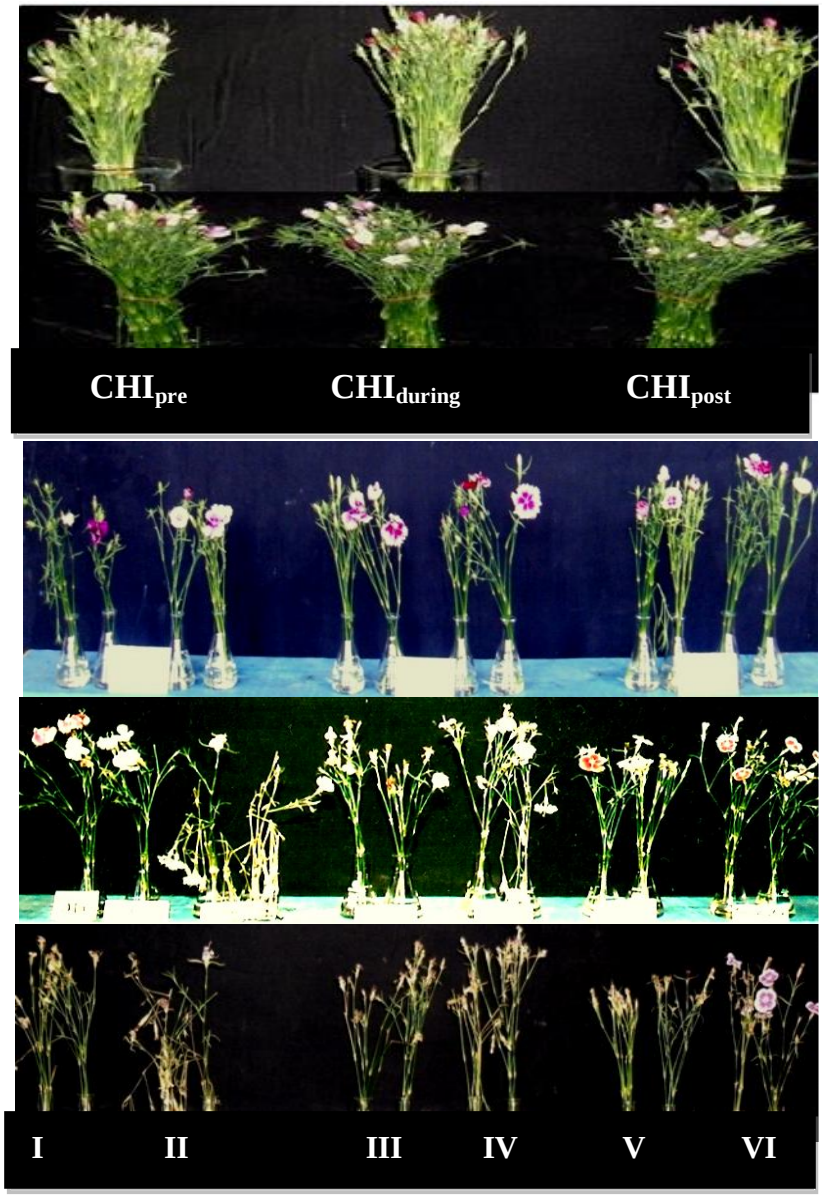


Plate 4.4.1

EXPERIMENT 4.5

Effect of different sugars on flower senescence in isolated flowers of *Dianthus caryophyllus* L.

Experimental

Isolated flowers of *Dianthus caryophyllus* were harvested from the plants growing in Kashmir University Botanic Garden (KUBG). The isolated flowers were brought to the laboratory, pedicels were cut to a uniform length of 2cms and were divided into 6 sets, each set comprising 35 vials with each vial containing one flower. The buds were held in 10ml glass vials containing 5ml of respective holding solutions. Each set was supplied with a different sugar source viz. glucose, sucrose, mannitol, inositol and polyethylene glycol (PEG), at 0.05 M concentration. A separate set of 35 vials containing buds held in distilled water represented the control. The treatment effects were evaluated by keeping the experimental set up under natural conditions in the laboratory with temperature ranging from 24±3°C. Floral diameter, fresh & dry mass, water content; besides soluble proteins, α-amino acids, total phenols and sugar fractions in petal tissues were recorded at periodic intervals.

Results

Visible effects

All the buds opened on the day1 of transfer to holding solution, except for those transferred to PEG. Flower senescence under laboratory conditions was comparable to that under field conditions. Flower senescence was characterized by the loss of petal turgidity followed by inrolling of petals. The flowers transferred to various vase solutions showed marked variation in color. Flowers transferred to SUC were intense in color followed by the flowers transferred to D-glucose. The flowers transferred to other sugar sources (such as inositol, mannitol and PEG) were comparable in their visible effects.

Longevity of flowers

Maximum longevity of 9 days was registered in the flowers held in SUC followed by the flowers transferred to D-Glucose (7days) as compared to control where it was only

5 days. The floral buds transferred to PEG did not open at all. PEG clearly arrested bud opening in *Dianthus caryophyllus* (Table 4.5.2; Fig. 4.5.1).

Fresh and dry mass

Maximum fresh mass and water content of flowers was recorded in the flowers held in SUC followed by the flowers held in D- Glucose, while as the maximum dry mass was recorded in the flowers transferred to SUC followed by the flowers transferred to Inositol. Lower fresh mass, dry mass and water content was recorded in the floral buds held in PEG as compared to control (Table 4.5.1; Fig.4.5.2-4).

Floral diameter

Generally a higher floral diameter was recorded in the samples held in SUC followed by the samples held in D-glucose. The floral diameter in other treatments (inositol and mannitol) was comparable to that control. In PEG as the floral buds did not open up. Floral diameter generally showed an increase with the progression in time from day 2 to day 4 but decrease in floral diameter was registered from day 4 onwards as senescence progressed in most of the cases irrespective of the nature of sugar source (Table 4.5.2; Fig.4.5.5.).

Volume of holding solution absorbed

Maximum vase uptake was recorded in the floral buds held in distilled water. The volume of holding solution absorbed increased with the progression in time from D2 to D8. The increase was more pronounced in the floral buds held in DW (Table 4.5.2; Fig 4.5.6)

Soluble proteins

The concentration of soluble proteins was highest in samples from floral buds transferred to polyethylene glycol (PEG) followed by the samples from floral buds transferred to sucrose. The soluble protein content showed a significant decrease with the progression in the time from day 2 to day 5. The decrease was more pronounced in the floral buds transferred to PEG (Table 4.5.2; Fig.4.5.7).

α- amino acids

Higher α-amino acid content was observed in the tissue samples held in mannitol, followed by the floral buds kept in D- glucose and PEG. α-amino acid content generally showed a significant decrease with the progression in time from day 2 to day 5. The decrease was pronounced in the samples from floral buds held in held in PEG (Table 4.5.3; Fig.4.5.8).

Total phenols

Maximum phenolic content was observed in the samples held in PEG. Total phenolic content showed an increase with the progression in time from day 2 to day 5. Lowest phenolic content was registered in the samples held in inositol on D2 (Table 4.5.3; Fig.4.5.9).

Total sugars

The tissue content of total sugars showed a decrease with the progression in time from day 2 to day 5. Maximum total sugars were registered in the samples held in D-glucose followed by the samples from buds held in sucrose (Table 4.5.3; Fig.4.5.10).

Reducing sugars

Highest reducing sugar content was observed in the samples held in D-glucose followed by the floral buds kept in sucrose. The reducing sugar content showed a significant decrease with the progression in time from day 2 to day 5 (Table 4.5.4; Fig.4.5.11).

Non-reducing sugar

Non- reducing sugars generally showed a significant decrease with the progression in time from day 2 to day 5. Highest non-reducing sugar content was observed in the samples held in D- glucose (Table 4.5.4; Fig.4.5.12).

Table 4.5.1: Effect of different sugars on vase life, fresh mass, dry mass and water content on the isolated flowers of *Dianthus caryophyllus* L.

Treatments	**Flower longevity (days)**	**Fresh mass (g)**	**Dry mass (g)**	**Water content (g)**
Control	4	0.157	0.078	0.079
D-glucose	7	0.208	0.095	0.113
Inositol	6	0.189	0.108	0.081
Mannitol	6	0.178	0.082	0.096
Sucrose	9	0.307	0.168	0.139
PEG	-	0.129	0.058	0.071
LSD at $P_{0.05}$	0.09	0.009	0.007	0.006

Table 4.5.2: Effect of different sugars on floral diameter, holding solution, and soluble proteins of the isolated flowers of *Dianthus caryophyllus* L.

Treatments	**Days of transfer**							
	Floral diameter (cm)			**Holding solution absorbed (ml)**			**Soluble proteins (mg/g fm)**	
	D2	**D4**	**D8**	**D2**	**D4**	**D8**	**D2**	**D5**
DW	2.06	3.65	--	1.8	2.5	2.75	1.76	--
D-glucose	3.24	4.21	--	1.06	1. 48	1.82	2.08	1.21
Inositol	2.78	3.27	--	1.23	1.46	1.74	2.08	1.27
Mannitol	2.82	3.36	--	1.6	1.71	1.82	2.16	1.35
Sucrose	3.90	4.91	4.07	1.32	1.87	2.06	2.24	1.41
PEG	--	--	--	1.20	1.35	1.56	4.320	1.03
LSD at $P_{0.05}$	0.051	0.047	--	0.014	0.011	0.021	0.013	0.007

Table 4.5.3: Effect of different sugars on α-amino acids, total phenols and reducing sugars on the isolated flowers of *Dianthus caryophyllus* L.

treatments	**α-amino acids (mg/g fm)**		**Total phenols (mg/g fm)**	
	D2	**D5**	**D2**	**D5**
control	0.59	--	3.73	--
D-glucose	2.26	1.07	2.73	2.95
Inositol	1.97	1.03	2.6	2.63
Mannitol	2.76	1.31	3.06	3.27
Sucrose	1.38	0.75	3.4	3.53
PEG	2.26	0.53	5.26	5.37
LSD at $P_{0.05}$	0.009	0.006	0.111	0.012

Table 4.5.4: Effect of different sugars on non reducing and total sugars on the isolated flowers of *Dianthus caryophyllus* L.

Treatments	**Total sugars (mg/g fm)**		**Reducing sugars (mg/g fm)**		**Non-reducing sugars (mg/g fm)**	
	D2	**D5**	**D2**	**D5**	**D2**	**D5**
control	17.14	--	9.64	--	7.5	--
D-glucose	87.85	40.65	72.85	32.33	15.0	8.83
Inositol	15.0	0.06	7.5	5.73	7.5	4.33
Mannitol	12.85	8.97	6.42	4.45	6.43	4.52
Sucrose	60.0	26.07	55.71	22.31	4.29	3.76
PEG	28.93	7.55	21.42	15.23	7.5	2.32
LSD at $P_{0.05}$	0.129	0.123	0.912	0.812	0.579	0.291

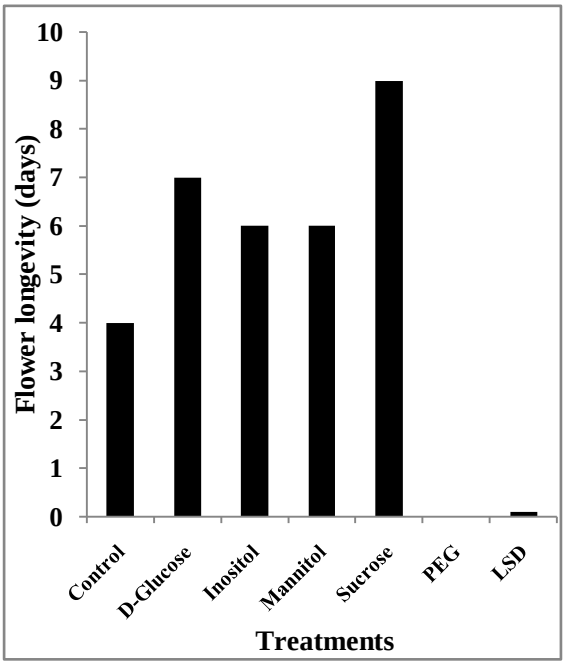


Fig. 4.5.1

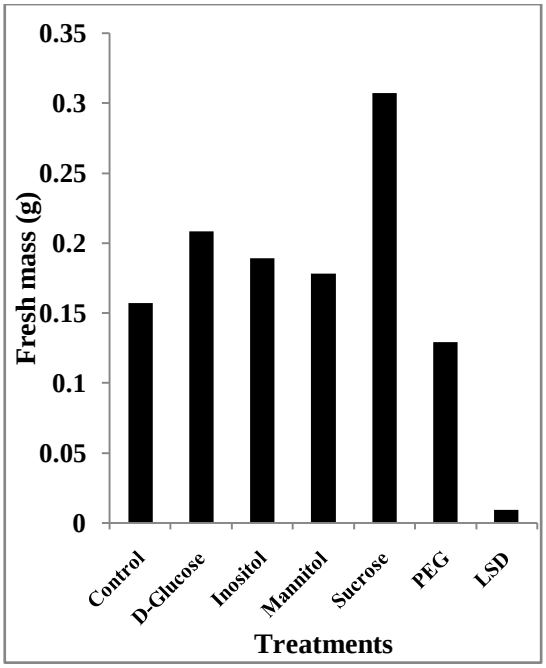


Fig. 4.5.2

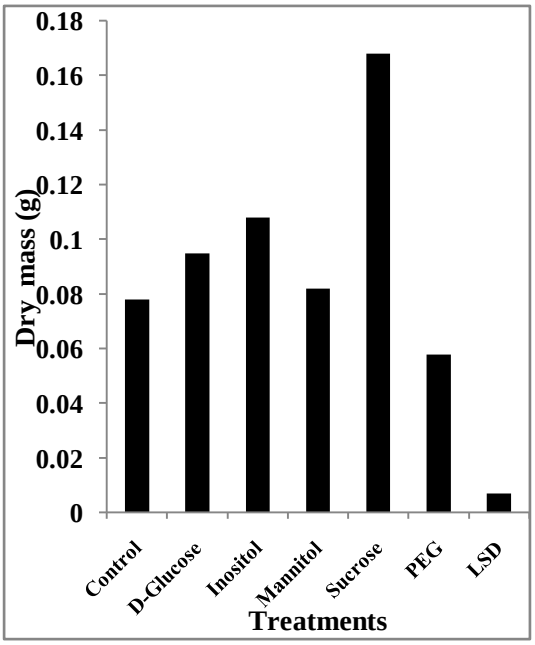


Fig. 4.5.3

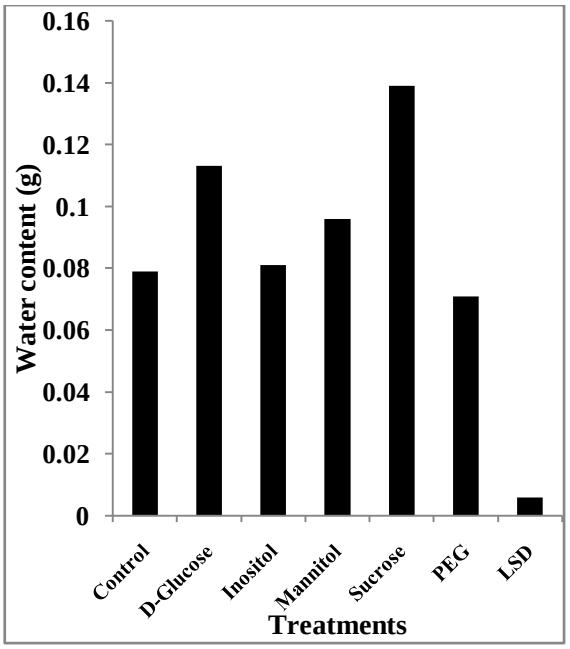


Fig. 4.5.4

Table 4.4.1: Effect of different sugars on the flower longevity, fresh mass, dry mass and water content in isolated flowers of *Dianthus caryophyllus.*L.

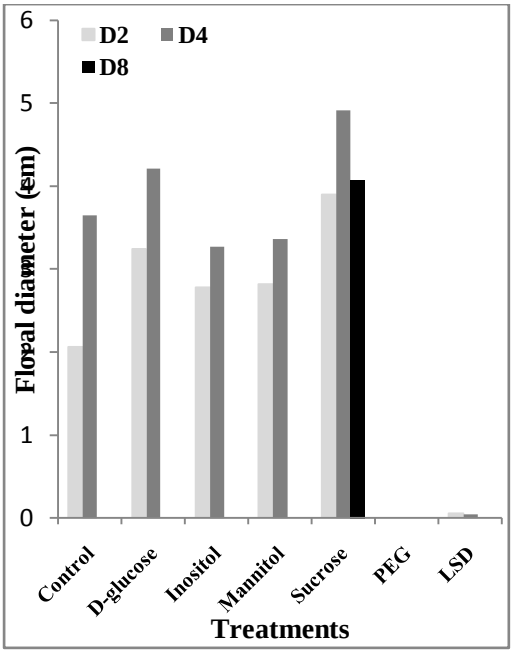


Fig. 4.5.5

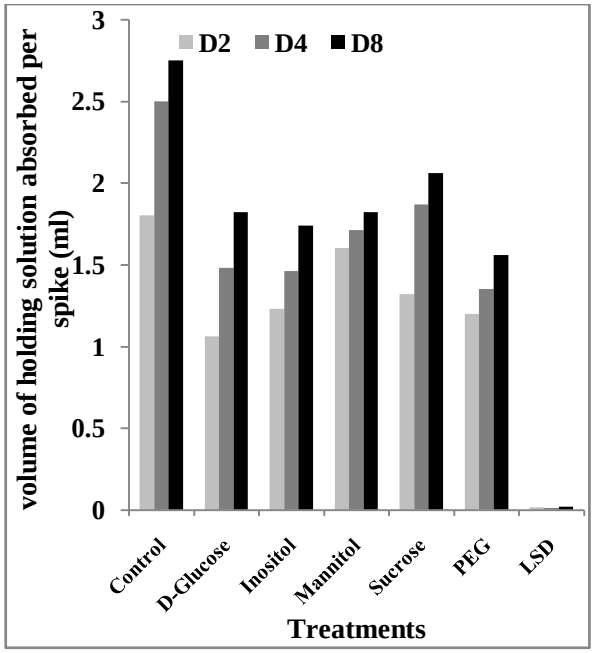


Fig. 4.5.6

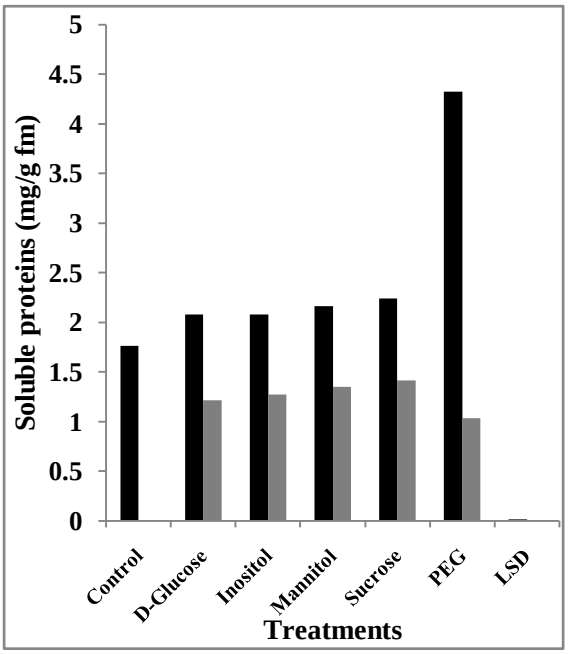


Fig. 4.5.7

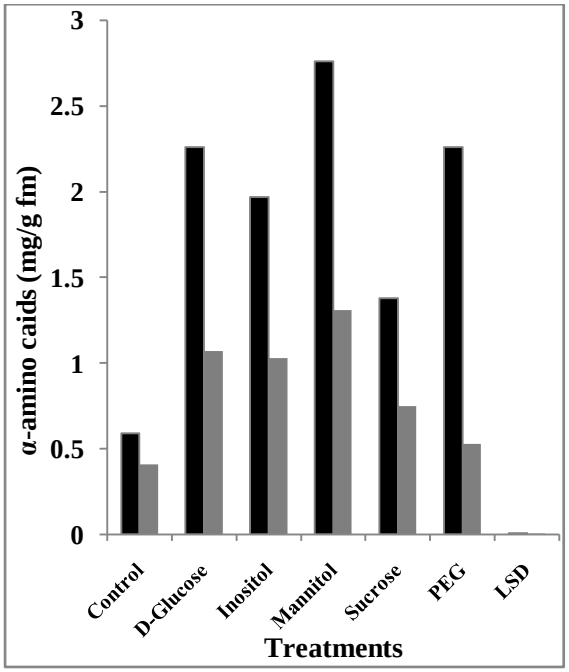


Fig. 4.5.8

Table 4.4.2: Effect of different sugars on the floral diameter, volume of holding solution absorbed, soluble proteins and α-amino acid content in isolated flowers of *Dianthus caryophyllus*.L

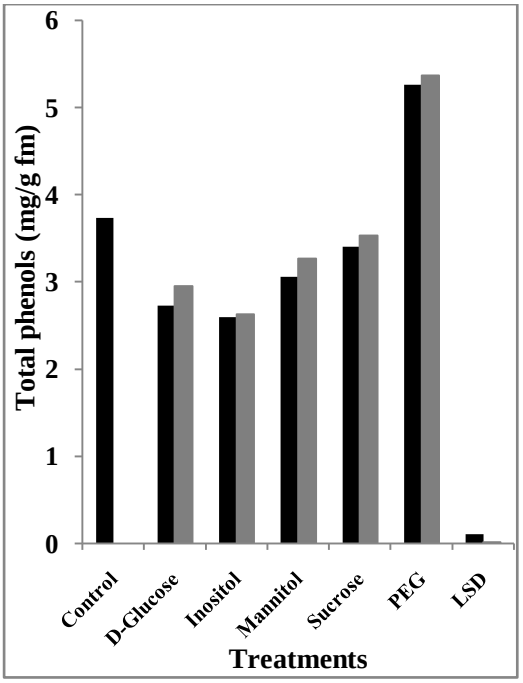


Fig. 4.5.9

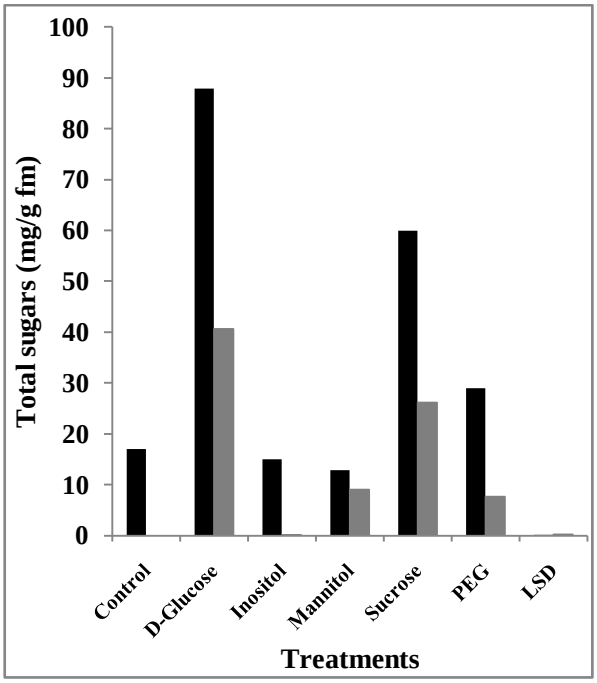


Fig. 4.5.10

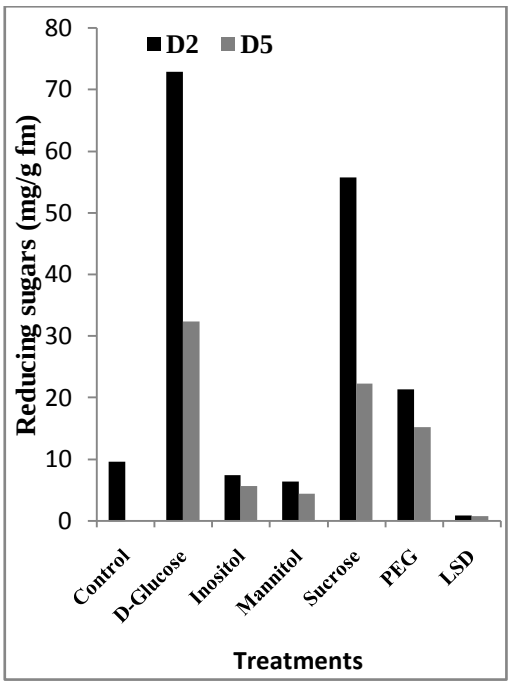


Fig. 4.5.11

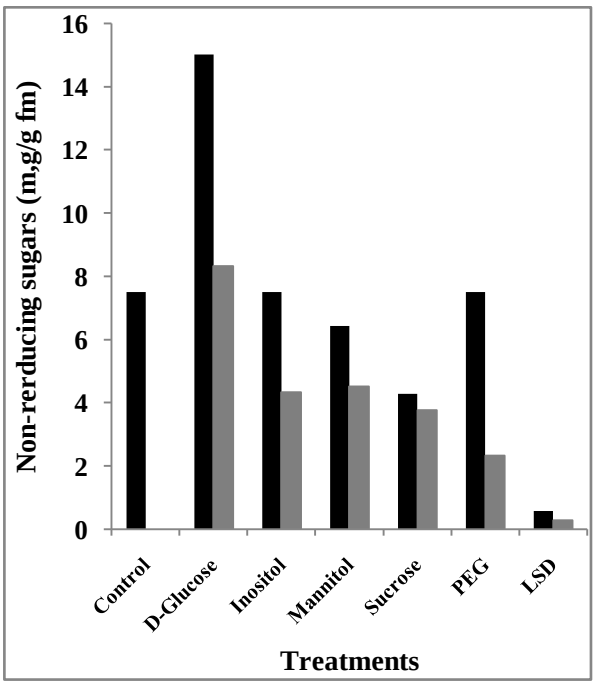


Fig. 4.5.12

Table 4.4.3: Effect of different sugars on the total phenols and sugar fractions in isolated flowers of *Dianthus caryophyllus* L.

Plate 4.5.1

From left to right are arranged the floral buds held in DW (control), D-glucose, mannitol, inositol, sucrose and PEG. Fig a, b and c correspond to day 0, day 2 and day 7 of transfer to the respective vases respectively.

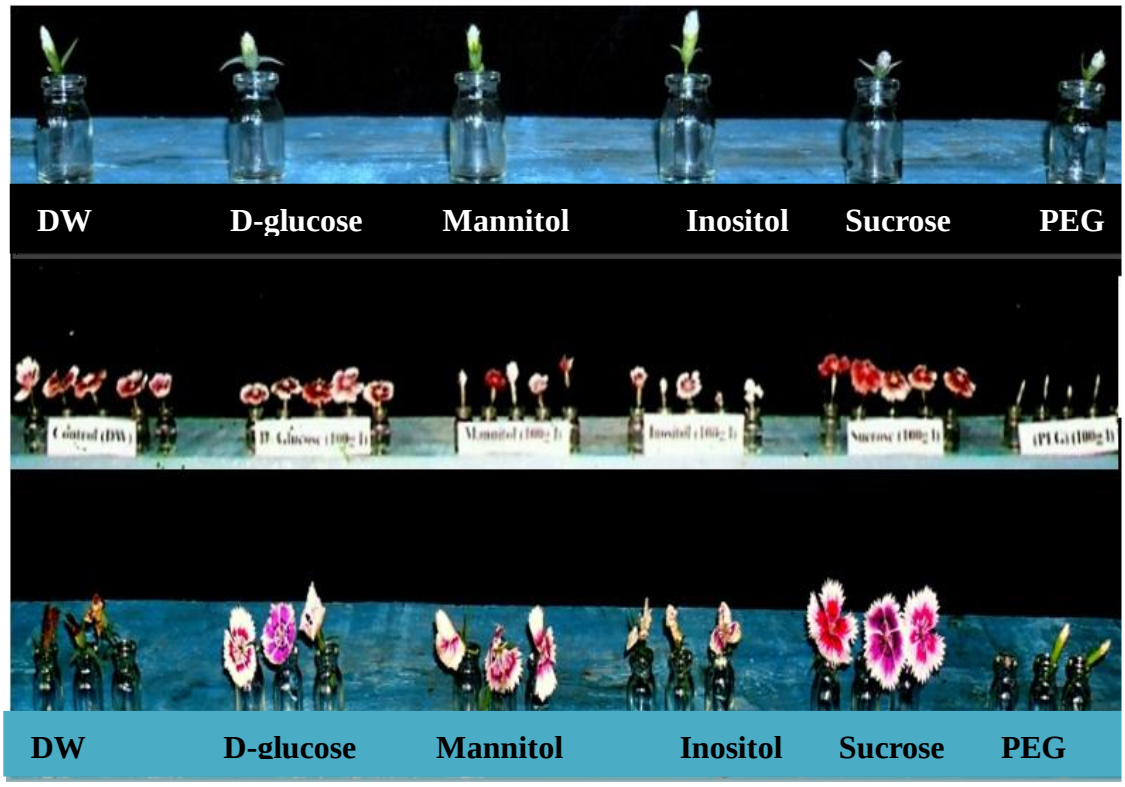

DW
D-glucose
Mannitol
Inositol
Sucrose
PEG
(a)
(b)
DW
D-glucose
Mannitol
Inositol
Sucrose
PEG
(c)

Plate 4.5.1

EXPERIMENT 4.6

Effect of silver thiosulphate-STS (0.25 and 0.5mM) on the flower senescence in isolated flowers of *Dianthus caryophyllus.*

Experimental

Isolated floral buds from the spikes of the *Dianthus caryophyllus* collected from Kashmir University Botanic Garden (KUBG) were used for the present study. The buds were harvested at 800h when buds were at stage III (one day before anthesis). The isolated flowers we immediately brought to the laboratory, cut to a uniform pedicel length of 5cm and divided into three sets. 1st set was pulse treated for 1 h with 0.25mM STS; 2nd set pulse treated for 1 h with 0.5 mM STS, whereas the 3rd set of floral buds was kept unpulsed and designated as control. After 1 h pulse treatment, the isolated flowers were transferred to 10ml glass vials containing vase solutions (DW and SUC). For each treatment there were 35 replicates (vials), each vial containing one flower. The day of transfer to vase solutions was designated as day zero (D0). The experiment was conducted under laboratory conditions under cool white fluorescent light with a mix of diffused natural light (10 Wm^{-2}) 12h a day and relative humidity (RH) of 60 ±10%. The longevity of flowers was regarded to be terminated when the visible signs of senescence (loss of petal turgidity and inrolling of petals) became apparent. The parameters tested during the present study include vase life, floral diameter, soluble proteins and conductivity of leachates.

Results

Visible effects

All the buds opened on the subsequent day (D1) of transfer to the holding solution in case of both STS treatments (0.25 and 0.5mM) including control. The floral buds transferred STS (0.25) were bright in color as compared to the control. Flower senescence was characterized by the loss of petal turgidity and inrolling of petals, but the floral buds remained persistent on the pedicel even after complete senescence (Plate 4.6.1)

Flower longevity

Maximum longevity of 9 days was registered in the flowers pulse treated for 1 h with 0.25mM STS and transferred to SUC. However, the longevity of floral buds pulse treated for 1 h with 0.5mM STS was approximately 7 days (Table 4.6.1; Fig.4.6.1).

Floral diameter

Floral diameter generally showed a decrease with the progression in time from day 2 to day 7 except for the floral buds pulse treated for 1 h with 0.25mM STS and transferred to SUC which showed an increase in floral diameter with the progression in time from day 2 to day 7. Maximum floral diameter of 4.65cm was recorded in the floral buds pulse treated for 1 h with 0.25mM STS and transferred to SUC on day 7. The floral diameter of all other buds pulse treated with either concentration of STS was comparable to that of control (Table 4.6.1; Fig.4.6.2).

Soluble proteins

Treatment of isolated flowers with STS (0.25mM) showed more soluble protein content than the flowers pulse treated with 0.5mM STS after transfer to sucrose. However, the soluble proteins showed a significant increase in tissue samples from flowers pulse treated and transferred to SUC as compared to control irrespective of concentration of STS treatment. The soluble protein content showed a decrease with the progression in time from day 2 to day 7 of transfer (Table 4.6.1; fig.4.6.3)

Conductivity of leachates

Pretreatment of flowers with 0.25mM STS resulted in a significant decrease in electrical conductivity as compared to control. However, the electrical conductivity of flowers pretreated with STS showed a significant increase with the progression in time from day 2 to day 7 (Table 4.6.1; fig.4.6.4).

Table 4.6.1: Effect of silver thiosulphate (STS 0.25 and 0.5mM) on vase life, floral diameter, soluble proteins and conductivity in isolated flowers of *Dianthus barbatus.*

STS Treatment (1 h pulse)							
Treatments	**Flower longevity (days)**	**Floral diameter (cm)**		**Soluble proteins (mg/g fm)**		**Conductivity of leachates (µS)**	
		D2	**D8**	**D2**	**D7**	**D2**	**D7**
Control to DW	4.00	2.40	--	1.235	--	4	--
Control to SUS	5.20	3.05	--	1.320	--	3	--
STS (0.25) to DW	8.05	3.05	3.70	3.405	2.63	2	3
STS (0.25) to SUC	8.99	4.27	4.65	3.480	2.07	2	4
STS (0.5) to DW	7.15	3.15	--	1.318	0.97	3	5
STS (0.5) to SU	7.33	3.02	--	1.329	0.76	3	6
LSD P=0.05	0.75	0.04	--	0.011	0.005	0.10	0.20

Each value is mean of 6 independent replicates.

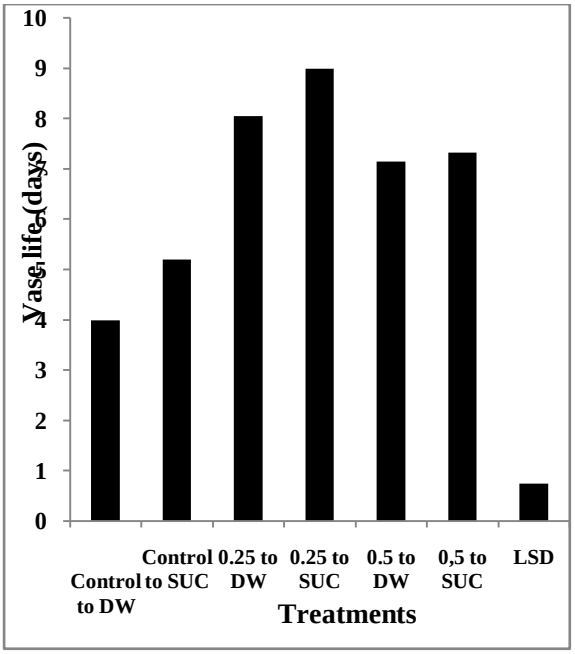


Fig.5.6.1

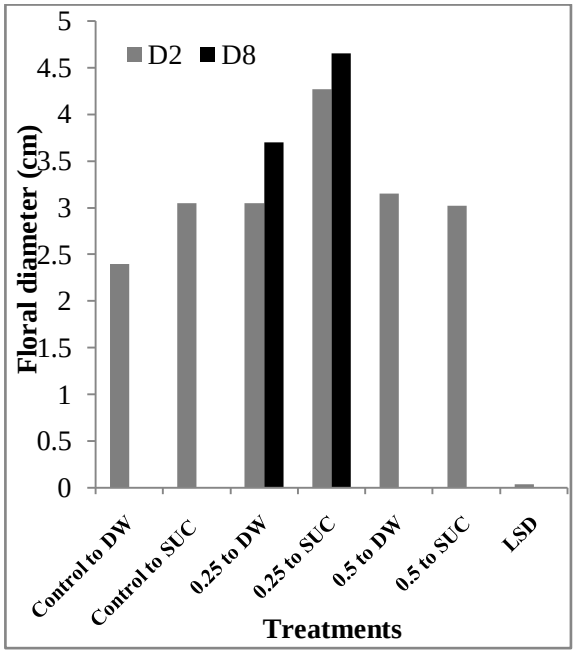


Fig.5.6.2

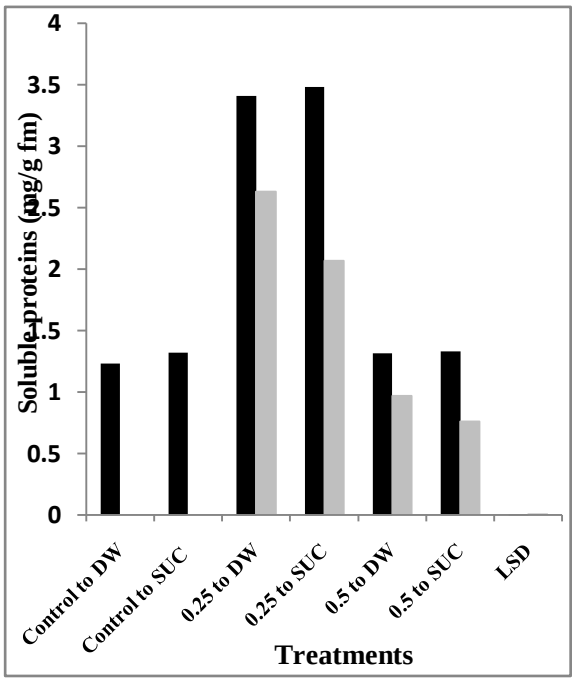


Fig.5.6.3

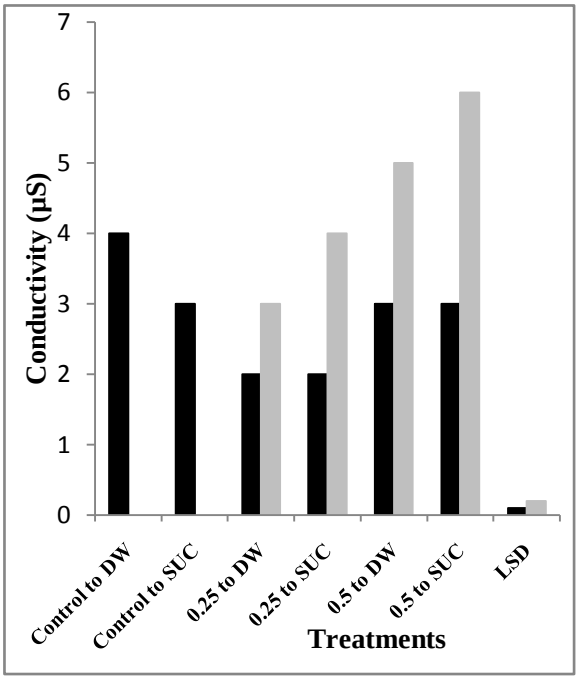


Fig.5.6.4

Fig.5.6.1-5.6.4: Effect of 1 h pulse of silver thiosulphate (STS, 0.25 and 0.5mM) on vase life, floral diameter, soluble proteins and conductivity in isolated flowers of *Dianthus caryophyllus.*

Plate 4.6.1

Isolated flowers of *Dianthus caryophyllus* held in DW, SUC on D0 (fig. a), D4 (fig b) and D8 (fig. c) after 1h pulse treatment with STS (0.25, 0.5mM).

Plate 4.6.1

EXPERIMENT 4.7

Effect of aminooxyacetic acid-AOA (0.25 and 0.5mM) on the flower senescence in isolated flowers of *Dianthus caryophyllus.*

Experimental

Isolated floral buds from the spikes of the Dianthus caryophyllus collected from Kashmir University Botanic Garden (KUBG) were used for the present study. The buds were harvested at 800h when buds were at stage III (one day before anthesis). The isolated flowers we immediately brought to the laboratory, cut to a uniform pedicel length of 5cm and divided into three sets. 1st set was pulse treated for 1 h with 0.25mM AOA; 2nd set pulse treated for 1 h with 0.5mM AOA. The 3rd set of floral buds was kept unpulsed and designated as control. After one hour pulse treatment, the isolated flowers were transferred to 10ml glass vials containing holding solutions (DW and SUC). For each treatment there were 35 replicates (vials), each vial containing one flower. The day of transfer to vase solutions was designated as day zero (D0). The experiment was conducted under laboratory conditions under cool white fluorescent light with a mix of diffused natural light (10 Wm^{-2}) 12h a day and relative humidity (RH) of 60 ±10%. The longevity of flowers was regarded to be terminated when the visible signs of senescence (loss of petal turgidity and inrolling of petals) became apparent. The parameters tested during the present study include vase life, floral diameter, soluble proteins and conductivity of leachates.

RESULTS

Visible effects

All the buds opened on the subsequent day (D1) of transfer to the holding solution in case of both AOA treatments (0.25 and 0.5mM) including control. The floral buds transferred AOA (0.5) were bright in colour as compared to the control. Flower senescence was characterized by the loss of petal turgidity and inrolling of petals, but the floral buds remained persistent on the pedicel even after complete senescence (Plate 4.6.1).

Flower longevity

Maximum life of 11 days was registered in the flowers pulse treated for 1 h with 0.5mM AOA and transferred to SUC. However, the life of floral buds pulse treated for 1 h with 0.25mM AOA was approximately 8 days (Table 4.6.1; Fig.4.6.1).

Floral diameter

Floral diameter generally showed an increase from 3.98 to 3.99 and 4.18 to 4.64 with the progression in time from D2 to D7 from the samples of floral buds pulse treated for 1 h with 0.5mM AOA and transferred to either 0.25 or 0.5mM AOA. Maximum floral diameter of 4.65cm was recorded in the floral buds pulse treated for 1 h with 0.5mM AOA and transferred to SUC on day 7. However, the floral diameter of floral buds pulse treated for 1 h with 0.25mM was comparable to that of control (Table 4.6.1; Fig.4.6.2).

Conductivity of leachates

Pretreatment of flowers with 0.5mM AOA resulted in a significant decrease in electrical conductivity as compared to control at day 2. However, the electrical conductivity of flowers pre-treated with AOA at 0.25 or 0.5mM concentration showed a significant increase with the progression in time from day 2 to day 7 (Table 4.6.1; Fig.4.6.3).

Soluble proteins

Treatment of isolated flowers with AOA (0.5mM) showed a higher soluble protein content than the samples from flowers pulse treated with 0.25mM AOA after transfer to sucrose. However, the soluble proteins showed a significant increase in samples from flowers pulse treated with either concentration of AOA and transferred to SUC at day 2. The soluble protein content generally showed a decrease with the progression in time from day 2 to day 7 of transfer of buds either to DW or to SUC (Table 4.6.1; Fig.4.6.4)

Table 4.6.1: Effect of aminooxyacetic acid (AOA 0.25 and 0.5mM) on vase life, floral diameter, soluble proteins and conductivity in isolated flowers of *Dianthus caryophyllus* L.

AOA Treatment (1 h pulse)							
Treatments	**Flower longevity (days)**	**Floral diameter (cm)**		**Soluble proteins (mg/g fm)**		**Conductivity of leachates (µS)**	
		D2	**D7**	**D2**	**D7**	**D2**	**D7**
Control to DW	4.00	2.40	--	1.296	--	4	--
Control to SUC	5.20	3.05	--	1.303	--	2	--
AOA (0.25) to DW	7.00	3.43	3.04	2.325	1.587	5	7
AOA (0.25) to SUC	8.33	3.69	3.02	3.355	1.98	6	8
AOA (0.5) to DW	9.15	3.98	3.99	4.309	3.53	2	5
AOA (0.5) to SUC	11.02	4.18	4.64	4.321	3.05	2	6
LSD at $P_{0.05}$	0.044	0.411	0.011	0.03	0.008	0.2	0.3

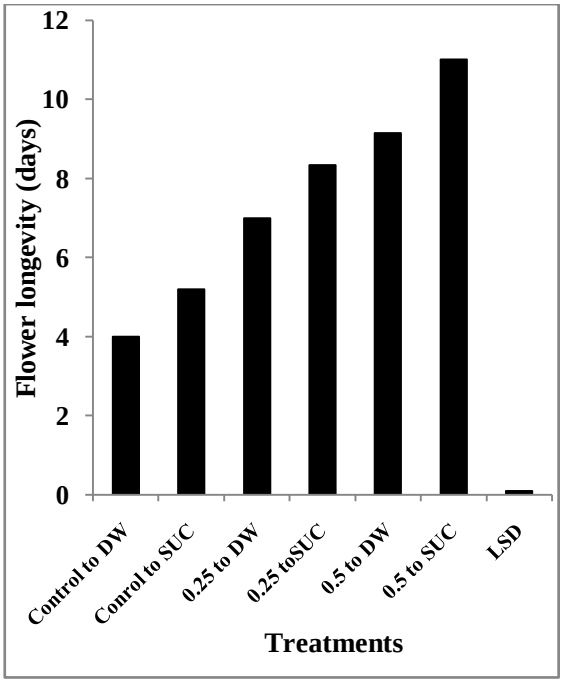


Fig.4.6.1

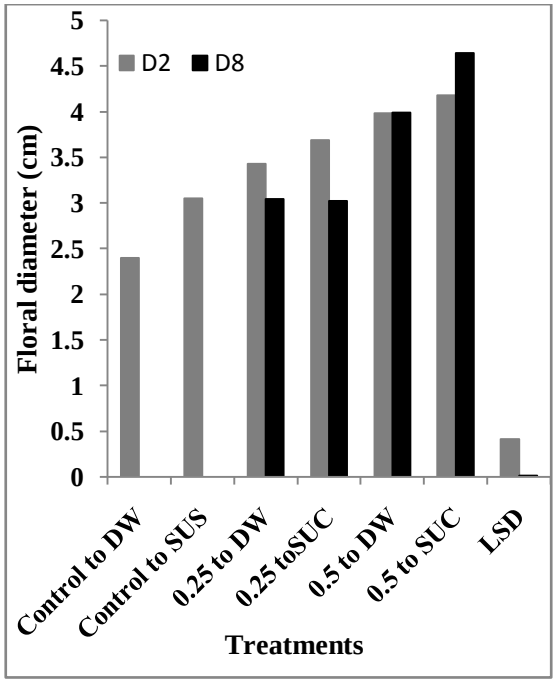


Fig.4.6.2

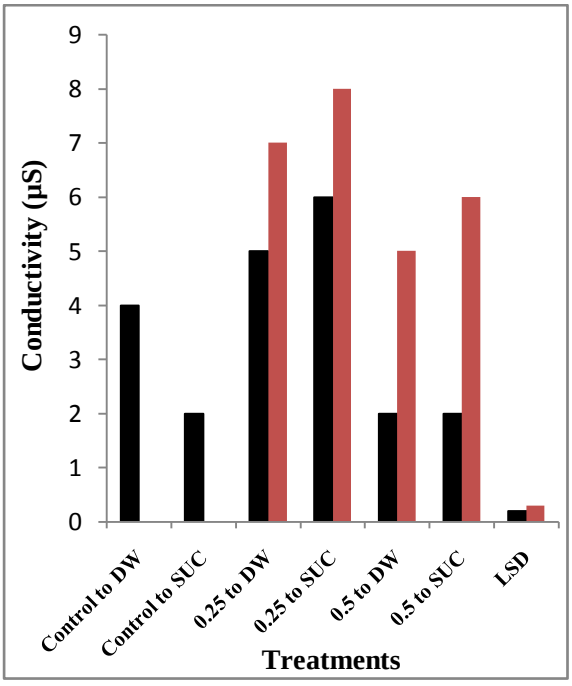


Fig.4.6.3

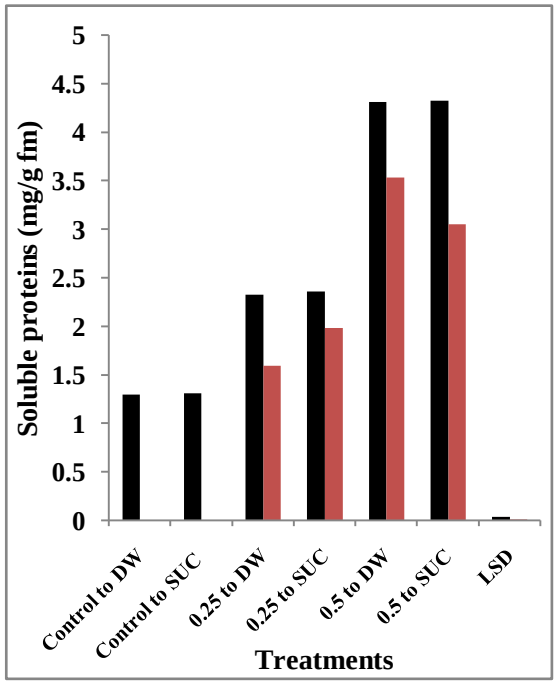


Fig. 4.6.4

Fig. 4. 6.1-4: Effect of 1 h pulse of aminooxyacetic acid-AOA (0.25 and 0.5 mM) on flower longevity, floral diameter, conductivity of leachates and soluble proteins in cut spikes of *Dianthus caryophyllus.*

Plate 4.7.1

Isolated flowers of *Dianthus caryophyllus* held in DW, SUC on D0 (fig. a), D4 (fig b) and D8 (fig. c) after 1h pulse treatment with AOA (0.25, 0.5mM).

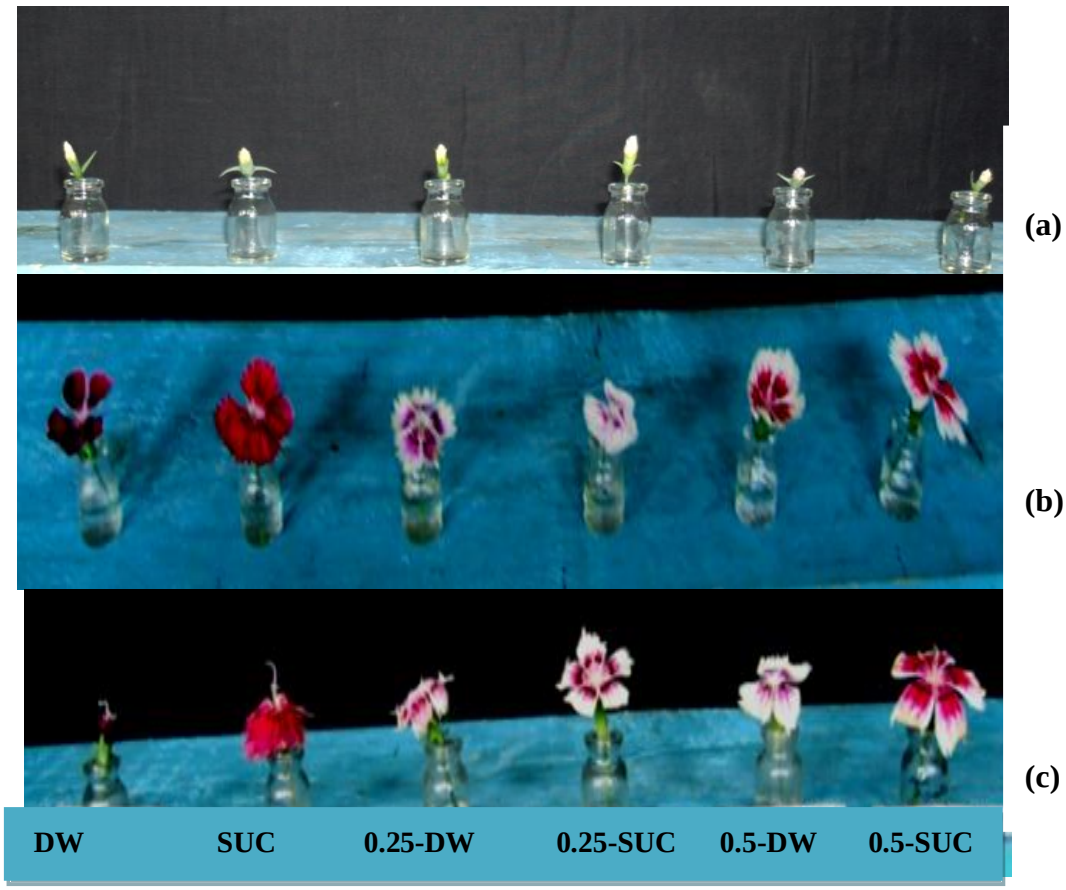


Plate 4.7.1

CHAPTER-5

FLOWER SENESCENCE AND CHEMICAL REGULATION OF VASE LIFE IN DIANTHUS BARBATUS

***Dianthus barbatus* L.**

Botanical name	*Dianthus barbatus*
Common name	Sweet William
Family	Caryophyllaceae
Flowering period	May-August
Plant height	30-50 cm
Number of sepals	5 sepals (connate)
Number of petals	5; usually differentiated into a distinct claw and a limb, with an appendaged joint between the two, often notched or deeply bilobed at tip.
Number of stamens	10
Number of carpels	2
Fruit	Loculicidal capsule
Flower longevity	4 days
Symptoms and pattern of senescence	Flower senescence in *D. barbatus* is characterized by loss of petal turgidity followed by abscission of stamens. Petals turn from pinkish red to pale yellow.

EXPERIMENT 5.1

Physiology and biochemical changes associated with flower development and senescence in *Dianthus barbatus.* L

Experimental

Flowers of *Dianthus barbatus* growing in Kashmir University Botanic Garden (KUBG) were selected for the present study. Flower development and senescence was divided into six stages (stage I-VI). These stages were deciphered as I-tight bud stages, II-mature bud stage, III-brush stage, IV-fully open stage, V-partially senescent stage and VI-senescent stage (plate 5.1.1). Visible changes were recorded throughout flower development and senescence. Floral diameter, fresh mass, dry mass and water content was determined at each stage.

For the estimation of tissue constituents from petal tissues, 1g chopped material was fixed in hot 80% ethanol at each stage of flower development and senescence. The material was macerated and centrifuged three times. The supernatants were pooled and used for the estimation of soluble proteins, α-amino acids, total phenols, reducing sugars, non-reducing sugars and total sugars from the suitable aliquots. Besides non-reducing sugars were calculated as the difference between total and reducing sugars. Soluble proteins were extracted from 1g of petal tissue drawn separately from five different flowers at each of the six stages. Protease activity has been expressed as µg tyrosine equivalents liberated per 100mg fresh mass, besides specific protease activity has been computed as µg tyrosine equivalents liberated per mg protein. At each stage the homogenates from the petal tissues were used for studying electrophoretic profiles of proteins by SDS-PAGE. The tissue was homogenized in 0.1M phosphate buffer (pH=7.2). Each value represented in the tables corresponds to the mean of five to ten independent replicates. The data has been analyzed statistically by computing standard deviation.

RESULTS

Visible changes

The senescence in *Dianthus barbatus* was marked by loss of petal turgidity, followed by their out rolling. Petals lose their color from pinkish red to pale yellow (Plate 5.1.1; Fig. a). The average life span of an individual flower after it opened fully was about 4 days.

Fresh mass, dry mass and water content

Fresh & dry mass and water content of flowers increased with flower development up to stage IV and declined thereafter as senescence progressed through stages V and VI (Table 5.1.1; Fig. 5.1.1, 5.1.2, 5.1.3).

Soluble proteins

The tissue concentration of soluble proteins showed a continuous decrease as the flower development progressed from stage I to stage V but showed an increase as flower development progressed towards senescence. However, when the data was expressed on per flower basis, the soluble protein content registered an increase upto stage IV and then decreased as the senescence progressed through stages V and VI (Table 5.1.2; Fig.5.1.4)

Specific protease activity

The specific protease activity (expressed as µg tyrosine equivalents per mg protein) showed an increase during the flower development from stage I to stage III, registered a slight decrease at stage IV and thereafter a sharp increase was recorded as the flower development progressed towards senescence (Table 5.1.2; Fig.5.1.5)

α- amino acids

The α- amino acid content was maintained as the flower development progressed upto stage IV and thereafter showed an increase as the senescence progressed through stages V and VI. Same trend was maintained when the data was expressed on per flower basis (Table 5.1.2; Fig.5.1.6).

Total phenols

The tissue content of total phenols showed a continuous increases upto stage III, a significant decrease at stage IV and then sharp increase as the senescence progressed through stages IV and VI. Same trend became more apparent when the data was expressed on per flower basis (Table 5.1.2; Fig. 5.1.7).

Total sugars

The total sugar content showed a continuous increase as flower opened from stage I to stage IV and thereafter a decrease was registered in the total sugar content as senescence progressed through stage V and VI. The trend became more apparent when the data was expressed on per flower basis (Table 5.1.3; Fig.5.1.9)

Reducing sugars

The tissue content of reducing sugars showed a continuous increase from sage I to stage III and thereafter declining trend was observed as senescence progressed through stage IV and VI. The trend became more apparent when the data was plotted on per flower basis (Table 5.1.3; Fig 5.1.8).

Non-reducing sugars

The tissue content of non-reducing sugars registered a decrease as flower development progressed from stage I to stage IV, the decrease was however, sharp when the flower development progressed from stage III to VI. However, when data was expressed on per flower basis, the non- reducing sugar content first decreased to stage III and was more or less maintained as the flower development progressed towards the partially senescent stage (Table 5.1.3; Fig.5.1.10).

Table 5.1.1: Fresh and dry mass, water content(in grams), soluble proteins and specific protease activity (expressed as mg g^{-1} fresh mass) during flower development and senescence in flowers of *Dianthus barbatus.* L

Stages	**Fresh mass**	**Dry mass**	**Water content**	**Soluble proteins**	**Protease activity**
I (Tight bud stage)	0.046±0.02	0.009±0.005	0.037±0.0021	10.24±0.84 (0.471)	0.17±0.03
II (Loose bud stage)	0.049±0.04	0.08±0.004	0.040±0.015	8.08±0.52 (0.395)	0.26±0.02
III (Brush stage)	0.067±0.03	0.002±0.006	0.047±0.003	7.2±0.0.13 (0.482)	0.38±0.03
IV (Open bud stage)	0.08±0.023	0.0018±0.004	0.060±0.015	6.4±0.43 (0.505)	0.30±0.01
V (Partially senescent stage)	0.056±0.025	0.0015±0.004	0.041±0.016	6.64±0.44 (0.371)	0.51±0.02
VI (Senescent stage)	0.049±0.013	0.0014±0.003	0.035±0.009	7.68±0.65 (0.376)	0.65±0.07

Each value is the mean of 6 independent replicates ± SD

Table 5.1.2: Soluble proteins, specific protease activity (expressed as µg tyrosine equivalents librated per mg protein) α-amino acids, total phenols, total, reducing and non-reducing sugars (expressed as mg g^{-1} fresh mass) during flower development and senescence in flowers of *Dianthus barbatus.*

Stages of flower development	α-amino acids	Total phenols	Total sugars	Reducing sugars	Non-reducing sugars
I (tight bud stage)	6.49±0.76 (0.298)	1.33±0.03 (0.061)	16.07±0.20 (0.739)	7.14±0.19 (0.328)	8.93±0.28 (0.410)
II (loose bud stage)	5.50±0.23 (0.269)	1.38±0.03 (0.067)	28.57±0.78 (1.399)	25.0±0.75 (1.225)	3.57±0.09 (0.174)
III (brush stage)	7.06±0.13 (0.473)	1.55±0.02 (0.104)	35.71±0.76 (2.392)	31.25±0.73 (2.093)	4.46±0.05 (0.303)
IV (open bud stage)	5.01±0.21 (0.395)	1.0±0.09 (0.079)	36.60±0.43 (2.891)	25.0±0.21 (1.975)	11.6±0.08 (0.916)
V (partially senescent stage)	8.62±0.4 (0.482)	2.65±0.04 (0.148)	33.03±0.32 (1.849)	21.42±0.15 (1.199)	11.61±0.05 (0.650)
VI (senescent stage)	12.73±0.54 (0.623)	2.83±0.043 (0.138)	28.02±0.6 (1.372)	18.09±1.08 (0.894)	9.93±0.078 (0.486)

Each value is the mean of 6 independent replicates ± SD.

Figures in parentheses represent values on per flower basis.

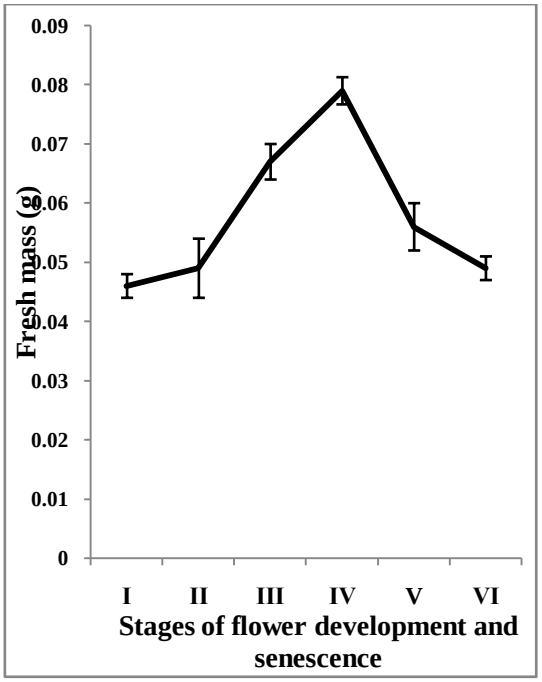


Fig. 5.1.1

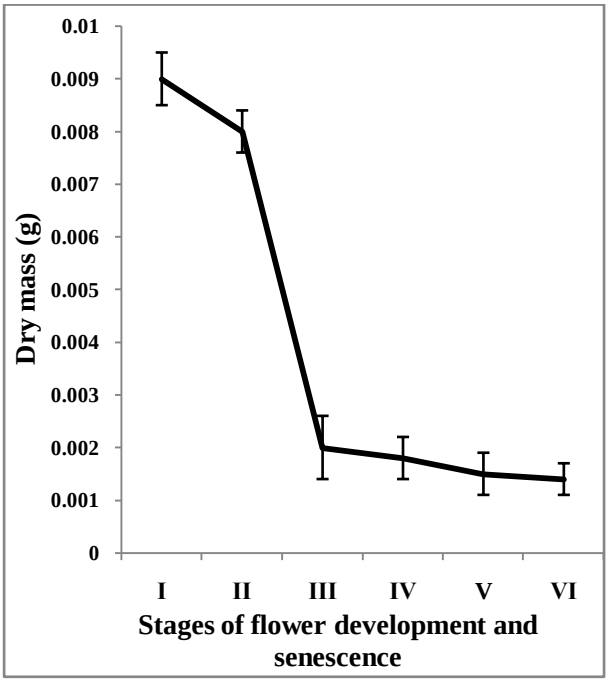


Fig. 5.1.2

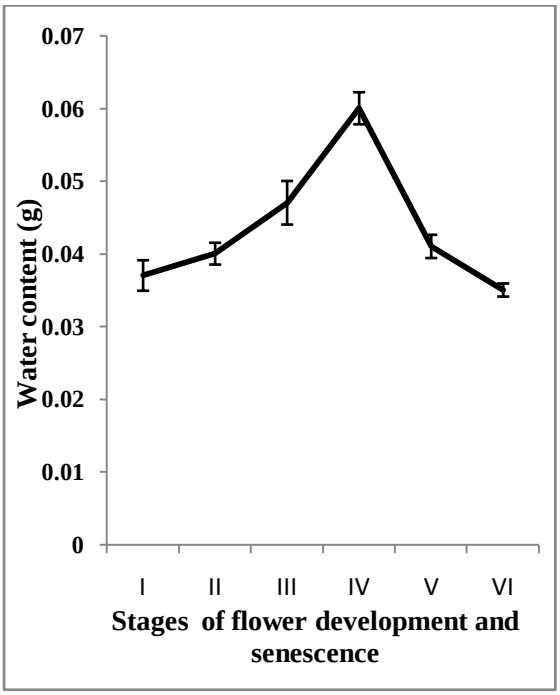


Fig. 5.1.3

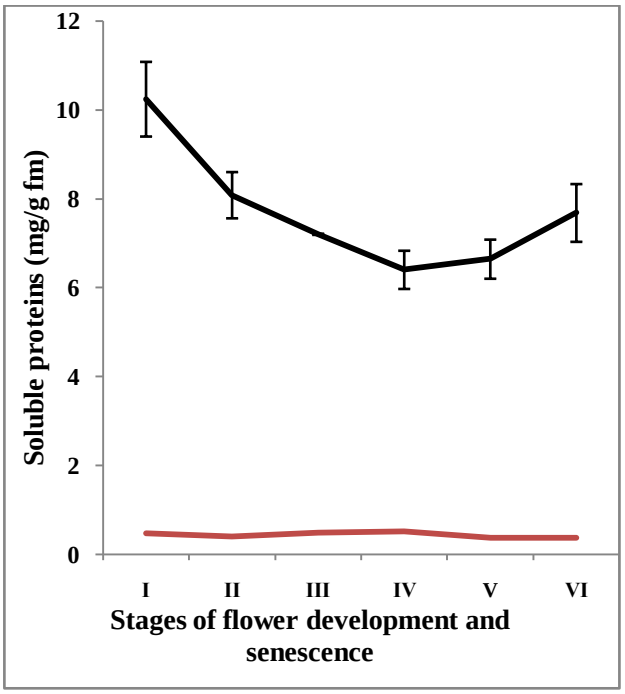


Fig. 5.1.4

Fig.5.1.1-5.1.4: Changes in fresh & dry mass, water content and soluble proteins of flowers at various stages of flower development and senescence in *Dianthus barbatus*.

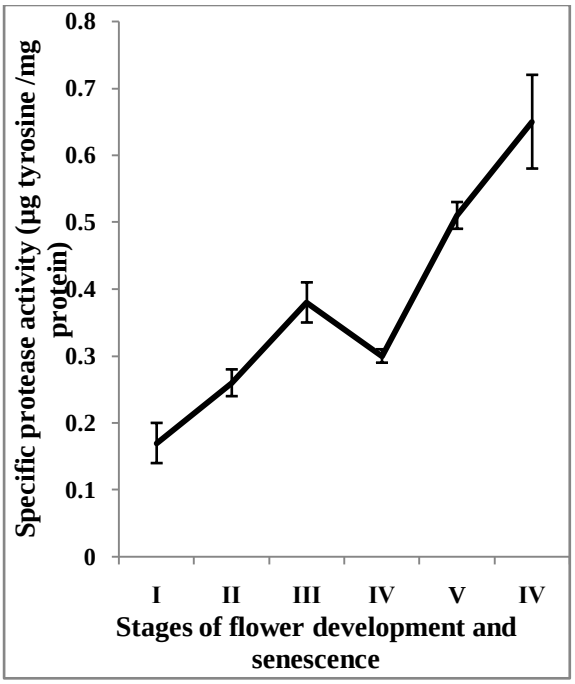


Fig. 5.1.5

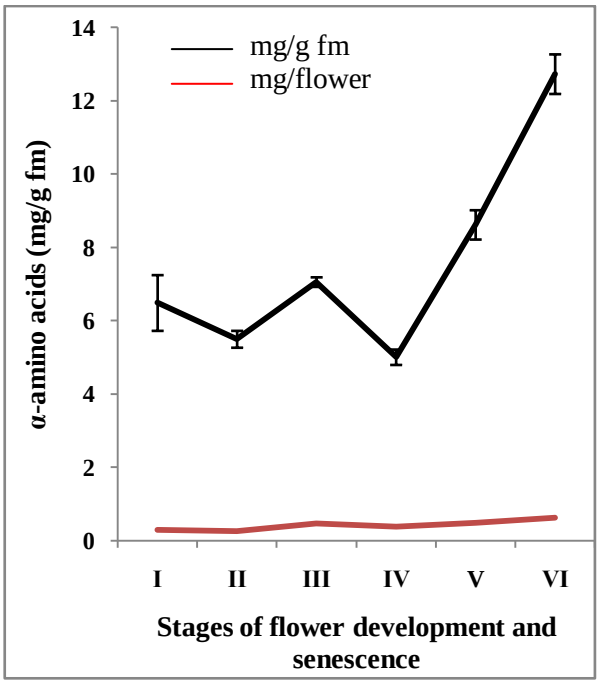


Fig. 5.1.6

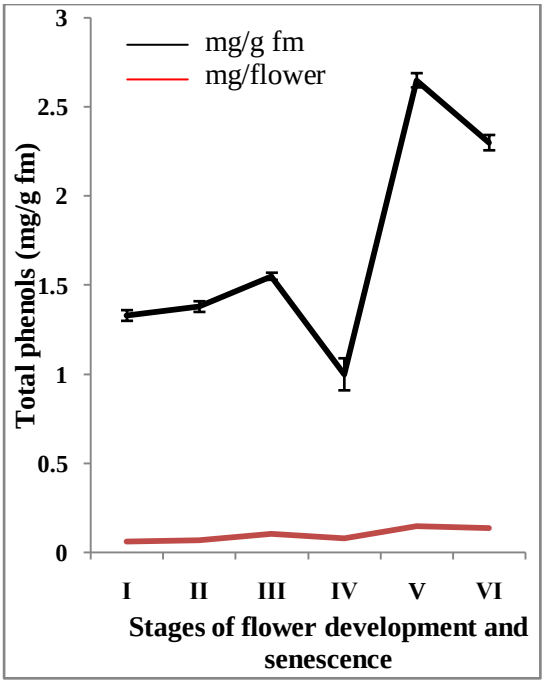


Fig. 5.1.7

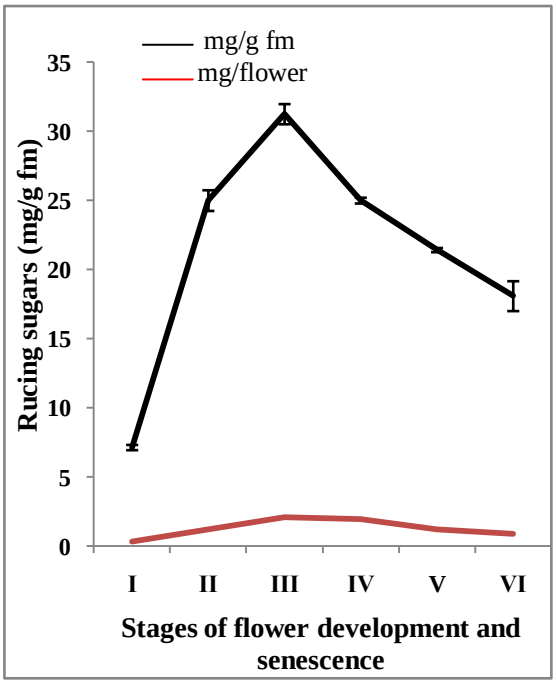


Fig. 5.1.8

Fig. 5.1.5 - 5.1.8: Changes in specific protease activity, α- amino acids, total phenols and reducing sugars at various stages of flower development and senescence in *Dianthus barbatus.*

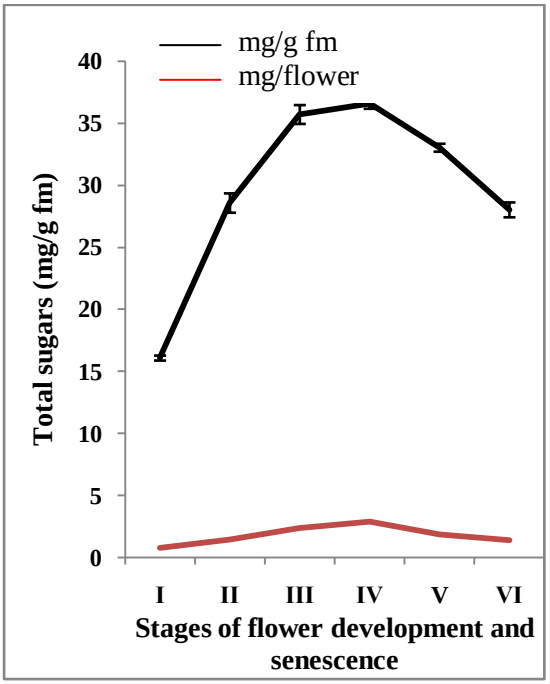


Fig. 5.1.9

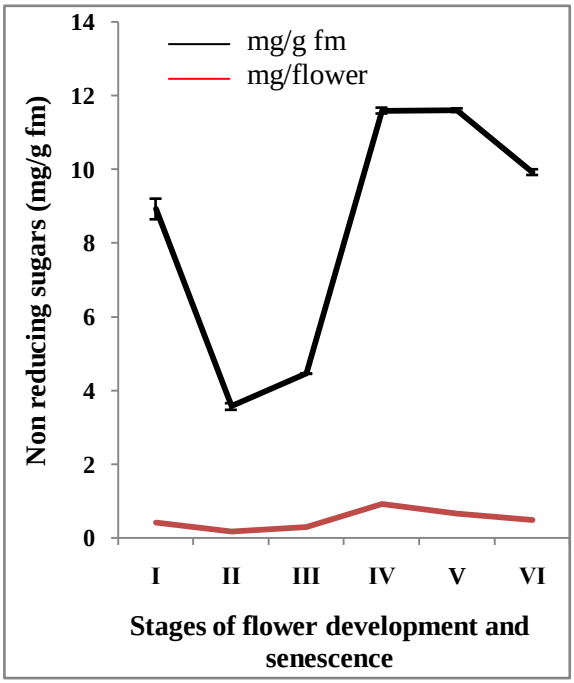


Fig. 5.1.10

Fig.5.1.9-5.1.10: Changes in total sugars and non reducing sugars at various stages of flower development and senescence in *Dianthus barbatus*.

Plate 5.1.1

Stages of flower development and senescence in *Dianthus barbatus.*

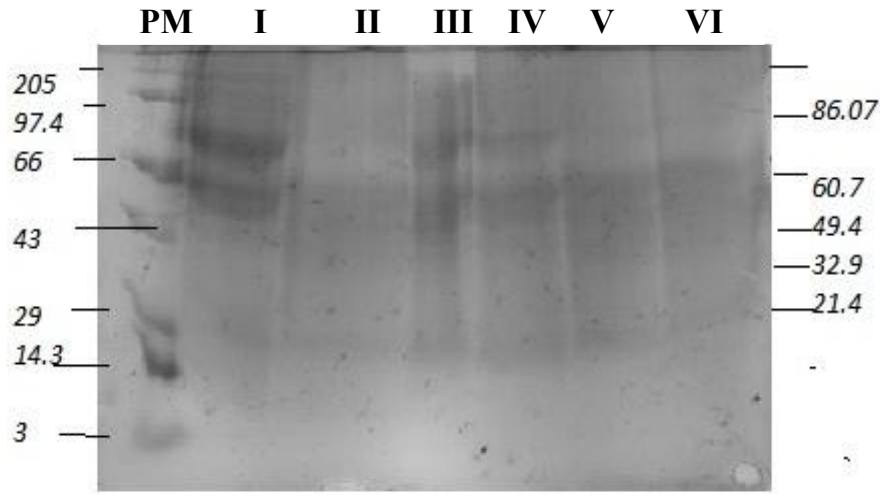
Fig. a: From left to right are arranged six stages of flower development and senescence in *Dianthus barbatus.* Stages I (Tight bud stage), II (loose bud stage), III (brush stage), IV (open stage), V (partially senescent stage) and VI (senescent stage).

Fig. b: SDS-PAGE of equal amounts of extractable protein at various stage (I-VI) of flower development and senescence from petal tissue of Dianthus barbatus. The gel was stained with coomassie blue. Numbers above lanes correspond to developmental stages.

Fig. c: Scanning electron microscopic analysis of the petal tissues from the different stages of flower development and senescence in *Dianthus barbatus* L. From left to right are arranged the scanning electron micrographs of the stages I to VI.

I II III IV V VI

Stages of flower development and senescnce in *Dianthus caryophyllus.*



Electophoretogram of the stages of flower development and senescence of *Dianthus caryophyllus.*

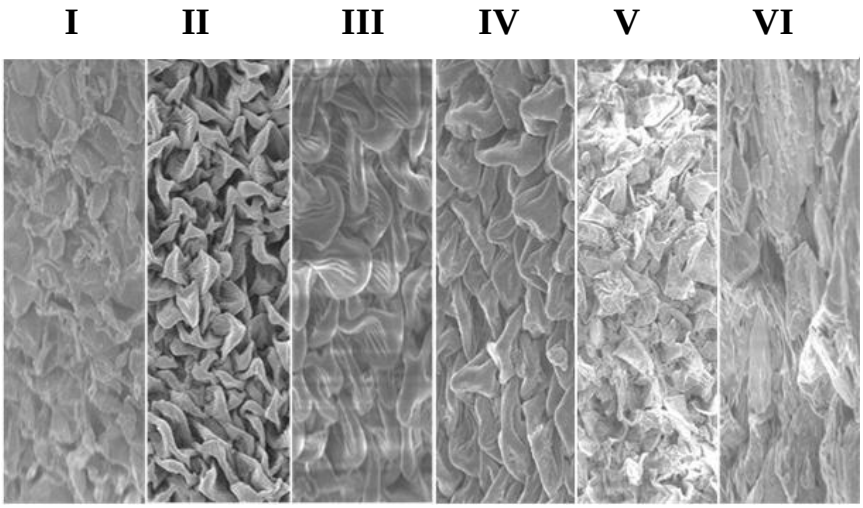


Plate 5.1.1

EXPERIMENT 5.2

Effect of postharvest wet storage (PHWS) at three different temperature regimes RT (20±2ºC), 10ºC and 5ºC) for 72 h and subsequent transfer to vase solutions {distilled water (DW) and sucrose (SUC)} on postharvest performance in cut spikes of *Dianthus barbatus* L.

Experimental

Uniform floral buds of *Dianthus barbatus* growing in the Kashmir University Botanic Garden (KUBG) were used for this study. The buds were harvested at 800 h when the buds were at brush stage. The harvested spikes were immediately brought to the laboratory and cut to a uniform length of 27 cm and divided into three sets containing equal number of spikes. One set of spikes was kept at 5ºC and other at 10ºC. The third set of spikes kept at room temperature (20±2ºC) were designated as control. The spikes were held in distilled water (DW) in separate glass beakers and kept at 5 and 10°C. A separate set of spikes was kept at room temperature (20±2°C). After 72 h the spikes were kept at room temperature after transferring them to 250 ml Ehrlenmeyer flasks containing 200 ml of vase solution {Distilled water (DW) and Sucrose (SUC) 0.05 M}. Each treatment was represented by 5 replicates (flasks) with each flask containing two spikes. The day of transfer of spikes to vase solutions (DW and SUC) was designated as day zero (D0). The average vase life of spikes was counted from the day of transfer (D0) to holding solution and was regarded to be terminated when the last flower lost its display value (marked by color change and loss of petal turgidity). Visual changes were observed at periodic intervals during the course of the experiment. Number of blooms per spike, floral diameter, and volume of holding solution absorbed per spike were estimated on Day 2, 6, 12 and 17. Changes in tissue constituents including soluble proteins, α- amino acids, phenolics and sugar fractions from petal tissue were estimated at day 4 and day 10 of transfer of spikes to vase solution (DW and SUC). The results have been analyzed statistically and LSD computed at $P_{0.05}$ using MINITAB (v15.1.2-EQUINOX_Softddl.net) software.

Results

Visible effects

The spikes previously wet stored for 72 h at RT ((20±2ºC) had already bloomed during storage as compared to the buds stored at 5 and 10 ºC which maintained the premature status (plate 5.2.1). Flower senescence was characterized by loss of petal turgidity, followed by their out rolling. Petals lost their color from pinkish red to pale yellow.

Vase life

The average vase life of spikes wet stored at various temperature regimes i.e. RT (20±2ºC), 10 ºC, 5 ºC before transfer to holding solution (DW) was approximately 8, 10 and 14 days as compared to 9,12 and 17 days in corresponding spikes transferred to SUC. The maximum vase life of 17 days was recorded in spikes wet stored at 5ºC and transferred to SUC (Table 5.2.1; Fig.5.2.1).

Number of blooms per spike

The rate of blooming as well as number of blooms per spike was appreciably higher in spikes previously wet stored at RT (20±2ºC) and 10ºC before transfer to vase solution as compared to corresponding spikes previously wet stored at 5ºC. The rate of blooming showed an increase with the progression in time from day 2 to day 17 of transfer of spikes irrespective of temperature treatment and vase solutions. However the rate of blooming was 91% in spikes previously wet stored for 72 h and transferred to SUC at 5ºC on day 17 of transfer as compared to spikes wet stored at RT (20±2ºC) and 10ºC which attained 68 % blooming and 73% respectively (Table 5.2.1;Fig.5.2.2).

Floral diameter

The spikes transferred to SUC showed an increased floral diameter as compared to the corresponding spikes transferred to DW. Floral diameter was almost maintained after various days of transfer to vase solutions. Maximum floral diameter was recorded in the flowers from spikes previously wet stored for 72 h at 5ºC and subsequently transferred to SUC on day 6 (Table 5.2.2; Fig. 5.2.3).

Volume of holding solution absorbed

The volume of holding solution absorbed per spike increased with the progression in time from day 2 to day 17. The spikes transferred to DW showed significantly higher

volume of the holding solution absorbed per spike as compared to the corresponding spikes transferred to SUC. Maximum volume of holding solution absorbed per spike was recorded in the spikes wet stored for 72 h at 5ºC and subsequently transferred to DW on day 17 (Table 5.2.2, Fig.5.2.4).

Soluble proteins

The soluble protein content of the samples from spikes previously wet stored at 10 °C and 5ºC for 72 h registered a significant increase as compared to the samples from spikes held at RT irrespective of vase solution. Soluble protein content in the petal tissues from spikes held in DW was generally comparable with the sample from spikes held in SUC. Soluble protein content generally registered an increase with the progression in time from day 4 to day 10. Maximum soluble protein content was registered in the samples from spikes previously wet stored for 72 h at 10ºC and transferred to DW on day 10 (Table 5.2.3;Fig.5.2.5).

α-amino acid

A higher α-amino acid content was registered in samples from spikes previously wet stored at RT(20±2ºC)and 10 °C as compared to samples from spikes wet stored at 5 °C irrespective of vase solution. However, the α-amino acids decreased with progression in time from day 4 to 10 of transfer irrespective of temperature regime and holding solution. The highest α-amino acid content was noticed in samples from spikes wet stored at RT and transferred to SUC on day 4 (Table 5.2.3; Fig.5.2.6).

Total phenolics

The phenolic content was significantly higher in the samples from spikes held in SUC as compared to the corresponding samples from spikes held in DW. The total phenolic content in the samples from spikes wet stored for 72 h at 10ºC and 5ºC and transferred to either DW or SUC was however, maintained after various days of transfer to vase solutions. Maximum total phenolic content was registered in the spikes previously stored at RT and transferred to SUC on day 4 (Table 5.2.3; Fig.5.2.7).

Total sugars

The total sugar content in samples from spikes previously wet stored for 72h at 10 and 5 °C registered a significant increase as compared to the samples from corresponding spikes held at RT irrespective of vase solution used. However, the spikes transferred to SUC showed an increased total sugar content in the petal tissue as compared to the

corresponding samples from transferred to DW irrespective of the temperature regime. The total sugar content showed a profound decrease with progression in time from day 4 to day 10 of transfer irrespective of vase solution used and temperature regime. Maximum total sugar content was recorded in the samples from spikes previously wet stored for 72 h at 10ºC and transferred to SUC on D4 (Table 5.2.4; Fig.5.2.10).

Reducing sugars

The reducing sugar content in the samples spikes previously wet stored for 72h at 10 and 5 °C registered a significant increase as compared to the samples from corresponding spikes held at RT irrespective of the vase solution used. However, the reducing sugar content was higher in samples from spikes transferred to SUC as compared to samples from spikes transferred to DW irrespective of the different temperature regime. The reducing sugar content showed a sharp decrease with the progression in time from day 4 to day 10 irrespective of the vase solution used. Maximum reducing sugar was recorded in the samples obtained from spikes previously wet stored at 5 °C for 72 h and transferred to SUC on D4 (Table 5.2.4; Fig 5.2.8)

Non-reducing sugars

The spikes previously stored at 5ºC and transferred to either DW or SUC showed considerably lower non-reducing sugar content as compared to the samples from spikes held at RT and 10ºC. The spikes held in the SUC showed significantly higher non-reducing sugar content in the petal tissues than the corresponding samples from spikes held in DW irrespective of the different temperature regime. The non-reducing sugar content showed a significant decrease with the progression in time from day 4 to day 10 irrespective of the different temperature treatments. The maximum non-reducing sugar content was recorded in the samples from spikes previously wet stored at RT and 10ºC and transferred to SUC (Table 5.2.4; Fig.5.2.9).

Table 5.2.1: Effect of postharvest wet storage (PHWS) at different temperatures (RT, 10ºC and 5ºC) for 72h and subsequent transfer to different holding solution (DW and SUC) on vase life and no. of blooms per spike of cut spikes on day 2, 6, 12 and day 17 of transfer in *Dianthus barbatus.*

Treatments	Vase life	Days of transfer

	(days)	Number of blooms per spike			
		D2	D6	D12	D17
SET A (DW)					
RT	8.03	8.5 (39.56)	12.33 (57.8)	13.66 (64)	13.66 (64)
10˚C	10.33	5.83 (27.33)	7.89 (37)	12.84 (60.6)	12.84 (60.6)
5˚C	14.66	4.16 (19.5)	6.0 (28.12)	15.83 (74.2)	17.89 (83.8)
SET B (SUC)					
RT	9.20	9.56 (44.8)	13.33 (62.5)	14.71 (69)	14.71 (69)
10˚C	12.33	6.12 (28.7)	8.26 (38.7)	13.33 (62.5)	13.33(62.5)
5˚C	17.66	5.07 23.7)	7.0 (32.8)	16.5 (77.3)	19.45 (91)
LSD at $P_{0.05}$	0.211	0.029	0.079	0.111	0.121

Each value is mean of 6 independent replicates

Values in parenthesis represent percent blooms

Room temperature= 22±2ºC

Table 5.2.2: Effect of postharvest wet storage (PHWS) at different temperatures (RT, 10ºC and 5ºC) for 72h and subsequent transfer to different holding solution (DW and SUC) on floral diameter and volume of holding solution absorbed per spike of cut spikes on D2, D6, D12 and D17 of transfer in *Dianthus barbatus.*

Treatments	**Days of transfer**	**Days of transfer**
	Floral diameter (cm)	**Volume of holding solution**

					absorbed per spike			
	D2	**D6**	**D12**	**D17**	**D2**	**D6**	**D12**	**D17**
Set A (DW)								
RT	1.80	1.82	--	--	1.27	2.22	3.5	3.83
10ºC	2.0	2.0	2.0	--	2.16	4.27	6.0	7.53
5ºC	1.66	2.1	2.0	--	2.05	3.94	6.27	7.92
Set B (SUC)								
RT	1.9	1.9	--	--	0.83	1.88	3.22	3.80
10ºC	2.02	2.02	2.0	--	1.27	2.38	4.5	6.2
5ºC	1.9	2.12	2.1	2	1.72	2.77	5.61	7.27
LSD at $P_{0.05}$	0.091	0.021	0.051	--	0.009	0.031	0.121	0.116

Each value is mean of 6 independent replicates

Room temperature= 22±2ºC

Table 5.2.3: Effect of postharvest wet storage (PHWS) at different temperatures (RT, 10ºC and 5ºC) for 72h and subsequent transfer to different holding solution (DW and SUC) on soluble proteins, α- amino acids and total phenols of cut spikes on day 4 and day 10 of transfer in *Dianthus barbatus.*

Treatments	**Days of transfer**					
	Soluble proteins (mg/g fm)		**α- amino acids (mg/g fm)**		**Total phenols (mg/g fm)**	
	D4	**D10**	**D4**	**D10**	**D4**	**D10**
Set A (DW)						
RT	1.72	--	7.39	--	1.53	--
10ºC	2.32	5.6	9.07	4.43	2.06	2.06
5ºC	3.0	3.88	7.09	6.40	2.26	2.20
Set B (SUC)						
RT	2.12	--	10.45	--	4.44	--
10ºC	2.8	3.6	9.26	4.60	2.27	2.33
5ºC	3.52	3.68	7.59	6.07	2.72	2.61
LSD at $P_{0.05}$	0.031	0.012	0.120	0.111	0.013	0.016

Each value is mean of 6 independent replicates.

Room temperature= 22±2ºC

Table 5.2.4: Effect of postharvest wet storage (PHWS) at different temperatures (RT, 10ºC and 5ºC) for 72h and subsequent transfer to different holding solution (DW and SUC) on total, reducing and non-reducing sugars of cut spikes on day 4 and day 14 of transfer in *Dianthus barbatus.*

Treatments	**Days of transfer**					
	Total sugars (mg/g fm)		**Reducing sugars (mg/g fm)**		**Non-reducing sugars (mg/g fm)**	
	D4	**D10**	**D4**	**D10**	**D4**	**D10**
Set A (DW)						
RT	8.57	--	4.28	--	4.29	--
10ºC	36.42	14.28	23.57	12.5	12.85	1.78
5ºC	34.28	17.85	32.14	16.71	2.14	1.14
Set B (SUC)						
RT	30.71	--	17.85	--	12.86	--
10ºC	60.0	29.46	47.14	32.14	12.86	2.68
5ºC	57.85	37.32	51.42	35.71	6.43	1.61
LSD at $P_{0.05}$	1.02	1.11	0.92	0.291	0.611	0.259

Each value is mean of 6 independent replicates.

Room temperature= 22±2ºC

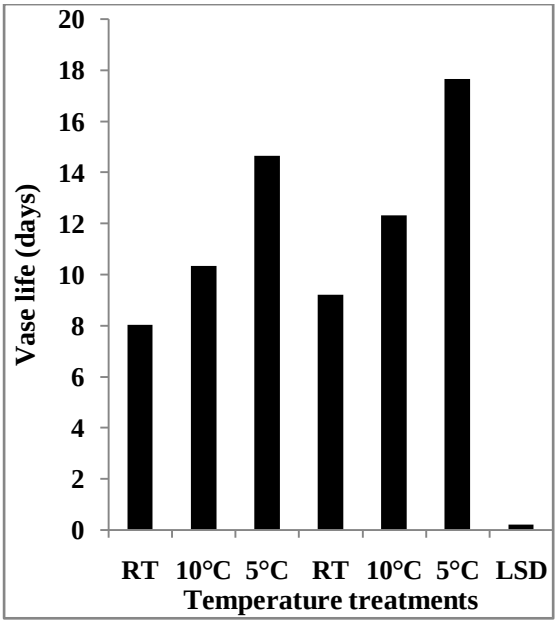
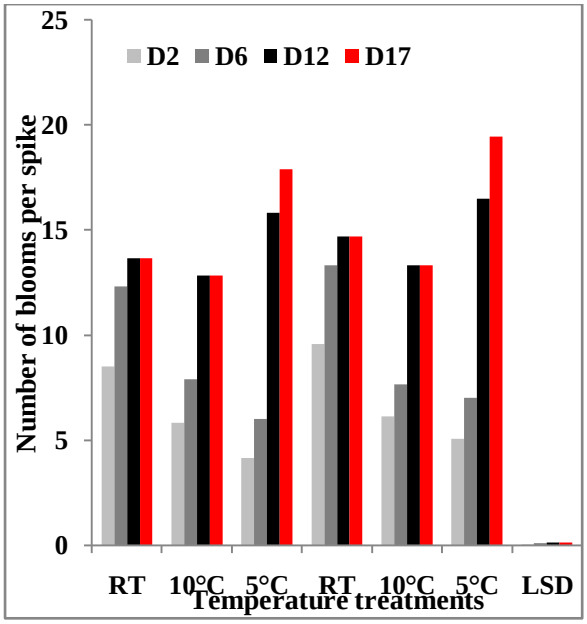


Fig. 5.2.1



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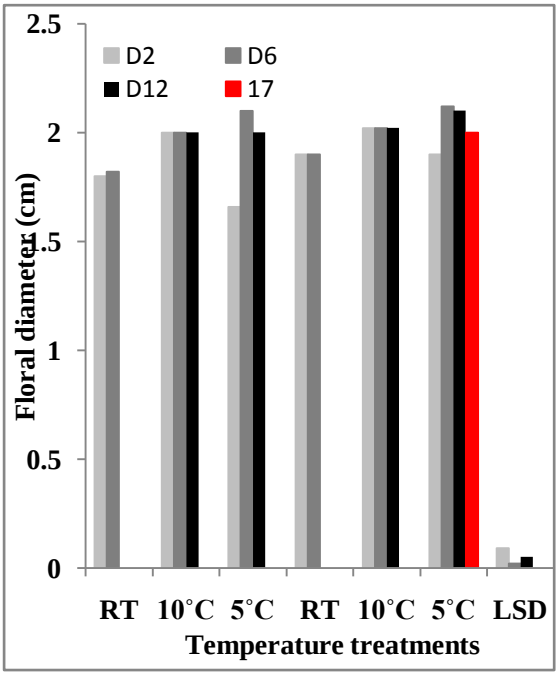


Fig. 5.2.3

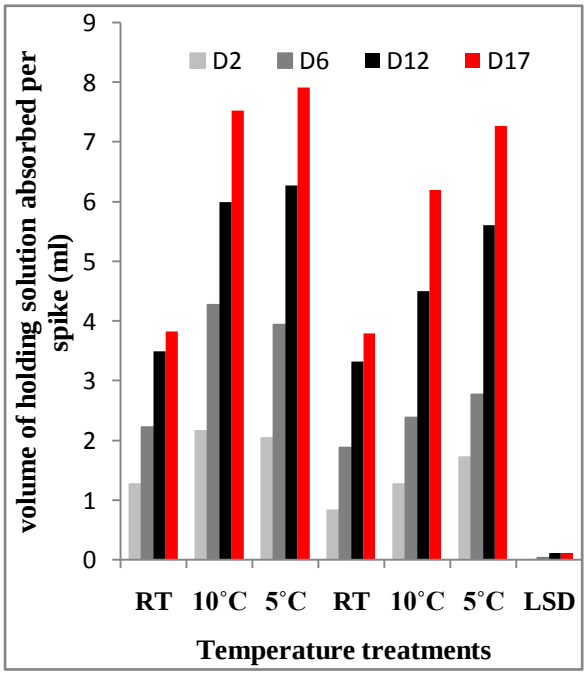


Fig. 5.2.4

Fig. 5.2.1-5.2.4: Effect of postharvest wet storage (PHWS) at different temperatures (RT, 10ºC and 5ºC) for 72 h and subsequent transfer to different holding solution (DW and SUC) on vase life, number of blooms per spike, floral diameter and volume of holding solution absorbed per spike at D2,D6,D12 and D18 in cut spikes of *Dianthus barbatus.*

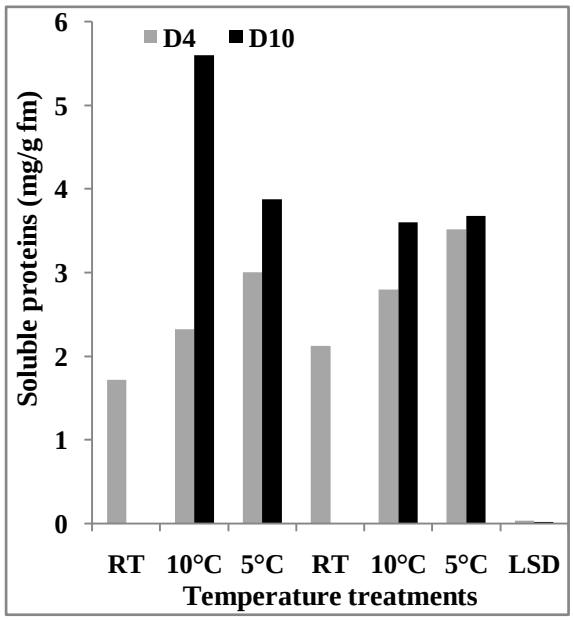


Fig. 5.2.5

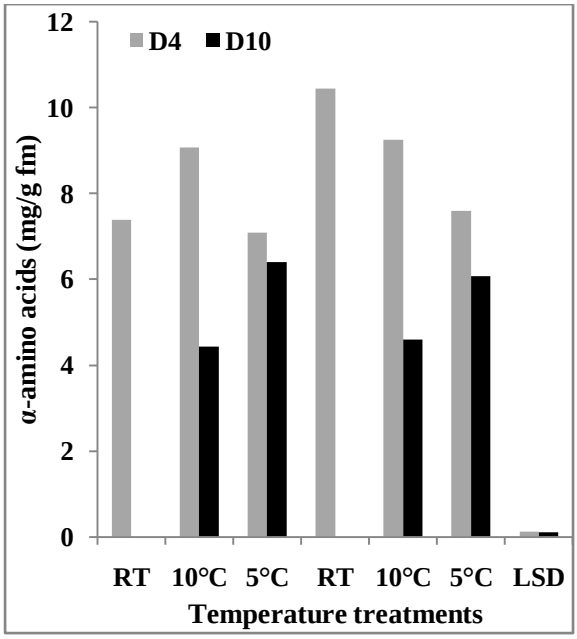


Fig. 5.2.6

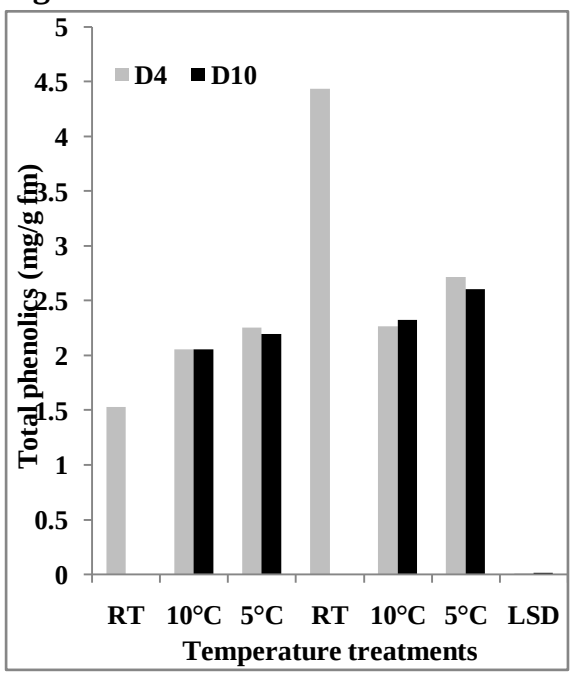


Fig. 5.2.7

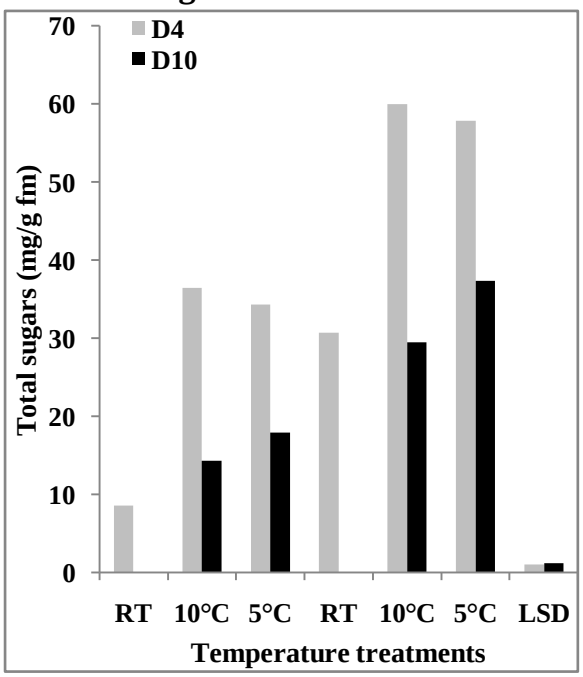
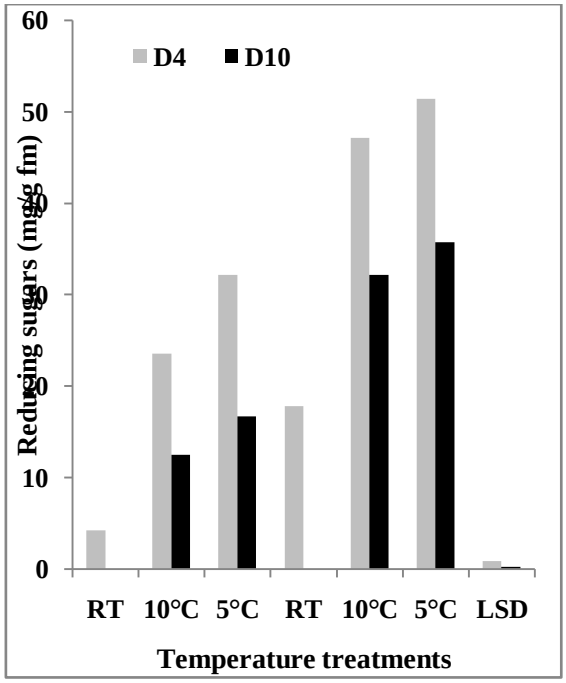


Fig. 5.2.8

Fig. 5.2.5-5.2.8: Effect of postharvest wet storage (PHWS) at different temperatures (RT, 10ºC and 5ºC) for 72 h and subsequent transfer to different holding solution (DW and SUC) on soluble proteins, α-amino acids, total phenols and total sugars at D4 and



D14 in cut spikes of *Dianthus barbatus.*

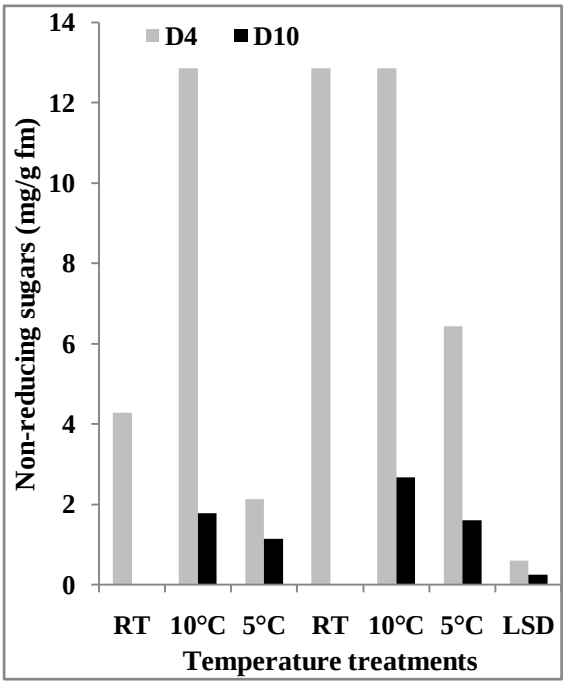


Fig.5.2.9 **Fig.5.2.10**

Fig. 5.2.9-5.2.10: Effect of postharvest wet storage (PHWS) at different temperatures (RT, 10ºC and 5ºC) for 72 h and subsequent transfer to different holding solution (DW and SUC) on reducing and non-reducing sugars at D4 and D14 in cut spikes of *Dianthus barbatus.*

Plate 5.2.1

Effect of postharvest wet storage (PHWS) for 72 h at room temperature (RT), 10°C and 5°C before transfer to DW and SUC (0.05M) in cut spikes of *Dianthus barbatus*

Fig. (a): From left to right are arranged spikes before wet storage for 72 h.

Fig. (b): From left to right are arranged spikes after wet storage for 72 h.

Spikes of *Dianthus barbatus* held in distilled water (DW) and sucrose (SUC) after 72 h wet storage at day 8 (Fig. c) and day 18 of transfer (Fig. d). From left to right are arranged flasks containing spikes which were previously held at different temperatures {(RT, 22±2˚C), 10˚C and 5˚C} and in different vase solutions {DW and SUC}.

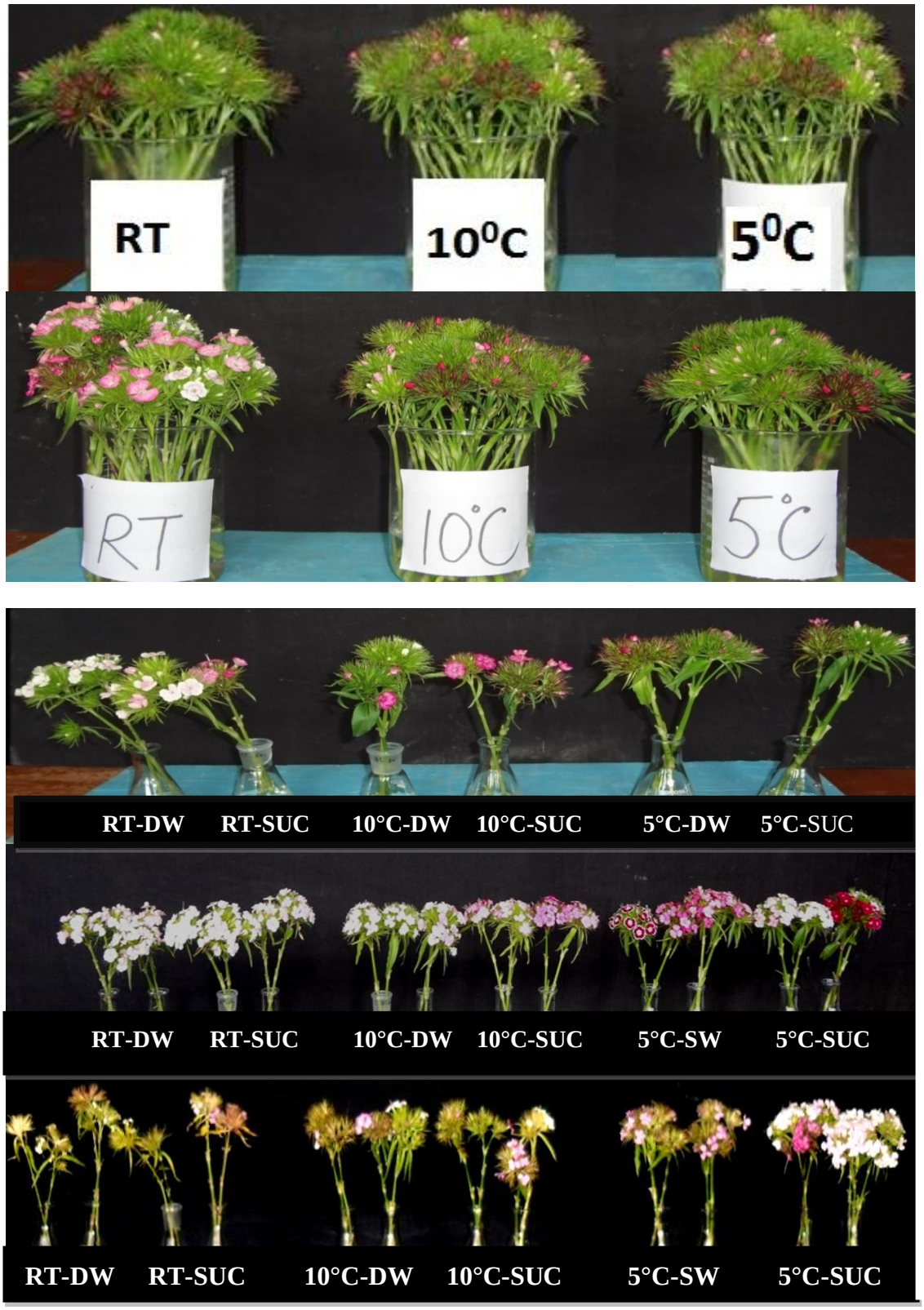

RT
10⁰C
5⁰C
(a)
RT
10°C
5°C
(b)
RT-DW RT-SUC 10°C-DW 10°C-SUC 5°C-DW 5°C-SUC
(c)
RT-DW RT-SUC 10°C-DW 10°C-SUC 5°C-SW 5°C-SUC
(d)
RT-DW RT-SUC 10°C-DW 10°C-SUC 5°C-SW 5°C-SUC
(e)

Plate 5.2.1

EXPERIMENT-5.3

Effect of postharvest dry storage (PHDS) at three different temperature regimes RT (20±2ºC), 10ºC and 5ºC) for 72 h and subsequent transfer to vase solutions {distilled water (DW) and sucrose (SUC)} on postharvest performance in cut spikes of *Dianthus barbatus* L.

Experimental

Uniform spikes of *Dianthus barbatus* growing in the Kashmir University Botanic Garden (KUBG) were used for the present study. The buds were harvested at 800 h when the buds were at loose bud stage (i.e. brush stage). The harvested spikes were immediately brought to the laboratory and cut to a uniform length of 27 cm and divided into three sets with equal number of spikes. The spike sets were wrapped in perforated polyethylene flower sleeves. One set of spikes was cool stored at 10ºC and the other at 5ºC for 72 h. The third set of spikes was kept at room temperature (20±2°C) and designated as control. After 72 h the spikes were kept at room temperature after transferring them to 250 ml Ehrlenmeyer flasks containing 200 ml of vase solution {Distilled water (DW) and Sucrose (SUC) 0.05 M}. Each treatment was represented by 5 replicates (flasks) with each flask containing two spikes. The day of transfer of spikes to vase solutions (DW and SUC) was designated as day zero (D0). The average vase life of spikes was counted from the day of transfer (D0) to holding solution and was regarded to be terminated when the last flower lost its display value (marked by color change and loss of petal turgidity). Visual changes were observed at periodic intervals. Floral diameter, number of blooms per spike and volume of holding solution absorbed per spike were estimated on Day 2, 7 and 15. Changes in tissue constituents including soluble proteins, α-amino acids, total phenolics and sugar fractions were estimated in the petal tissue at day 4 and day 9 of transfer of spikes to vase solution (DW and SUC). The results have been analyzed statistically and LSD computed at $P_{0.05}$ using MINITAB (v15.1.2-EQUINOX_Softddl.net) software.

Visible effects

The spikes previously dry stored for 72 h at different temperature regimes (RT, 10 and 5ºC) were fresh and had maintained their premature status (1 day before anthesis). However, the spikes dry stored at RT had slightly lost their turgor as compared to buds dry stored at 5 and 10ºC. The spikes previously dry stored at 10ºC and RT opened on day 1 of transfer to holding solution (DW and SUC). While as the spikes previously dry stored at 5ºC for 72 h opened on day 2 of transfer to vase solutions (DW and SUC). The flower senescence was characterized by loss of petal turgidity and change in petal color from pink to yellow. The pattern of flower senescence was comparable with the flowers growing in plants under field conditions.

Vase life

The vase life of spikes previously dry stored at RT, 10ºC and 5ºC was 7, 10 and 16 when transferred to DW as compared to the corresponding spikes transferred to SUC where it was 9, 14 and 19 days respectively. Maximum vase life of 19 days was observed in the spikes previously dry stored at 5ºC for 72 h and transferred to SUC (Table 5.3.1; Fig.5.3.1).

Floral diameter

The floral diameter of the spikes increased with the progression in time from day 2 to day 7 but thereafter showed a decrease with the progression in time from day 7 to day 15. The floral diameter of the spikes previously held at various temperature regimes was almost comparable irrespective of the nature of the vase solution. While as floral diameter of the spikes previously dry stored at 10ºC & 5ºC was comparatively more than the spikes previously dry stored at RT irrespective of the nature of the vase solution. Maximum floral diameter of 2.5cm was exhibited by the spikes dry stored at 5ºC and transferred to SUC on day 7 (Table 5.3.1; Fig 5.3.2)

Holding solution absorbed per spike

The volume of holding solution absorbed was significantly higher in spikes previously dry stored at various temperature regimes before transfer to DW as compared to the corresponding spikes transferred to SUC. The volume of holding solution absorbed per spike increased significantly with the progression in time from

day 2 to day 15. Maximum holding solution was absorbed by the spikes previously dry stored for 72 at 5ºC and transferred to DW on day 15 (Table 5.3.1; Fig.5.3.3).

Number of blooms per spike

The rate of blooming as well as number of blooms per spike was appreciably higher in spikes previously dry stored at RT (20±2ºC) before transfer to DW or SUC as compared to corresponding spikes previously dry stored at 10ºC and 5ºC. The rate of blooming showed a significant increase with the progression in time from day 2 to day 15 in spikes dry stored at RT, 10ºC and 5ºC irrespective of the vase solutions. However the rate of blooming was 76.73, 81 and 94 % in spikes previously dry stored RT, 10ºC and 5ºC on day 15 in spikes transferred to SUC respectively. Maximum rate of blooming, therefore was observed in case of spikes dry stored at 5ºC and transferred to SUC on day 15 (Table 5.3.1; Fig.5.3.4).

Soluble proteins

The soluble protein content of the petal tissue from spikes increased with the progression in time from day 4 to day 9 irrespective of the different temperature regimes and nature of the vase solution. Higher soluble protein content on day 4 was registered in the samples from spikes previously kept at 10ºC irrespective of the nature of the vase solution. But higher soluble protein content on day 9 was registered in the samples from spikes previously kept at RT irrespective of the nature of the vase solution as compared to the samples from corresponding spikes previously kept at 10ºC and 5ºC. Maximum soluble protein content was registered in the spikes previously dry stored at RT for 72 and transferred to SUC on day 9 (Table 5.3.2; Fig 5.3.5).

α-amino acids

The petal tissue samples from spikes previously dry stored for 72 h at 10ºC and 5ºC showed significantly lower α-amino acid content as compared to the spikes previously dry stored at RT (20±2ºC) irrespective of the nature of the vase solutions. The α-amino acid content increased with the progression in time from day 4 to day 9 irrespective of temperature regime and vase solution. Maximum α-amino acid content

was registered in the spikes previously dry stored at RT (20±2ºC) and transferred to DW on day 4 (Table 5.3.2; Fig.5.3.6).

Total phenolics

The total phenolic content of the samples from the spikes previously dry stored at 10 °C & 5ºC for 72 h was comparatively more than the spikes dry stored at RT irrespective of nature of vase solution. The total phenolic content of the petal tissue samples decreased with the progression in time from D4 to D9 in the spikes previously dry stored at 10 and 5 °C while as considerable decrease was registered with the progression in time from day 4 to day 9 in the samples from spikes previously dry stored at RT (20±2ºC) irrespective of nature of vase solution. Minimum total phenolic content was registered in the samples from spikes previously dry stored at 5ºC & transferred to SUC on day 9 (Table 5.3.2; Fig.5.3.7).

Total sugars

The total sugar content of the samples from the spikes previously dry stored at RT (20±2ºC) was considerably higher than the spikes stored at 10ºC & 5ºC irrespective of nature of vase solution. However, on day 9, the spikes held in DW after 72 h dry storage at 5ºC maintained higher total sugar content in the petal tissues as compared to the samples from corresponding spikes held at RT and 10ºC. While as the samples from spikes previously dry stored at RT showed higher total sugar content as compared to the samples from corresponding spikes dry stored at 10ºC and 5ºC when transferred to SUC. The total sugar content generally showed a marginal increase with the progression in the time from day 4 to day 9 irrespective of the nature of the vase solution (Table 5.3.3; Fig 5.3.10).

Reducing sugars

The reducing sugar content of the samples from spikes previously dry stored at RT for 72 h was higher as compared to samples from the spikes dry stored at 10 and 5ºC irrespective of the vase solution. However, the samples from spikes previously dry stored for 72 h and transferred to SUC showed higher reducing sugar concentration as compared to spikes transferred to DW irrespective of temperature regimes. Reducing sugar content showed a significant decrease with the progression in time from day 4 to

day 9 irrespective of the nature of vase solution. Maximum reducing sugar content was registered in the spikes previously dry stored for 72 h at RT and transferred to SUC on day 4 (Table 5.3.3; Fig 5.3.8).

Non-reducing sugars

The non-reducing sugar content of the samples from the spikes previously dry stored for 72 h at different temperature regimes and transferred to SUC was generally higher than the samples from the corresponding spikes held in DW. However, the non-reducing sugar content on day 9 was significantly higher in the samples from spikes previously kept at 5ºC & transferred to DW. While as the samples from spikes previously stored at 5ºC and transferred to SUC showed significantly lower non-reducing sugar content as compared to the samples from corresponding spikes kept at RT (20±2ºC) and 10ºC. Maximum non-reducing sugar content was registered in the samples from spikes dry stored at 5ºC and transferred to DW on day 9 (Table 5.3.3; Fig 5.3.9).

Table 5.3.1: Effect of postharvest dry storage (PHDS) at different temperatures (RT,10ºC and 5ºC) for 72h and subsequent transfer to different holding solution (DW and SUC) on vase life, floral diameter and volume of holding solution absorbed on D2, D7 and D15 of transfer in *Dianthus barbatus* L.

Treatments	**Vase life (days)**	**Days of transfer**					
		Floral diameter (cm)			**Volume of holding solution absorbed per spike (ml)**		
		D2	**D7**	**D15**	**D2**	**D7**	**D15**
Set A (DW)							
RT	7.2	1.77	2.1	--	1.52	2.90	4.33
10°C	10.0	1.9	2.3	--	2.2	4.36	8.09
5°C	16.0	1.5	2.3	2.2	2.3	4.30	8.32
Set B (SUC)							
RT	9.33	1.78	2.33	--	1.02	1.93	4.02
10°C	14.66	2.0	2.3	--	1.38	2.54	6.4
5°C	19.33	1.5	2.5	2.3	1.87	2.90	7.80
LSD at $P_{0.05}$	0.431	0.021	0.032	--	0.009	0.011	0.012

Each value is mean of 6 replicates.

Room temperature (RT= 20±2ºC)

Table 5.3.3: Effect of postharvest dry storage (PHDS) at different temperatures (RT,10ºC and 5ºC) for 72h and subsequent transfer to different holding solution (DW and SUC) on number of blooms per spike (D2, D7 and D15) and soluble proteins of cut spikes on day 4 and day 9 of transfer in *Dianthus barbatus* L.

Treatments	**Days of transfer**				
	Number of blooms per spike			**Soluble proteins (mg/g fm)**	
	D2	**D7**	**D15**	**D4**	**D9**
Set A (DW)					
RT **10°C** **5 °C**	8.66 (28.66) 6.0 (20) 3.33 (11.1)	13.21 (44.03) 12.1 (40.33) 18.99 (63.3)	21.87 (72.9) 18 (60) 22.32 (74.4)	1.2 1.68 1.04	-- 2.72 1.28
Set B (SUC)					
RT **10°C** **5 °C**	9.0 (30) 7.6 (25.33) 7.21 (24)	14.02 (46.73) 16.7 (55.66) 21.0 (70)	23.02 (76.73) 24.3 (81) 28.21 (94)	1.12 1.84 1.04	3.68 2.24 1.76
LSD at $P_{0.05}$	0.037	0.062	0.281	0.009	0.011

Each value is the mean of 6 replicates

Room temperature= (20±2ºC)

Table 5.3.3: Effect of postharvest dry storage (PHDS) at different temperatures (RT, 10ºC and 5ºC) for 72h and subsequent transfer to different holding solution (DW and SUC) on α–amino acids and total phenols of cut spikes on day 4 and day 9 of transfer in *Dianthus barbatus* L.

Treatments	**Days of transfer**			
	α–amino acids (mg/g fm)		**Total phenols (mg/g fm)**	
	D4	**D9**	**D4**	**D9**
Set A (DW)				
RT	11.46	--	1.5	--
10°C	8.38	10.84	2.58	1.25
5 °C	6.40	7.92	2.08	0.58
Set B (SUC)				
RT	7.39	8.32	1.75	4.80
10°C	4.80	6.48	3.33	1.83
5 °C	5.54	6.66	2.58	0.83
LSD at $P_{0.05}$	0.021	0.032	0.061	0.047

Each value is the mean of 6 replicates

Room temperature= (20±2ºC)

Table 5.3.4: Effect of postharvest dry storage (PHDS) at different temperatures (RT, 10ºC and 5ºC) for 72 h and subsequent transfer to different holding solution (DW and SUC) on total, reducing and non-reducing sugars on day 4 and day 9 of transfer in *Dianthus barbatus* L.

Treatments	**Days of transfer**					
	Reducing sugars (mg/g fm)		**Non reducing sugars (mg/g fm)**		**Total sugars (mg/g fm)**	
	D4	**D9**	**D4**	**D9**	**D4**	**D9**
Set A (DW)						
RT	13.39	--	5.36	--	18.75	--
10 °C	5.35	3.67	8.04	11.38	13.39	15.05
5 °C	11.42	6.69	5.38	30.81	16.78	37.5
Set B (SUC)						
RT	21.42	10.71	8.04	16.07	29.46	26.78
10 °C	16.07	8.03	8.03	14.73	24.10	22.76
5 °C	19.63	12.05	8.07	9.37	27.70	21.42
LSD at $P_{0.05}$	0.022	0.041	0.014	0.237	0.511	0.120

Each value is the mean of 6 replicates

Room temperature=(20±2ºC)

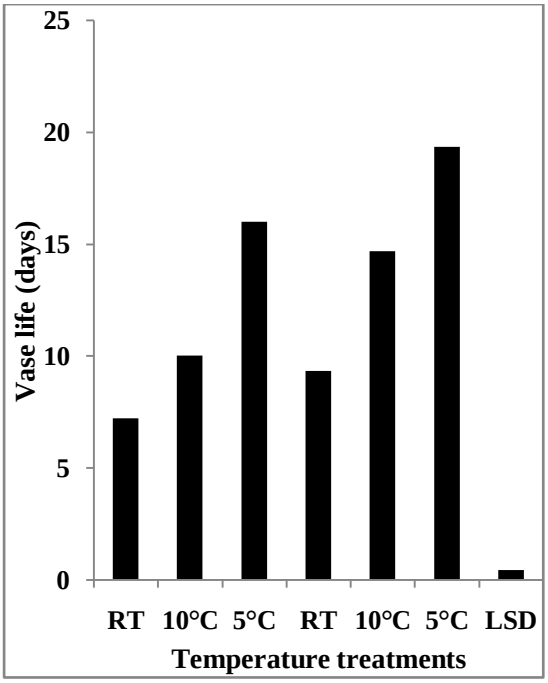


Fig. 5.3.1

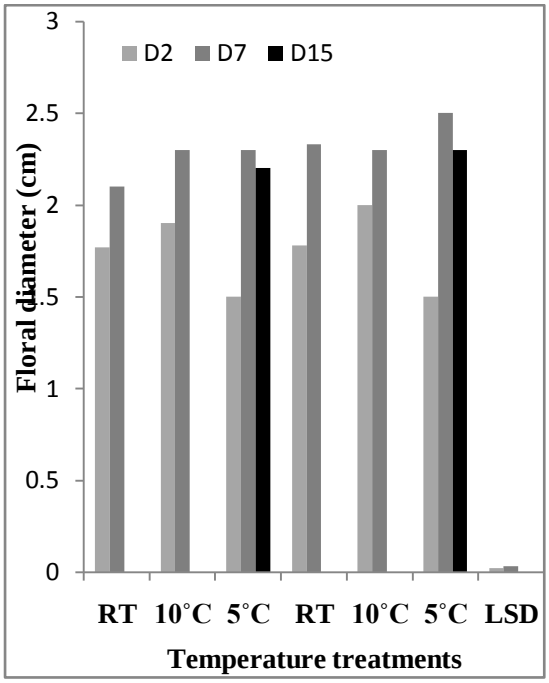


Fig. 5.3.2

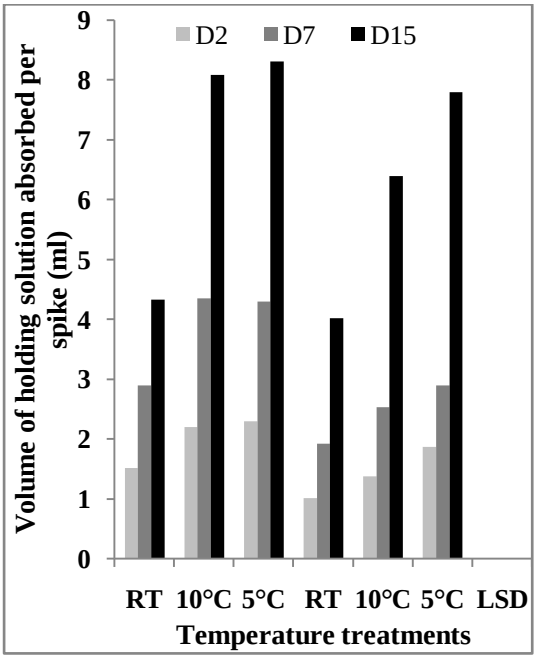


Fig. 5.3.3

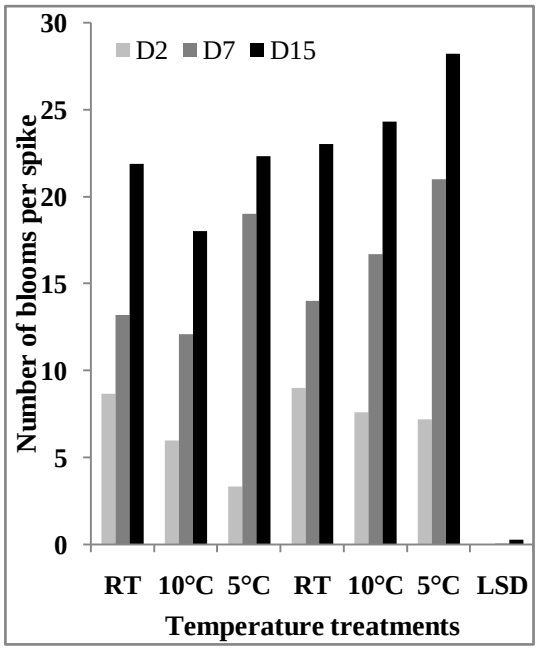


Fig. 5.3.4

Fig.4.3.1-4.3.4: Effect of postharvest dry storage (PHDS) at different temperatures (RT, 10°C and 5°C) for 72 h and subsequent transfer to different holding solutions (DW and SUC) on vase life, floral diameter, volume of holding solution absorbed and number of blooms per spike in cut spikes of *Dianthus barbatus L.*

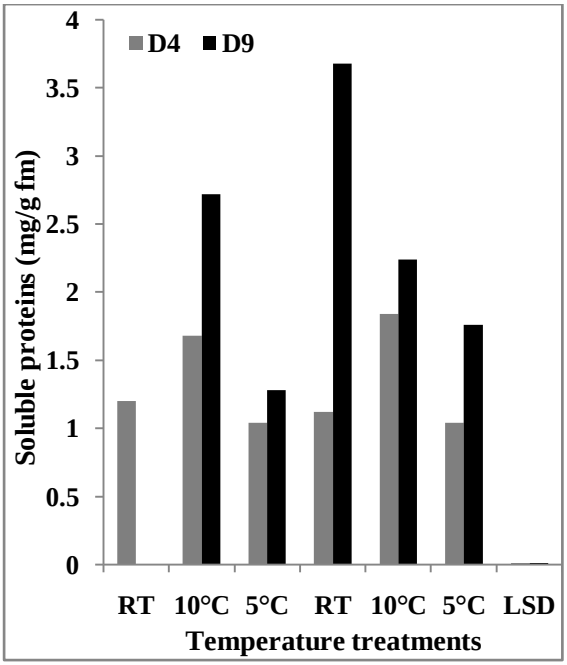


Fig. 5.3.5

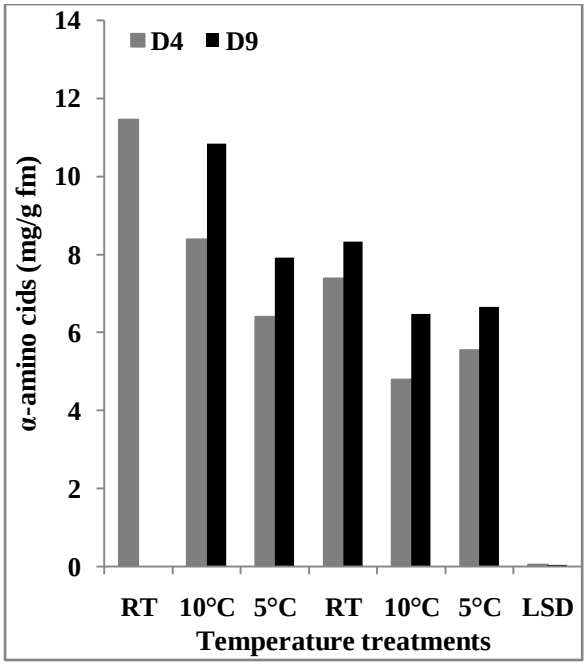


Fig. 5.3.6

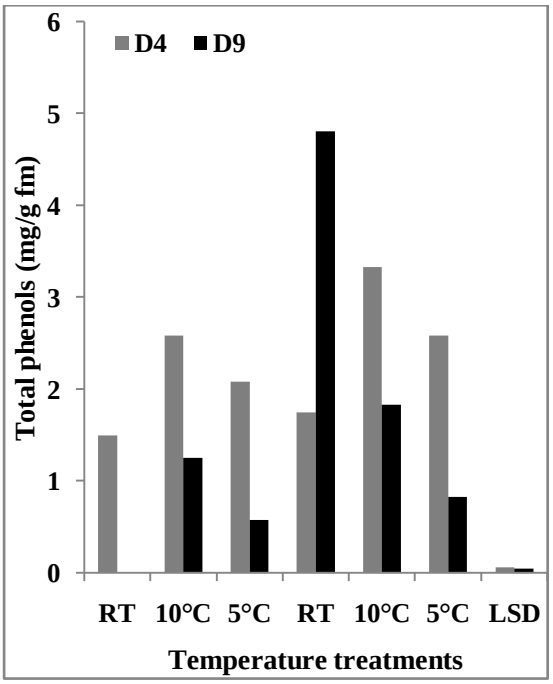


Fig. 5.3.7

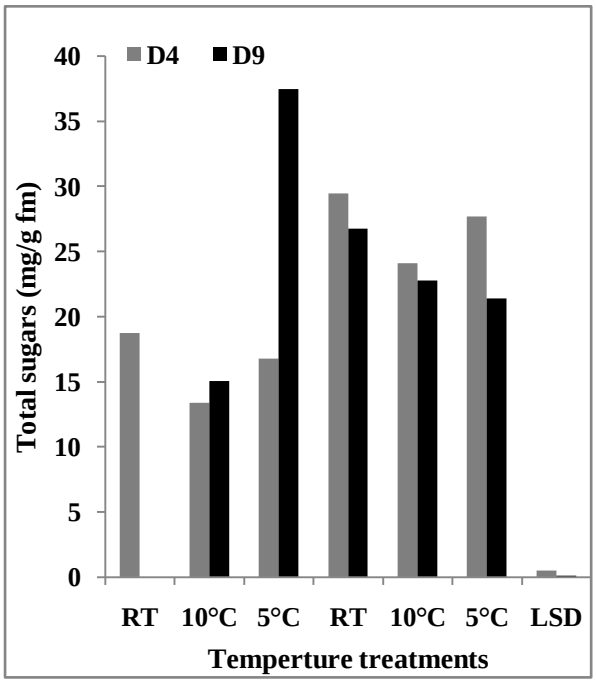


Fig. 5.3.8

Fig.4.3.1-4.3.4: Effect of postharvest dry storage (PHDS) at different temperatures (RT, 10°C and 5°C) for 72 h and subsequent transfer to different holding solutions (DW and SUC) on soluble proteins, α-amino acids, total phenols and total sugars in cut spikes of *Dianthus barbatus L.*

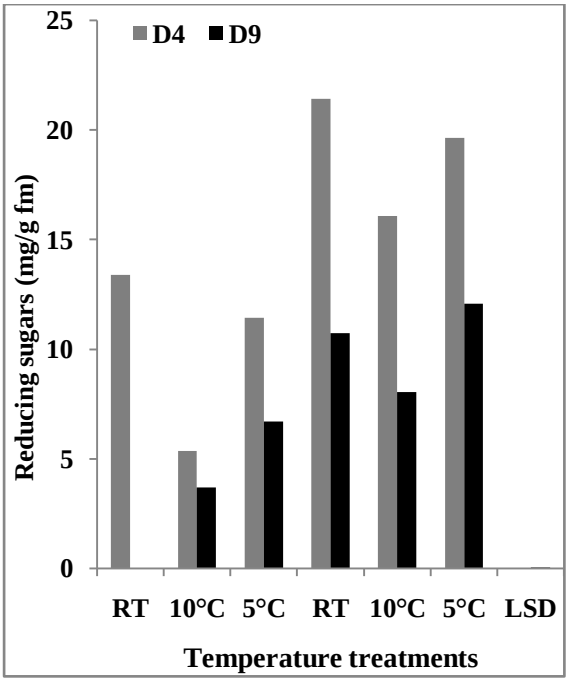


Fig. 5.3.9

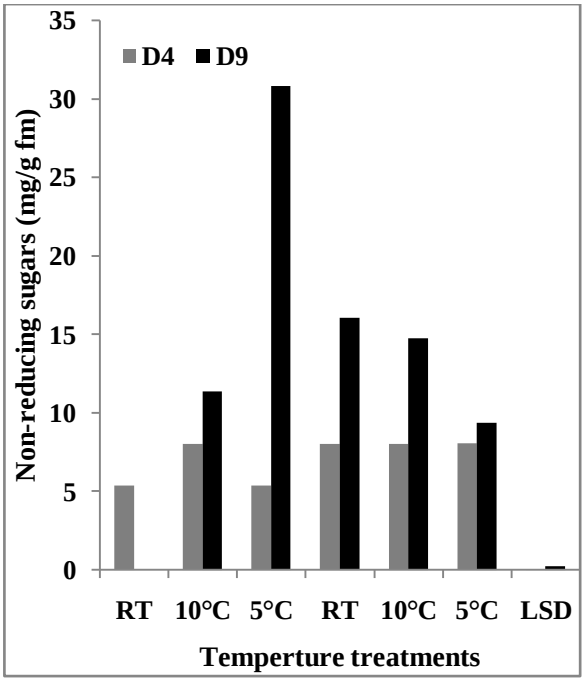


Fig. 5.3.10

Fig.4.3.1-4.3.4: Effect of postharvest dry storage (PHDS) at different temperatures (RT, 10°C and 5°C) for 72 h and subsequent transfer to different holding solutions (DW and SUC) on reducing and non-reducing sugars in cut spikes of *Dianthus barbatus L.*

Plate 5.3.1

Effect of postharvest dry storage (PHDS) for 72 h at room temperature (RT), 10˚C and 5˚C before transfer to DW and SUC (0.05M) in cut spikes of *Dianthus barbatus*.

Fig. (a): From left to right are arranged spikes before dry storage for 72 h.

Fig. (b): From left to right are arranged spikes after dry storage for 72 h.

Spikes of Dianthus barbatus held in distilled water (DW) and sucrose (SUC) after 72 h dry storage at day 2 (Fig. c), day 12 (Fig. d) and day 17 of transfer (Fig. e). From left to right are arranged flasks containing spikes which were previously held at different temperatures {RT (20±2˚C), 10˚C and 5˚C} and in different vase solutions DW and SUC.

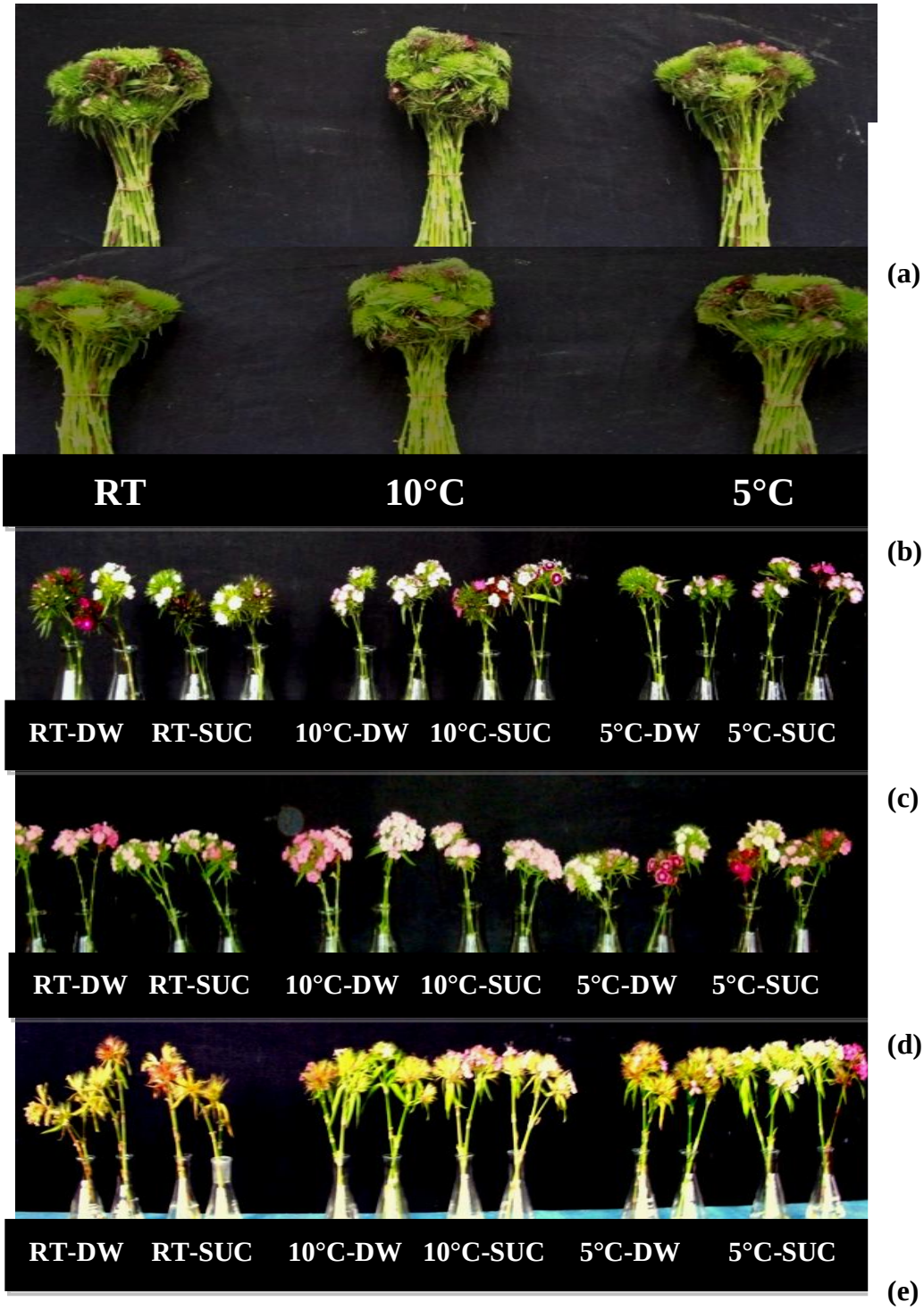

RT
10°C
5°C
(a)
RT-DW
RT-SUC
10°C-DW
10°C-SUC
5°C-DW
5°C-SUC
(b)
RT-DW
RT-SUC
10°C-DW
10°C-SUC
5°C-DW
5°C-SUC
(c)
RT-DW
RT-SUC
10°C-DW
10°C-SUC
5°C-DW
5°C-SUC
(d)
(e)

Plate 5.3.1

CHAPTER-6

REGULATION OF FLOWER DEVELOPMENT AND SENESCENCE IN SILENE ARMERIA

EXPERIMENT 6.1

Physiological and biochemical changes associated with flower development and senescence in *Silene armeria.* L.

Experimental

Flowers growing in Kashmir University Botanic Garden (KUBG) were used for the present study. Flower development and senescence was divided into five stages. These stages were deciphered as I-tight bud stage, II-mature bud stage, III-loose bud stage, IV- fully open stage and V-senescent stage. Visible changes were recorded throughout the flower development and senescence in the field. Floral diameter, fresh mass and dry mass were estimated at each stage. For the estimation of tissue constituents, 1 g of petal tissue was fixed in hot 80% ethanol at each stage of flower development and senescence. The material was macerated and centrifuged three times. The supernatants were pooled and final volume of all the replicates at different stages of senescence was made uniform with 80% ethanol. Suitable aliquots were used for the estimation of reducing sugars, total sugars, α-amino acids and total phenols. Non reducing sugars were calculated as the difference between total and reducing sugars. Soluble proteins were extracted from 1g of fresh petal tissue obtained from five different flowers at each stage of flower development and senescence. Specific protease activity has been computed as µg tyrosine equivalents librated per mg protein. At each stage the homogenates from the petal tissue were used for studying electrophoretic profiles of proteins by SDS-PAGE. The tissue was homogenized in 0.1 M phosphate buffer (pH= 7.2) and centrifuged, supernatant so obtained was processed for SDS-PAGE. Each value represented in the tables corresponds to the mean of five independent replicates. The data has been analyzed statistically by computing standard deviation.

RESULTS

Visible changes

The flower senescence and development in *Silene armeria* is characterized by the loss of petal turgidity, followed by inrolling of petal margins. During senescence, the color changes from pink to yellow. Corolla abscises about 72 h after senescence leaving behind the ovary, along with calyx that develops into capsule (Plate 6.1.1).

Fresh mass, dry mass and water content

Fresh & dry mass and water content of flowers increased with flower development up to stage IV and declined thereafter as the flower senescence progressed to stage V. A sharp increase in fresh mass and water content was recorded from stage III to IV (Table 6.1.1; Fig; 6.1.1-6.1.3).

Soluble proteins

The concentration of soluble proteins showed a sharp increase from stage I to stage II and was then more or less maintained as the flower development progressed from stage II to stage V. However, when data was expressed on per flower basis, the soluble protein content showed an increase upto stage IV followed by a decline as the senescence progressed to stage V (Table 6.1.2; Fig. 6.1.4).

Specific protease activity

The specific protease activity (expressed in µg tyrosine equivalents per mg protein) registered a sharp decrease from stage I to stage II and thereafter the protease activity showed an increase as the senescence progressed through stages II to V (Table 6.1.2; Fig.6.1.5).

α- amino acids

The α- amino acid content per gram fresh mass registered a sharp increase from stage I to stage II. Thereafter the α- amino acid content was more or less maintained as the flower development progressed through various stages . When data was expressed on per flower basis, the α- amino acid content registered a sharp increase from stage I to II, the increase in α- amino acid content on per flower basis was generally maintained as the senescence progressed through various stages (Table 6.1.2; Fig.6.1.6).

Total phenols

The concentration of total phenols registered an increase from stage I to stage II and then showed a decrease from stage II through stage IV of the flower development. An increase in total phenols was however recorded thereafter as flower senescence progressed to stage V. However when the data was plotted on per flower basis, the total phenolic content showed an increase from stage I to II, then generally maintained as the flower development progressed through various stages of flower development (Table 6.1.2; Fig.6.1.7).

Total sugars

The tissue content of total sugars increased continuously from stage I to stage III and declined thereafter as the flower development progressed through stages IV and V. The trend became more apparent when data was expressed on per flower basis (Table 6.1.3; Fig.6.1.8).

Reducing sugars

The tissue content of reducing sugars showed an increase as the flower development progressed through stages I to IV and declined sharply as the senescence progressed through stage V. The trend become more apparent when data was expressed on per flower basis (Table 6.1.3; Fig.6.1.9)

Non reducing sugars

The tissue content of non-reducing sugars showed an increase from stage I to stage III and thereafter, a decline was registered as the flower development progressed through stages IV and V. However, when data was expressed on per flower basis, the non-reducing content showed a continuous increase upto stage IV as the flower opens and thereafter a sharp decline was registered as senescence progressed through stage V (Table 6.1.3; Fig.6.1.10)

Table 6.1.1: Fresh mass, dry mass and water content during flower development and senescence in flowers of *Silene armeria. L*

Stages of development	Fresh mass (mg)	Dry mass (mg)	Water content (mg)
I (Tight bud stage)	5.1±0.0006	4±0.0003	1±0.0009
II (Mature bud stage)	18±0.008	6±0.00021	11±0.001
III (Loose bud stage)	20±0.002	7±0.00034	12±0.001
IV (Fully open stage)	33.5±0.003	8±0.0004	25±0.003
V (Senescent stage)	20.4±0.003	7±0.00033	13±0.002

Each value is a mean of 6 independent replicates±SD

Table 6.1.2: Soluble proteins, specific protease activity (expressed as µg tyrosine equivalents librated per mg protein), α-amino acids and phenols (expressed as mg g^{-1} fresh mass) during flower development and senescence in flowers of *Silene armeria. L*

Stages of development	**Soluble proteins**	**Specific protease activity**	**α-amino acids**	**Total phenols**
I (Tight bud stage)	3.84±0.437 (0.019)	0.81±0.02	3.79±0.54 (0.02)	3.88±0.276 (0.098)
II (Mature bud stage)	11.52±0.245 (0.207)	0.05±0.005	8.28±0.098 (0.194)	6.30±0.428 (0.113)
III (Loose bud stage)	10.4±1.22 (0.208)	0.35±0.02	6.32±0.09 (0.126)	4.50±0.132 (0.09)
IV (Fully open stage)	8.4±0.324 (0.281)	0.37±0.021	3.91±0.098 (0.130)	3.81±0.437 (0.127)
V (Senescent stage)	10.96±0.245 (0.223)	0.48±0.032	8.97±0.009 (0.182)	6.92±0.135 (0.141)

Each value is a mean of 6 independent replicates±SD

Figures in the parentheses represent values on per flower basis.

Table6.1.3: Total, reducing and non-reducing sugars and (expressed as mg g^{-1} fresh mass) during flower development and senescence in the flowers of *Silene armeria.*

Stages of flower development	**Total sugars**	**Reducing sugars**	**Non-reducing sugars**
I (Tight bud stage)	11.25±1.25 (0.038)	3.75±0.04 (0.019)	7.5±0.3 (0.057)
II (Mature bud stage)	37.5±1.98 (0.382)	16.25±1.125 (0.292)	21.25±0.75 (1.095)
III (loose bud stage)	63.75±1.762 (0.80)	23.75±0.07 (0.475)	40.0±0.8 (1.275)
IV (fully open stage)	46.25±3.88 (0.628)	27.5±0.23 (0.921)	18.75±0.02 (1.549)
V (Senescent stage)	27.5±1.08 (0.267)	14.37±1.43 (0.293)	13.1±1.175 (0.561)

Each value is a mean of 6 independent replicates±SD

Figures in the parentheses represent values on per flower basis.

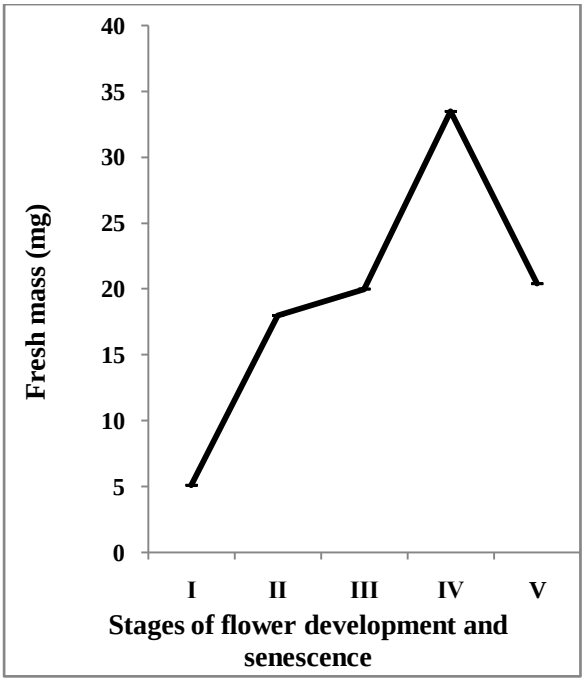


Fig. 6.1.1

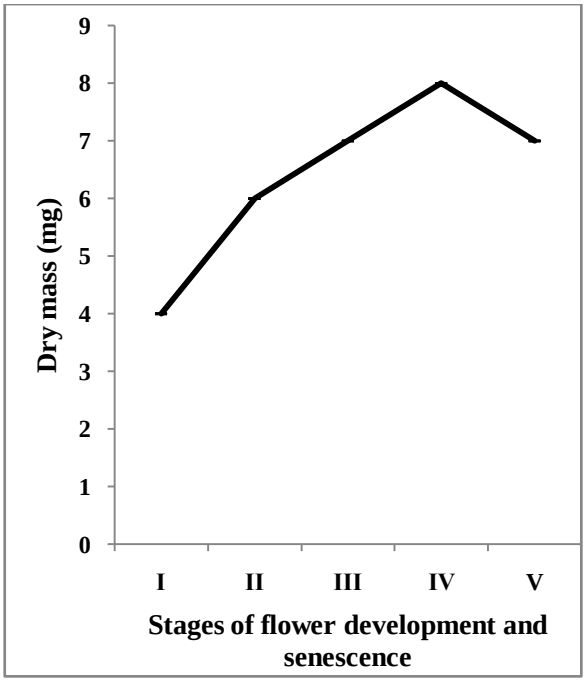
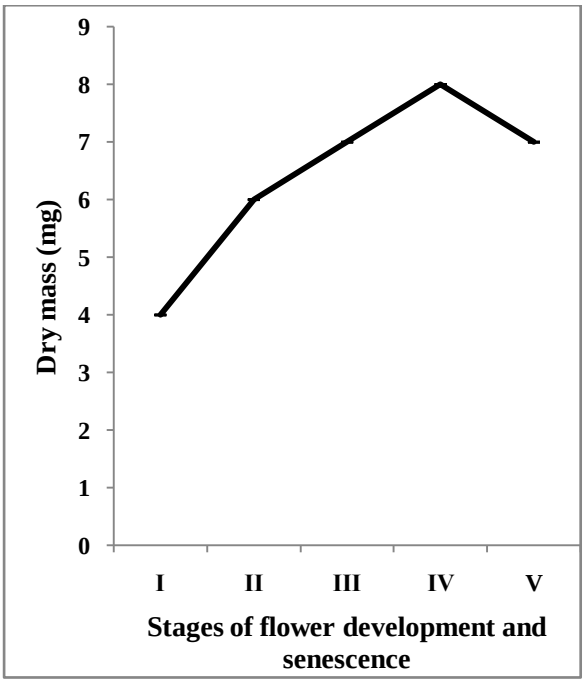


Fig. 6.1.2



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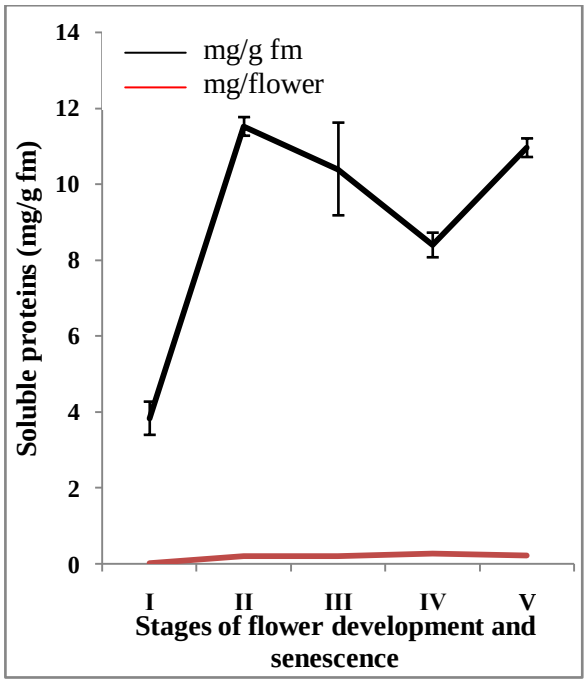


Fig. 6.1.4

Fig.6.1.1-6.1.4: Changes in fresh and dry mass and water content of flowers and floral diameter during different stages of flower development and senescence in *Silene armeria* L.

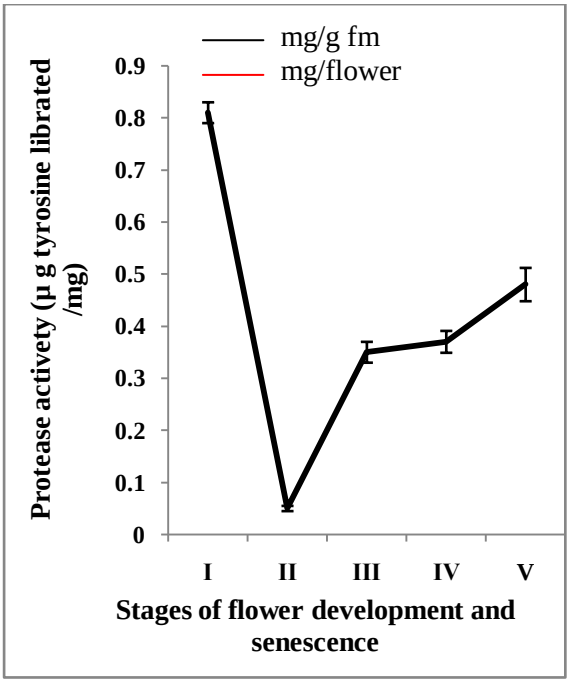
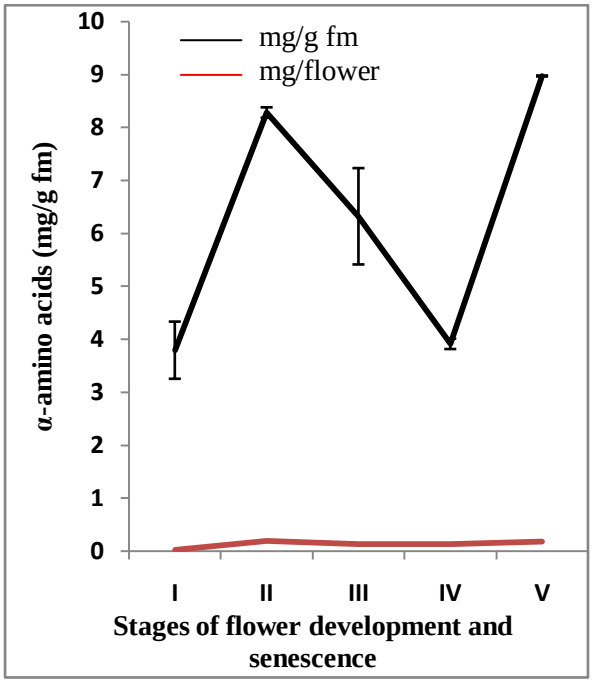


Fig. 6.1.5



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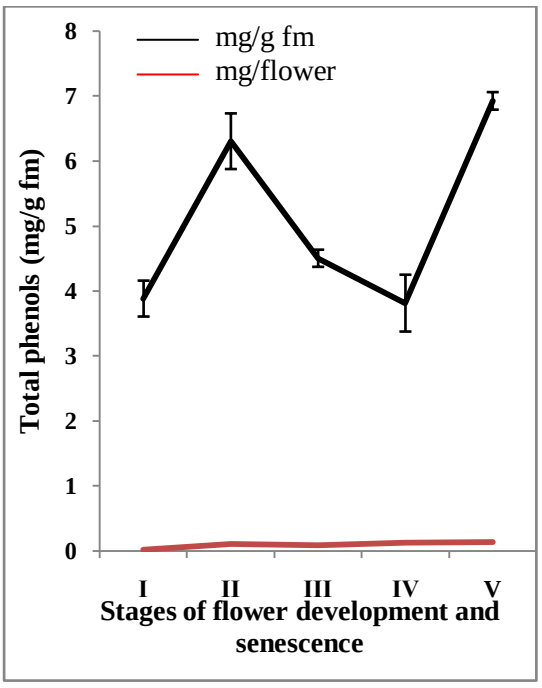

Fig. 6.1.7

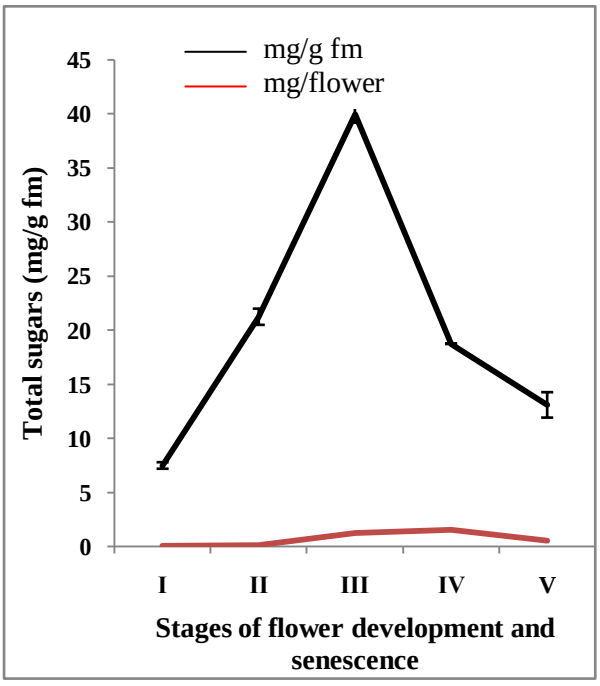

Fig. 6.1.8

Fig.6.1.5-6.1.8: Changes in protease activity, α-amino acids, total phenols and total sugars during different stages of flower development and senescence in *Silene armeria*. L.

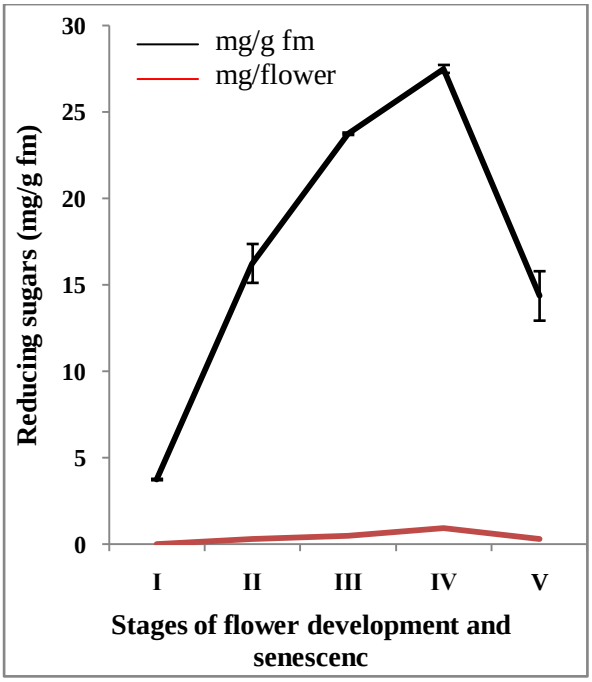

Fig. 6.1.9

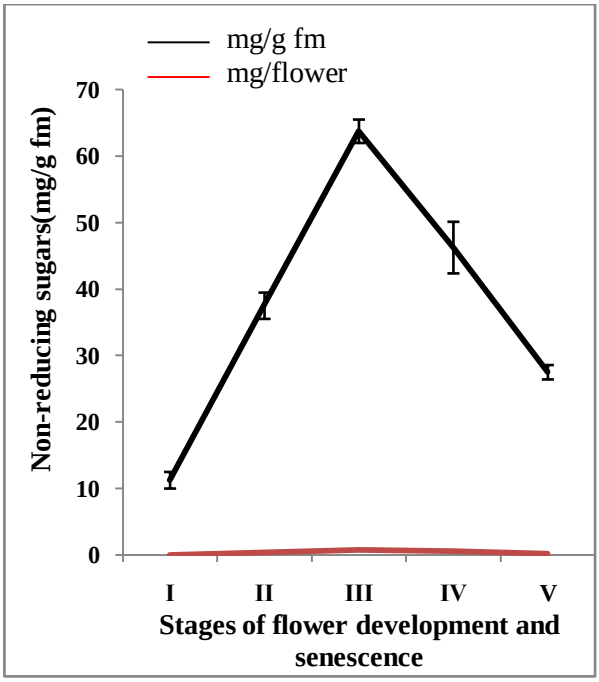

Fig. 6.1.10

Fig.6.1.5-6.1.8: Changes in reducing and non-reducing sugars during different stages of flower development and senescence in *Silene armeria.* L.

Plate 6.1.1

Stages of flower development and senescence in *Silene armeria* L.

Fig. a: From left to right are arranged five stages of flower development and senescence in *Silene armeria.* Stages I (Tight bud stage), II (mature bud stage), III (loose bud stage), IV (fully open stage) and V (senescent stage).

Fig. b: SDS-PAGE of equal amounts of extractable protein at various stages (I-VI) of flower development and senescence from petal tissue of Dianthus caryophyllus. The gel was stained with coomassie blue. Numbers above lanes correspond to developmental stages. Molecular weight standards are indicated on the left (kDa) and the ca molecular weights of major polypeptides to the right of the gel (kDa).

Fig. a: Stages of flower development and senescnce in *Silene armaria.*

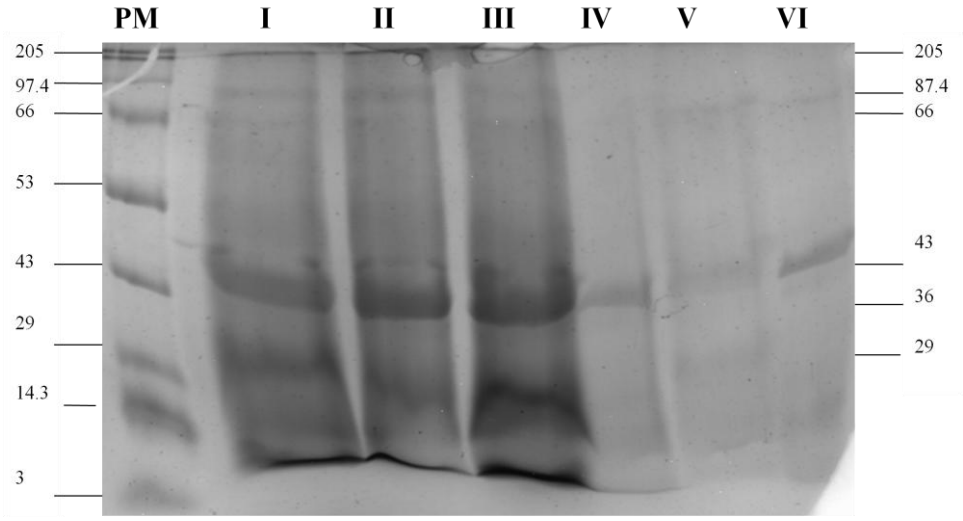

Fig. b: Electrophoretogram of stages of flower development and senescence in *Silene armeraia.*

Plate 6.1.1

CHAPTER-7

DISCUSSION

The present investigation was undertaken to elucidate the mechanism of flower/petal senescence with the fundamental aim to delay it and to devise a cost effective and eco-friendly handling and storage technique to improve the quality of some select ornamentals from Caryophyllaceae. Despite being a dicotyledonous family harbouring a number of flowering plant species, only a few species from Caryophyllaceae have ornamental value which include *Dianthus caryophyllus* (Pink), *D. barbatus* (sweet william), *Gypsophila* (baby's breath), *Agrostemma* (corn cockle), *Saponaria* (soapwort), *Lychnis* (firepink), and *Silene* (campions). During the present work, an attempt was made to study the mechanism of flower senescence in *Dianthus caryophyllus, Dianthus barbatus* and *Silene armeria*; besides, developing adequate postharvest handling and storage techniques to improve vase life of *Dianthus* caryophyllus and *Dianthus barbatus*.

Senescence in flowers/petals is a genetically programmed and developmentally regulated process and involves degradation of cellular biomolecules (Zhou *et al* 2005; Shibuya *et al.*, 2008 van Doorn and Woltering, 2008; Fisher, 2012). Flower senescence leads to the death of cells or organs regulated by an array of intrinsic and extrinsic cues. For the development of adequate postharvest techniques, studies pertaining to regulation of flower senescence is imperative. To achieve the aim of developing the adequate postharvest handling techniques, the mechanism and the factors regulating senescence are to be viewed. Ethylene is thought to be the main regulator of flower senescence among the plant growth regulators. Plants respond differently to ethylene and as such a distinction is made between ethylene sensitive and an insensitive flower system is made (Stead and van Doorn 1994; van Doorn 2001). The members of family Caryophyllaceae are generally considered to ethylene sensitive.

The flower systems studied during the present investigation showed petal wilting rather than the petal abscission to be important in flower senescence. It was followed by striking colour change (from pinkish red to yellow). The petals lost their turgidity, structural integrity and finally abscised leaving behind the pistil with a marked

increase in size finally changing into loculicidal capsule (fruit). Senesced flowers in all the three species remained persistent even after complete wilting and senescence.

During the present study on members of Caryophyllaceae the process of flower senescence was found to be associated with a decrease in fresh and dry mass of flowers, protein degradation accompanied by increased protease activity and a reduction in the pool of respiratory sugars. Besides an increase in the content of amino acids and total phenolics; a decrease in the cell protein content has been reported in various flower systems such as *Alstromeria, Petunia*, *Hemerocallis, Ranunculus* (van Doorn and Stead, 1994; Wagstaff, 2002; van Doorn 2008; Shahri and Tahir, 2011). During the present investigation it was found that decrease in the soluble protein content towards senescence was because of increased protease activity. The expression of transcript encoding proteases has been suggested to be one of the earliest senescence related gene changes (Eason 2002). The increase in protease activity during senescence has also been reported in the flowers of *Hemerocallis fulva, Iris germanica, Ranunculus asiaticus* and *Petunia hybrida* (Stephenson and Rubenstein, 1998; Shahri and Tahir 2011; Shahri *et al*., 2011).

In order to investigate the ultra structural changes during the various developmental stages in the flowers of *Dianthus caryophyllus* and *Dianthus barbatus*, the scanning electron microscopic studies were carried out. The petal tissues of *Dianthus caryophyllus* and *Dianthus barbatus* revealed a significant difference in the cellular architecture at different stages of development. The cells at stage IV look convex-faced, tightly packed, rounded in outline and were fully turgid. During senescence loss of cellular integrity can be visualised because the cells lose their convexity and turgidity as the flower development progressed towards senescence. Nevertheless, it is not possible to study and compile the complete sequence of events which characterize the process of senescence (Smith *et al.,* 1991). Our results corroborate with the earlier findings pertaining to ultra structural changes during the petal senescence of *Dianthus caryophyllus* (de Vetten and Huber, 1990; Smith et al., 1991). In *Dianthus caryophyllus* and *Dianthus barbatus* flowers the breakdown of cellular architecture would appear to limit complete mobilization, that would be the possible cause of

cellular disorganisation observed during the present investigation. Although evidence suggests that senescence is a highly regulated process, it may be that the combined activities of membrane associated lipoxygenase, phospholipases, phosphatidic acid, diacylglycerols and free fatty acids may contribute to increasing membrane vesiculation and destabilization as suggested earlier (Paliyath, Lynch and Thompson, 1987), so that ultimately an 'error catastrophe' befalls cells in spite of attempts to tightly regulate the process (Holliday, 1984).

SDS-PAGE of proteins from petal tissues of *Dianthus caryophyllus* and *Silene armeria* revealed a gradual decrease in both high and low molecular weight proteins; while as in *Dianthus barbatus*, SDS PAGE of proteins from petal tissues did not reveal any gradual decrease in the qualitative expression of proteins however some high molecular weight proteins were found to show differential expression during various stages (I-VI) of flower development and senescence. This corroborates with the finding on the flowers of *Ranunculus asiaticus* (Shahri and Tahir, 2011). Moreover, a polypeptide having molecular weight of approximately 14.3 kDa gets popped up during the late stages of senescence in *Dianthus caryophyllus*. But at the stage it is difficult to postulate whether this polypeptide has any role in flower senescence. The protein patterns clearly suggest that the regulation flower senescence is directly coupled with protein turnover and some proteins most oftenly called as suicidal or death proteins orchestrate petal senescence.

During the present course of investigation it was observed that the content of that reducing, non-reducing and total sugars showed a decrease as the flower development progressed towards senescence. These results corroborate with similar findings in *Hemerocallis fulva, Iris germanica, Ranunculus asiaticus*, rose and *Helleborus orientalis* (lay-Yee *et al.,* 1992; Sultan and Farooq, 1997; Mwangi *et al.*, 2003; Shahri and Tahir, 2011; Shahri *et al.*, 2011). An important reason of the decline in sugar levels may be the enhanced rate of respiration which results in rapid utilisation of the available sugar (Mwangi *et al*., 2003; Shahri and Tahir, 2011). Total phenolics content generally increased with the onset of senescence in all the three species. Mwangi *et al.* (2003) suggested that higher phenolic content is associated with longer vase life in cut roses. Present study revealed that the increase in the phenolics content during

senescence is indicative of cell death because of the increased membrane permeability. These results corroborate with the findings on *Helleborus orientalis* and *Cosolida ajacis* (Shahri and Tahir 2011; Shahri *et al* 2011).

Flowers being delicate are prone to postharvest losses and thus need to be handled with care. Once detached from the plant, cut flowers are deprived of food, minerals and hormones. Postharvest technology involves the steps taken for the improvement of the vase life of flowers. Vase life and flower longevity are the determinant factors for the expression of potential life of cut flowers. The termination of the vase life is marked by the wilting or abscission of individual petals or the whole flower. Preharvest and harvest conditions of cut flowers have a direct bearing on the postharvest quality and longevity of flowers (Singh *et al*., 2001). Being an important attribute, postharvest longevity is an important quality in agricultural products and it can satisfy not only distributors but also consumers. Vase life is a quality parameter to estimate the longevity of cut flowers and longer vase life is an important objective for maintaining flower quality either by chemical treatment or plant breeding. Petal senescence in mature flowers is a contributing factor towards to vase-life as has been emphasized by various workers (van Doorn and Woltering, 2008). Postharvest physiology deals with the functional processes in intact plants or plant parts after these have been harvested and kept in storage for marketing (Yamada *et al*., 2003). Postharvest physiology spans over the time period from harvest or removal of plant from its normal growing environment to the time of its ultimate utilization (Stanley, 1991). Various factors pertaining to preharvest, harvest and postharvest have a direct influence on postharvest attributes and as such these factors are considered to be vital components of postharvest utilization of cut flowers and foliage. Postharvest quality characteristics most importantly include flower longevity, changes during handling, storage, packaging, transportation and marketing operations. As far as cut flowers are concerned, the flower size, condition, maturity, shape; besides size and shape of spikes at postharvest are regarded to be important attributes which determine their display value and hence the acceptability of the product to the retail buyer and to the ultimate consumer. The handling, storage, transportation and marketing of plants and plant parts has therefore been one of the major preoccupations of human societies in

this regard. But much of the harvested plant material never reaches the point of utilization and is discarded because of deterioration either due to natural senescence, stress responses, pathogen activity or even due to lack of proper storage and transport facilities (Singh *et al.*, 2001; Shahri and Tahir, 2010a; Shahri, and Tahir, 2011a).

As long as flowering shoot or an inflorescence is attached to a healthy mother plant, nutrients are continuously supplied by the plant leading to normal development. After a flowering shoot or inflorescence is detached from the plant, the supply of nutrients is cut off and the physiological events leading to senescence are hastened. Therefore, the thermal or chemical regulation of senescence in enhancing the vase life of cut flowers assumes considerable significance in ornamental horticulture (Gul *et al*., 2007; Gul and Tahir, 2009; Shahri and Tahir, 2010a). The developmental events from bud into bloom are associated with considerable mobilization of metabolites towards blooms. It has been reported that flower opening in many species is accompanied by the mobilization of storage carbohydrates and the import of sucrose (Bieleski *et al*., 2000). The young petals of many species have been shown to contain considerable amount of starch which, shortly before opening is rapidly hydrolyzed to glucose and fructose by the enzyme machinery (Hammond, 1982; Collier, 1997; Bieleski *et al*., 2000). The exogenous supply of sugars is necessary for sustained development and opening of cut flowers (Halevy and Mayak, 1979). The exogenous application of sugar supplies the flower with much needed substrates for respiration and not only prolongs vase life but enables cut flowers harvested at the bud stage to open, which otherwise could not occur naturally (Pun and Ichimura, 2003). Sucrose has been reported to increase the vase life of certain cut flowers like *Iris, Nerine, Narcissus* (Gul and Tahir, 2009; Gul and Tahir; 2012). In the present study, sucrose (0.05M) alone or in combination improves the postharvest performance of cut spikes *Dianthus caryophyllus* and *Dianthus barbatus* along with isolated flowers of *Dianthus caryophyllus*.

In the present discourse, different sets of storage experiments were conducted on cut spike of *Dianthus caryophyllus* and *Dianthus barbatus.* The spikes were stored under dry or wet conditions at three different temperature regimes (5, 10°C and Room

temperature (RT=22±2ºC) for 72 h and then assessed for their postharvest performance in vase solutions containing either distilled water or sucrose (alone or in combination with CHI). The postharvest performance of cut spikes, wet stored at 5°C was found to be comparatively much better as compared to the corresponding spikes dry or wet stored at 10°C and RT in case of *Dianthus caryophyllus*. However, in case of *Dianthus barbatus* spikes dry stored for 72 h was comparatively much better than the spikes previously dry stored at 10°C and Room temperature (RT=22±2). The deleterious effects of dry storage at cool temperatures (including chilling injury, spike bending, bud abortion, petal curling, and colour change) as reported earlier in plants such as *Curcuma alismatifolia*, *Amaryllis belladonna* were also observed during the present investigation in cut spikes of *Dianthus caryophyllus* transferred to SUC (Bunya-Atichart *et al*., 2004; Gul *et al*., 2007; Shahri and Tahir, 2011a). Temperature is considered to be an important abiotic factor which can influence the respiration rate, response to ethylene, moisture loss and physical damage in various flowers (Cevallos and Reid, 2001; Leonard *et al*., 2001; Celikel and Reid, 2002; Gul *et al*., 2007). Low temperature is the most important factor in the successful storage of cut flowers by reducing both metabolic processes and microbial growth rates; besides delaying the symptoms of senescence through regulation at biochemical level (van Doorn and de Witte, 1991; Page *et al*., 2001). In the spikes of *Dianthus caryophyllus*, a set of storage experiments was carried out under wet conditions at 5°C before, during and after pretreatment with 0.1mM cycloheximide (CHI). The postharvest performance was significantly improved in spikes pretreated with CHI after 72 h wet storage at 5°C as compared to untreated spikes. We suggest that this significant improvement in the postharvest life of the spikes in *Dianthus caryophyllus* may be due to the synergistic effect of cool temperature and the protein synthesis inhibitor such as CHI. CHI has previously been found to delay senescence *Hemerocallis, Gladiolus, Narcissus, Ranunculus, Hemerocallis* and *Iris* (Wulster *et al*., 1982; Lukaszewski and Reid, 1989; Jones *et al*., 1994; van Doorn *et al*., 1995; Sultan and Farooq, 1997; Shahri and Tahir, 2010; Islam *et al*., 2011; Gul *et al*., 2012; Gul and Tahir, 2012). The ability of cycloheximide to delay senescence of flowers has been attributed to the fact that senescence requires active gene expression and *de novo* protein synthesis (Dilley and

Carpenter, 1975; Wulster *et al*., 1982; Lukaszewski and Reid, 1989; Lay-yee *et al*., 1992; van Doorn *et al*., 1995; Williams *et al*., 1995). Sultan and Farooq (1997) have reported that CHI while delaying flower senescence maintained a higher protein content in the perianth tissue of *Iris* flowers as compared to untreated flowers suggesting selective degradation of certain proteins to be an important factor contributing to the senescence of flowers. It has been suggested that CHI maintained a higher protein content presumably by inhibiting the synthesis of some specific proteases responsible for protein degradation (Celikel and van Doorn, 1995; Sultan and Farooq, 1997). Our results corroborate with the findings of Gul and Tahir (2012), who proposed that pulse treatment of spikes with CHI maintained higher fresh and dry mass of flowers, with decreased protein content and increased α-amino acids besides improving postharvest performance.

In the present study, the influence of inhibitors of ethylene synthesis (AOA) or ethylene action (STS) were also evaluated on the isolated flowers of *Dianthus caryophyllus* to assess their sensitivity to ethylene and action on their longevity. Silver ions have been found to be efficient in blocking the action of ethylene in various flowers especially in the form of silver thiosulphate (STS). A treatment with STS has been found to prolong the vase life of many cut flowers such as *Consolida, Petunia, Lathyrus* and *Delphinium* (Borochov *et al*., 1997; Ichimura and Hiraya, 1999; Kikuchi *et al*., 2003; Finger *et al*., 2004). Likewise pretreatment of flowers with AOA has been found to be effective in reducing respiration, ethylene production and increasing the vase life (Goh *et al*., 1985; Woltering, 1987; Bichara and van Staden, 1993; Porat *et al*., 1995a; Uda *et al*., 1997). van Altvorst and Bovy (1995) suggested that AOA inhibits the expression of ACC synthase, the enzyme responsible for the conversion of S-adenosyl methionine (SAM) to 1-aminocyclopropane-1- carboxylic acid (ACC). In the present study, ethylene antagonists (AOA and STS) were found to be effective in enhancing the longevity of isolated flowers of *Dianthus caryophyllus.* These results corroborate with the finding of Marandi *et al*. (2011) that pretreatment of STS in combination with sucrose improves the vase life of cut Gladiolus flowers. In addition, Zemin *et al*. (2001) showed that the pulsing treatment with sucrose and STS prolonged the vase life of Gentian flowers compared with the water control. They

suggest that sucrose and STS act similarly at least on soluble sugar changes and ethylene production that are associated with inhibiting flower senescence. However, among these two ethylene antagonists, AOA was found to be more effective than STS. This finding is in contrast to the finding of Wawrzyńczak and Goszcyńska (2003), who proposed that STS was the most effective in enhancing the longevity of *Dianthus caryophyllus* flowers. Fujino *et al*., (1980) found that *Dianthus caryophyllus* flowers when continuously kept in a solution containing AOA, the production of ethylene was suppressed and wilting was delayed.

During the present course of investigation the present study, it seems to be evident that protein turnover (degradation and synthesis) plays a central role in all the plants under study and that the loss of membrane integrity, decline in fresh and dry mass or reduction in the pool of respiratory sugars, being its important consequences. The observation that cycloheximide delays senescence in this ethylene sensitive flower system (*Dianthus caryophyllus*) confirms the fact that it could be used as effective modulator of senescence to study the expression of senescence-associated proteins and that it has a lot of potential to chemically regulate the vase life of these flowers.

The study can be undertaken at the molecular level so that the role of protein synthesis inhibitors in orchestrating the regulation of death proteins can be elucidated and subsequently modulation programmes can be standardized to improve the flower life in these important and beautiful cut flowers. The study although of a preliminary nature, identifies a potential area of investigation in these flower systems and can be pursued as a viable research programme.

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