CHAPTER 3
PHYSIOLOGICAL CHANGES OF POLLINATED *DENDROBIUM* POMPADOUR

3.1 INTRODUCTION

Among the earliest observations made on pollination-induced senescence (PIPS) in orchid flowers were done by Conrad Sprengel in 1983 (Arditti, 1979) followed by a number of scientists including Darwin in the late 1900s. However, the most significant documentation was by Hans Fitting who in 1921 was the person to use the word "hormone" with regards to the regulation of post pollination senescence in *Phalaenopsis* (for reviews see Arditti, 1971, 1975). Since then it has continued to receive great attention. Many literature have focused on different aspects of PIPS in flowers ranging from the relationship of pollinators and flowers to the post-pollination events that follows (van Doorn, 1997,2002; Yu and Goh, 2001).

In most flowers where pollination results in senescence such as digitalis (Stead and Moore, 1979), carnations (Larsen *et al.,* 1995), petunias (Xu and Hanson, 2000) and orchids (Ketsa and Rugkong, 2000), physiological changes erode the attractiveness of the flowers and eventually lead to death. They include perianth withering and hyponasty, colour change, thinning of petal, water and weight loss. In most flowers, senescence is characterized by the in-rolling of petals that eventually results in permanent flower closure.

Permanent flower closure has been documented as a distinct characteristic of pollination- induced senescence in different species of orchids such as in *Arachnis* (Hew *et al.,* 1989), *Phalaenopsis* (Porat *et al.,* 1994) and *Dendrobium* (Ketsa and Luangsuwalai, 1996; Chandran *et al.,* 2007).

Ethylene production in these flowers is that of a climacteric pattern where a burst of ethylene coincides with the onset of petal senescence. Work done by Woltering *et al.,* (1995) elicited that ethylene expression was modulated from the receptive stigma and subsequently translocated to the petals and the sepals. The most obvious physiological change observed in *D.* Pompadour is the complete closure of the perianth. Alongside this movement are other physiological changes such as weight loss, thinning of petals and discolouration.

Studies on the physiological changes that occur post pollination in flowers have reported that changes are similar to that of ageing (Doorn and Woltering, 2008; Jones *et al.,* 2009; Attri and Nayyar, 2011). However, pollination accelerates these processes and significantly shortens the vase life and severely affects the quality of the flowers when compared to unpollinated flowers. All these changes can be attributed to the production of ethylene, a hormone that is also implicated in the process of ripening in fruits. Furthermore, carbohydrate content and water balance in pollinated flowers also undergo an accelerated rate of decrement, adversely affecting the quality of flowers such as fresh weight and thickness of petals (Ferreira *et al.,* 2010).

Pollination-induced senescence can be circumvented by blocking the production of pollination-induced ethylene and suppressing the sensitivity of flowers towards ethylene (Liao *et al.,* 2000). Ethylene inhibitors have been extensively studied and proven to be effective in prolonging vase life of cut flowers. Compounds such as 1-aminooxyacetic acid (AOA) and aminoethoxyvinylkglycine (AVG) effectively delay senescence of climacteric flowers by inhibiting the action of 1-aminocyclopropane-1 carboxylate synthase (ACCS) On the other hand, compounds such as silver thiosulphate (STS) and 1-methylcyclopropane (1-MCP) competes with ethylene by binding to ethylene receptor thus blocking the signaling pathway. These chemicals have been widely applied to orchid flowers as well as other flowers such as carnations, roses, orchids, and snapdragons (Serek *et al.,* 2006).

Supplying cut flowers with exogenous sugars have proven to prolong vase life of many cultivars; roses, carnations and orchids (Ichimura *et al.,* 2002). Furthermore, the application of sugar has been reported to improve the physiological aspects of flowers which include fresh weight, water balance and. It has been speculated that exogenous sugar can also play a role in ethylene signal transduction (Leon and Sheen, 2003). In fact the use of sugars in ethylene sensitive flowers has been shown to suppress sensitivity to ethylene at the transcription level.

The aim of the experiments carried out in this chapter is to profile the morphological and physiological changes that occur during pollination-induced senescence of *D.* Pompadour and the effects of treatment solutions on this phenomenon.

3.2 MATERIALS AND METHODS

3.2.1 Plant Material

Dendrobium Pompadour flowers were obtained from the glasshouse in *Rimba Ilmu*, University of Malaya. Flowers harvested were aged 10-15 days after anthesis. Individual flowers were cut at the proximal end of the peduncles in water and placed in 20 ml water vials containing distilled water or treatment solutions. Flowers were self-pollinated by placing the pollinia onto the stigma. Ten flowers were pollinated and another ten remained unpollinated (control). Physiological and morphological changes were observed and recorded from the beginning of the experiment (day 0) until the flowers died. To show the visual changes, photographs were taken at different stages of flower senescence. Experiments were maintained at room temperature (24 ± 3^{o} C). All experiments were carried out in triplicates.

3.2.2 Treatment Solutions

Pollinated flowers were placed in vials containing 20 ml of treatments solution. Sucrose and glucose solutions were tested at 2% (w/v), 4% (w/v) and 6% (w/v) (Rattanawisalanon *et al.,* 2003). 0.1mg l^{-1} chloramphenicol was added to the sugar solutions as an antimicrobial agent. AOA solutions were used at 0.1, 0.05 and 0.025 mM (Rattanawisalanon *et al.,* 2003) while STS solutions of 0.2, 0.4, 0.6, 0.8 and 1.0 mM (Roein *et al.,* 2009) were used. All solutions were prepared at the beginning of the experiments and were not renewed.

3.2.3 Ethylene Measurement

Individual, weighed flowers were placed in 30 ml Corex tubes covered with air tight rubber stoppers. The tubes were sealed for two hours daily and 1 ml gas samples were withdrawn from the tubes for ethylene determination by Hewlett-Packard 5890 Gas Chromatograph fitted with a Flame Ionization Detector (FID). Helium was used as a carrier gas and the oven temperature was kept at 120°C. Ethylene, 0.04 ppm was used as standard. Production of ethylene was calculated as $\mu l\ h^{-1}g^{-1}$.

3.2.4 Morphological and Physiological Observations

3.2.4.1 Flower senescence

Levels of senescence were determined visually and described in terms of five stages.

Stage I: Flowers are fresh, open and turgid. Colour of the perianth appears bright.

Stage II: Upward movement of the perianth begins.

Stage III: Perianth is fully close.

Stage IV: First signs of venation are visible.

Stage V: Browning and necrosis are visible. Colour fades and perianth appears transluscent.

3.2.4.2 Colour measurements

The colour of the flowers was measured using a chromameter (Minolta CR-200). Measurements were done by placing the centre of the petals on the stage of the chromameter (Chandran *et al.,* 2005). The L*a*/b* colour system closely represents human sensitivity to colour. Thus, it can be used to measure the perceived colour of the flowers as they senesce. Lightness (brightness), hue (shade of colour) and chrome

(colour saturation or vividness) are values of L*, a* and b* respectively. The L*a*/b* value is obtained using the formula:

$$\frac{L^* \times a^*}{b^*}$$

3.2.4.3 Petal thickness

Thickness of petals was measured using a micrometer (Mitutoyo). Measurements were taken by placing the petal between the anvil and the spindle of the micrometer. Care was taken while measuring to ensure that no injury is inflicted onto the petal.

3.2.4.4 Water uptake and flower fresh weigh

The difference between consecutive weighing of the vials plus solution (without the flower) was used to determine water uptake. Vials were covered with aluminium foil and the flowers were inserted through a small hole into the vials. Flowers were weighed daily to measure their fresh weight.

3.2.4.5 Water loss

After determining their fresh weight (FW), the flowers were oven dried at 80^{o}C until they reached a constant dry weight (DW). Water content was determined by subtracting the DW from the FW. Water loss was finally expressed as percentage.

3.2.5 Statistical Analysis

Experiments were carried out in triplicates where n= 30. Standard error for means of the measurements was computed using Excel (Microsoft Office) and measurements were statistically tested using the Duncan's Multiple Range Test by an SPSS programme (Duncan, 1955).

3.3 RESULTS

Following pollination, rapid changes to the flower appearance occurred and five distinct stages of senescence were observed and determined (Fig 3.1). At Stage I, the flowers were completely open and petals turgid, with the perianth showing deep purple colour. At stage II, the upward movement of the petal was observed. This was the onset of flower closure. At stage III, the perianth reached full closure. At this stage, vase life was considered as terminated as deemed commercially. At stage IV, at a more advanced stage of senescence after full flower closure, the perianth showed apparent venation and discoloration. Owing to the drying out of the tissue, the veins on the petals of the pollinated flowers were clearly visible. At stage V, the perianth showed browning, shriveling and necrosis.

Table 3.1 shows the vase life of unpollinated and pollinated flowers held in distilled water. Unpollinated flowers maintained a longer vase life, reaching Stage III only after 19 days. In contrast, pollinated flowers showed a drastic reduction of vase life of only two days. This translated into an approximate 90% loss in vase life when *D.* Pompadour flowers were pollinated.

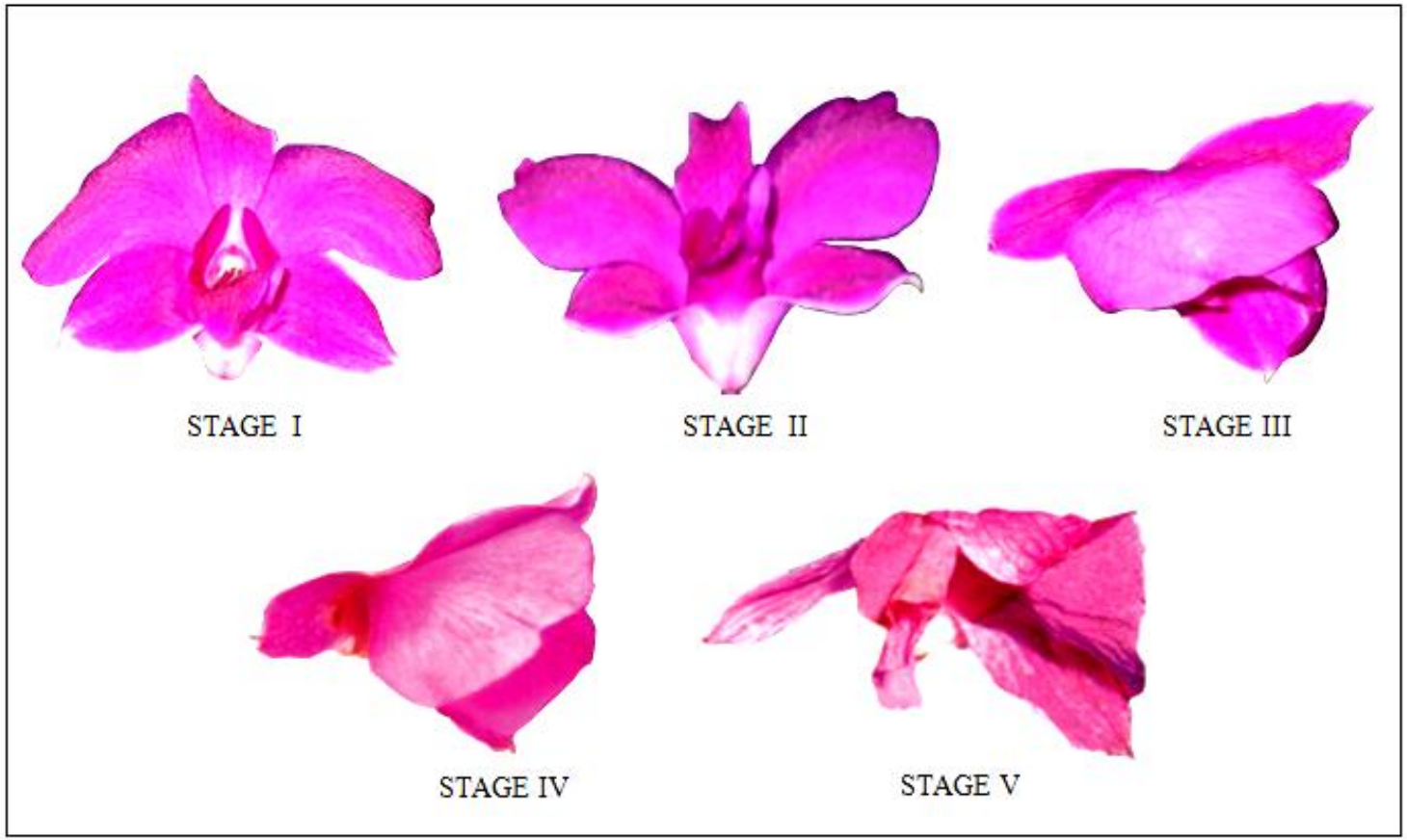

Fig 3.1: Flower appearance observed in pollinated *Dendrobium* Pompadour. Five distinct stages were identified from fresh open to fully closed, shrivelled flowers. A total of 30 flowers were used for this observation.

Table 3.1 Stages of senescence and vase life of unpollinated and pollinated *D.* Pompadour. Pollinated flowers reached full closure after 2 days compared to 19 days for unpollinated flowers. Values are shown with SE where n=30.

	Stage I	Stage II	Stage III	Stage IV	Stage V	Vase Life
Unpollinated	Day 0	Day 17±0.7	Day 19±0.8	Day 22±0.5	Day 24±0.8	19 days±0.8
Pollinated	Day 0	Day 1±0	Day 2±0.4	Day 5±0.9	Day 7±1.2	2 days±0.4

Pollination in *D.* Pompadour induced production of ethylene. Ethylene production was detected as early as 6 hours after pollination and continued to increase with a peak production of 12.28µl $g^{-1}h^{-1}$ at 12 hours and gradually decreased thereafter. At 28 hours after pollination, no ethylene was detected. On the other hand, unpollinated flowers produced no detectable ethylene throughout the observation.

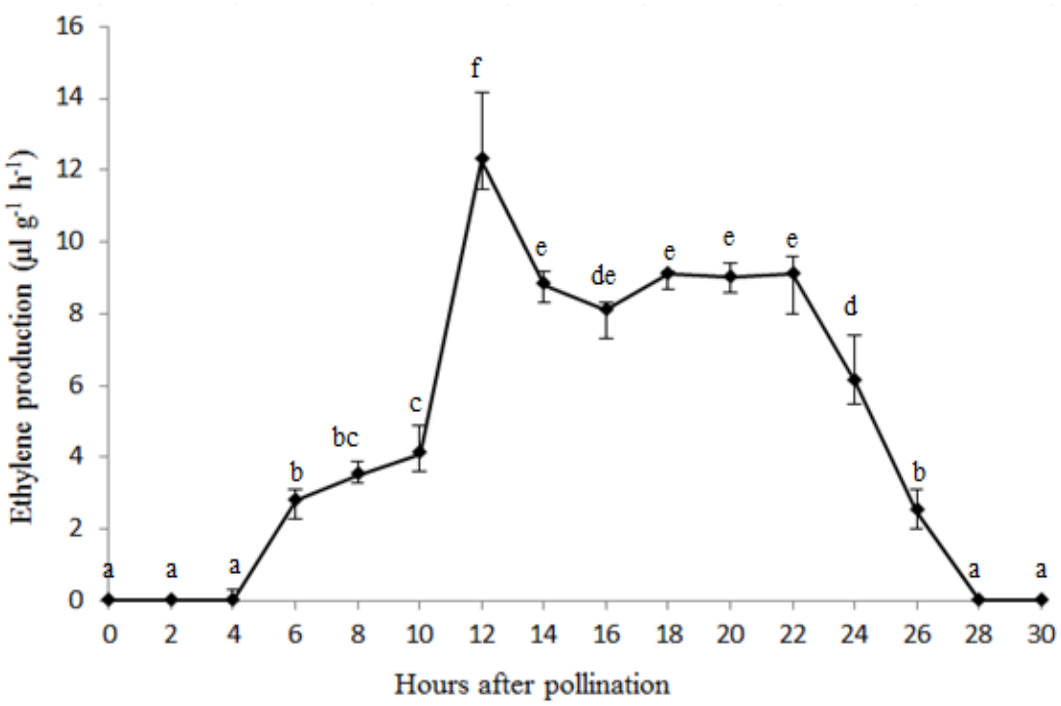

Figure 3.2: **Ethylene profile of pollinated *D.* Pompadour**. Peak ethylene production was observed 12 hours after pollination ethylene was not detected in unpollinated flowers.

Table 3.2 shows that treatment with ethylene inhibitors proved to be more effective in extending the vase life of pollinated flowers. Among the three different concentrations of glucose solutions, 4% glucose was most effective, extending vase life more than four folds. 2% sucrose treatments proved to be the best concentration of sucrose to use in extending vase life resulting in similar results with 4% glucose. The rest of the sucrose concentration did not give any positive results. Treatment with AOA resulted in two to six folds of vase life extension. AOA solution with a concentration of 0.05 mM was chosen as the best AOA treatment as it successfully extended the vase life to 13 days. Finally, STS treatments extended vase life of flowers ranging from three to four folds. 0.6 mM STS was chosen as the best STS treatment as it resulted in extension of vase life of up to nine days.

Table 3.2: Vase life of *Dendrobium* Pompadour flowers held in distilled water and different treatment solutions. As observed from the table above, some concentrations of treatment solution did not yield positive results while other concentrations of treatment solutions resulted in successful extension of vase life ranging from approximately 4 to 13 days. Treatment solutions printed in bold are the most effective results for each treatment solution which will be used for further analysis. Means within the column not sharing the same letter were significantly different at *P=0.01* by Duncan's Multiple Range Test (DMRT).

Treatment	Vase life (days)
Distilled water	2.0a
Chloramphenicol	2.0a
2% Glucose + CHP	2.4ab
4% Glucose + CHP	**4.7de**
6% Glucose + CHP	2.5b
2% Sucrose + CHP	**4.7de**
4% Sucrose + CHP	2.0a
6% Sucrose + CHP	2.0a
0.025 mM AOA	4.1c
0.05 mM AOA	**13.2gh**
1.0 mM AOA	2.0a
0.1 mM AOA	3.7bc
0.2 mM STS	2.2ab
0.4 mM STS	2.5ab
0.6 mM STS	**9.5g**
0.8 mM STS	6.8def
1.0 mM STS	6.2de

*CHP= chloramphenicol

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Figure 3.3 shows the L*a*b changes in pollinated, unpollinated and treated flowers. The L*a*b values increased as the colours of the flowers fade from a dark purple to light purple. In pollinated flowers, loss in colour was hastened and more rapid compared to unpollinated flowers as reflected by the early increase in L*a*b value. Colour loss in pollinated flowers was -62.55 at the beginning of the experiment, began increasing to -58.3 at day 3 and by day 8 L*a*b value reached -18.5. In contrast, very little change in L*a*b value was measured in the unpollinated flowers. The L*a*b value for unpollinated flowers was -64 at the beginning of the experiment and reached -54.2 at the end of the experiment. This meant that the increase in L*a*b value of pollinated flowers was three times more than unpollinated flowers.

Increase in L*a*b values in treated flowers were lower than pollinated flowers held in distilled water. However, treatment in 0.05 mM AOA and 0.6 mM STS proved to be more effective in maintaining flower colour compared to 2% sucrose and 4% Glucose. Furthermore, the trend of colour change in flowers held in 0.05 mM AOA and 0.6 mM STS was similar to unpollinated flowers. Flowers held in 0.05 mM AOA began with an L*a*b value of -62 and by the end of the experiment, L*a*b value was reached -50.6. Flowers held in 0.6 mM AOA had an L*a*b value of -62 at the beginning of the experiment and -53.4 at the end of the experiment. Flowers treated with 2% sucrose showed a reading of -61 at the beginning of the experiment and -40.5 at the end of the experiment. Lastly, flowers held in 4% glucose had an L*a*b value of -62.3 in the beginning of the experiment and -33.8 at the end of the experiment.

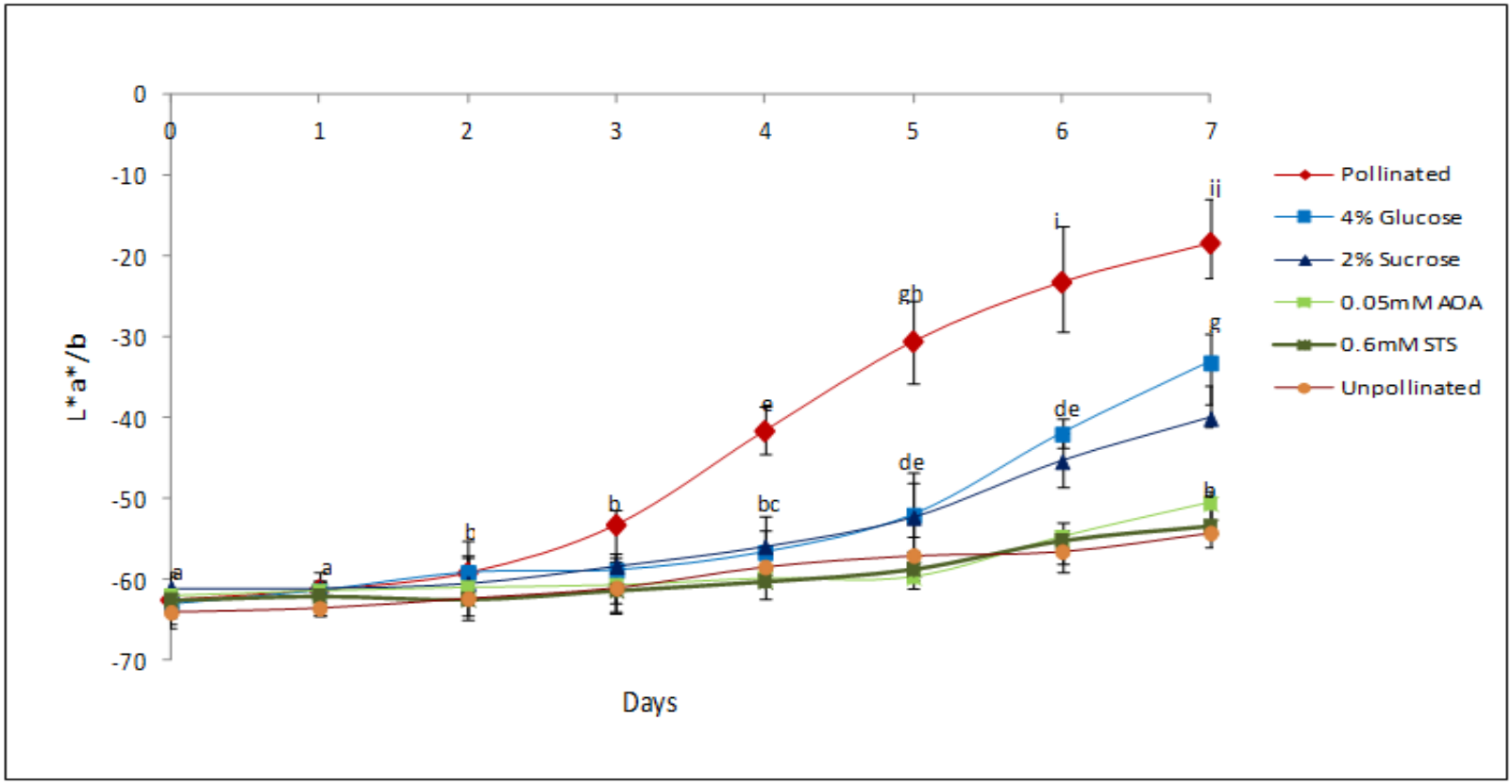

Figure 3.3 L*a*b values of unpollinated, pollinated and treated *D.* Pompadour flowers. Each value is the mean ± SE (n = 30), and values with different letter(s) are significantly different according to the Duncan's Multiple Range Test ($P < 0.05$).

Loss in petal thicknessss was observed in all flowers albeit at different rates, most apparent and rapid in pollinated flowers (Fig 3.4). Loss in petal thickness for pollinated flowers held in distilled water was 5% and continued rapidly to reach a percentage of 58% at the end of the experiment. On the contrary, 1.5% of loss was observed in unpollinated flowers in the beginning of the experiment, and by the end of experiment a total loss in thickness of 15% was recorded. This was less than half of what was observed in pollinated flowers held in distilled water.

Treatment with ethylene inhibitors and sugars also proved to be effective in reducing loss in petal thickness. Flowers held in 0.05 mM AOA and 0.6 mM STS were better in reducing loss in thickness compared to 2% sucrose and 4% glucose with a total loss of 18% and 20% respectively by the end of the experiment. Meanwhile, total loss in petal thickness for flowers held in 2% sucrose and 4% glucose was 35% and 38% respectively.

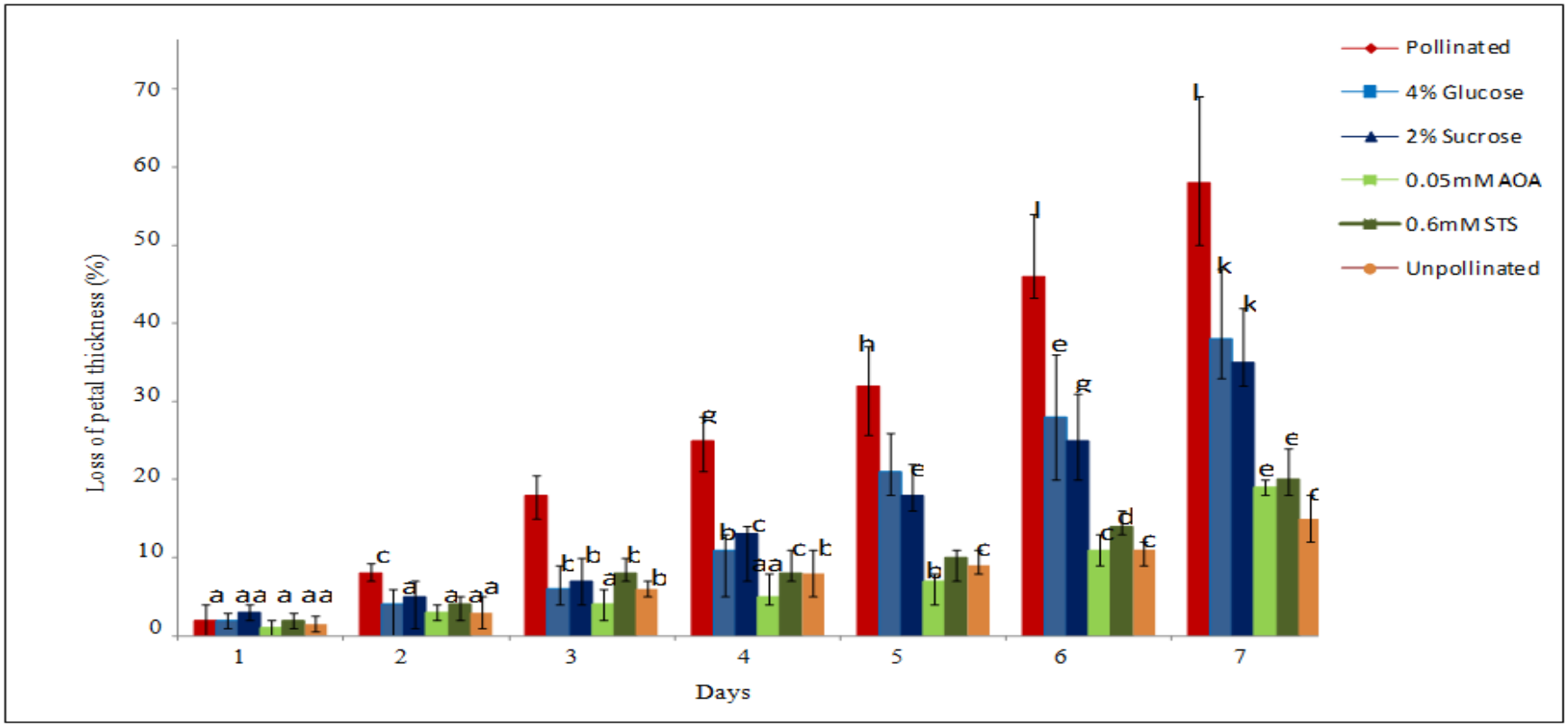

Figure 3.4 Loss in petal thickness of unpollinated, pollinated and treated *D.* Pompadour flowers. Each value is the mean ± SE (n = 30), and values with different letter(s) are significantly different according to the Duncan's Multiple Range Test ($P < 0.05$).

Water uptake declined rapidly in flowers held in distilled water compared to unpollinated and treated flowers (Fig 3.6). An obvious decline in water uptake in pollinated flowers held in distilled water was observed after day 2 when water uptake reduced from 0.36 ml to 0.33 ml. The rapid decline continued thereafter and by the end of the experiment water uptake was a mere 0.01 ml. In contrast, unpollinated flowers showed a higher and more stable water uptake compared to pollinated flowers. From day 1 to day 3 no significant reduction in water uptake was recorded. On day 4, a small reduction was recorded and by the end of the experiment water uptake in unpollinated flowers was 0.32 ml compared to 0.36 ml in the beginning of the experiment.

Treatments with ethylene inhibitors and sugars managed to improve water uptake in pollinated flowers. Treatments with 0.05 mM AOA and 0.6 mM STS were more effective in maintaining water uptake compared to 4% glucose and 2% sucrose and showed a trend similar to that of unpollinated flowers. At the beginning of the experiment, water uptake was 0.34 ml and 0.36 ml respectively for 0.05 mM AOA and 0.6 mM STS. By the end of the experiment, flowers held in the two treatments showed water uptake of 0.26 ml and 0.28 ml. Meanwhile in flowers held in 4% glucose and 2% sucrose an obvious decline in water uptake was evident after day 4. This trend continued and by the end of the experiment water uptake for 4% glucose and 2% sucrose was 0.14 ml and 0.18 ml respectively.

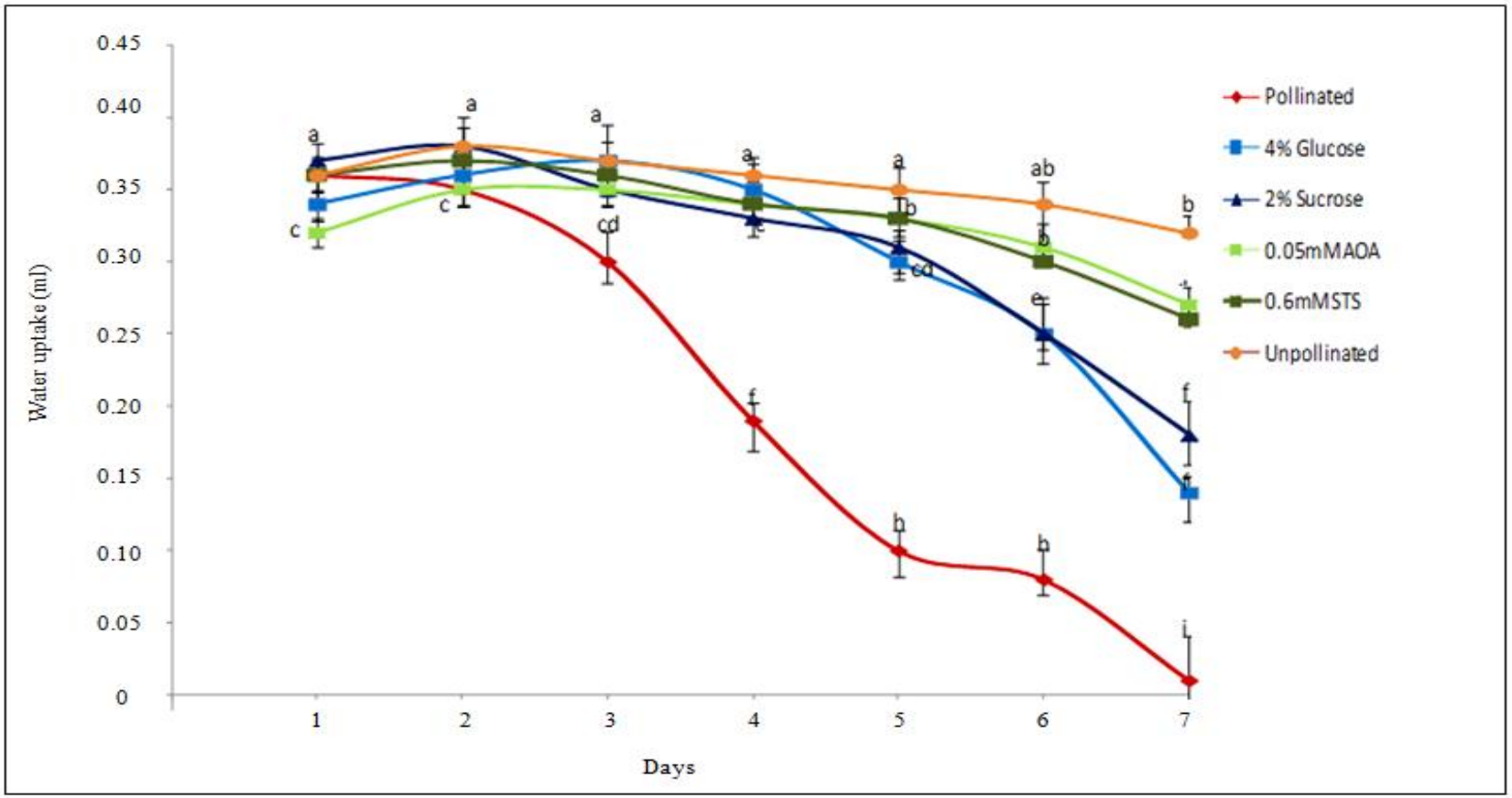

Figure 3.5 Water uptake of unpollinated, pollinated and treated *D.* Pompadour flowers. Each value is the mean ± SE (n = 30), and values with different letter(s) are significantly different according to the Duncan's Multiple Range Test ($P < 0.05$).

Loss in fresh weight was observed in pollinated, unpollinated and treated flowers at varying degrees. The most rapid loss occurred in pollinated flowers held in distilled water. Loss in fresh weight began on day 2 at 11.92% and continued rapidly to reach 50.9% at the end of the experiment. On the contrary, a much lesser loss in fresh weight was observed in unpollinated flowers. On day 2, a 2.9% loss was recorded and this value fluctuated until day 5. By the end of the experiment, fresh weight loss was in unpollinated flowers was 8.4%. This meant that pollinated flowers held in distilled water experienced fresh weight loss which was approximately 6 folds more than that of unpollinated flowers.

Treatments using ethylene inhibitors and sugars also managed to reduce fresh weight loss in pollinated flowers. However, 0.05 mM AOA and 0.6 mM STS were more effective compared to the 4% glucose and 2% sucrose in maintaining fresh weight of pollinated flowers. In flowers treated with 0.05 mM AOA, 3.3% of loss in fresh weight began on day 3 and by the end of the experiment loss recorded was 10.6%. Flowers treated with 0.6 mM STS lost 1.8% fresh weight beginning of day 2 and ended with 12% fresh weight loss at the end of the experiment. These weight losses were approximately 5 folds less than untreated pollinated flowers. Meanwhile, flowers held in 4% glucose and 2% sucrose recorded initial weight loss on day 3 with 37% and 28% respectively. By the end of the experiment fresh weight loss percentage recorded was 37% for flowers held in 4% glucose and 28% for flowers held in 2% sucrose.

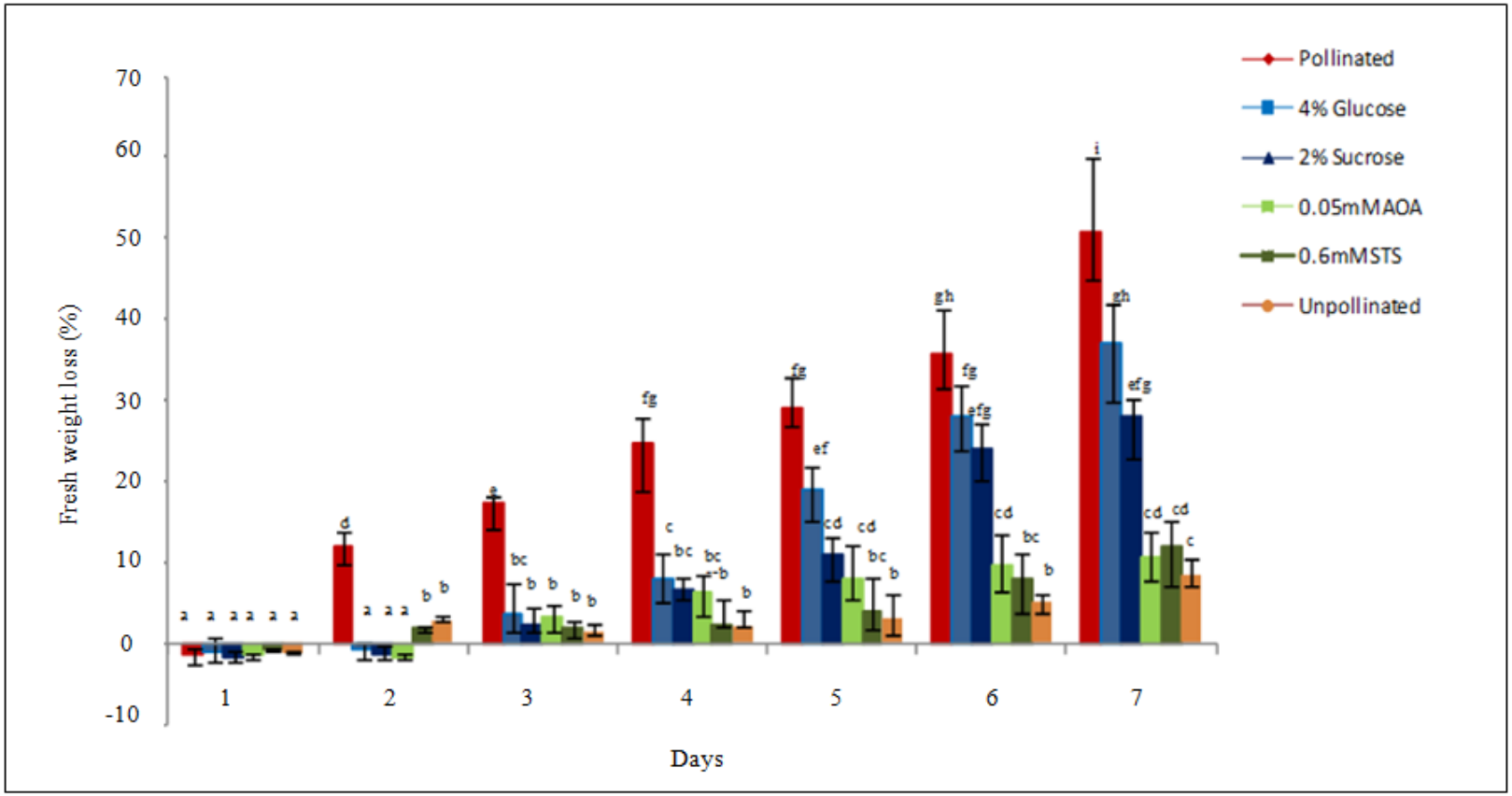

Figure 3.6 Fresh weight loss in unpollinated, pollinated and treated *D.* Pompadour flowers. Each value is the mean ± SE (n = 30), and values with different letter(s) are significantly different according to the Duncan's Multiple Range Test ($P < 0.05$).

Pollinated flowers of *D.* Pompadour exhibited loss in water content beginning day 2 after pollination. Water loss continued to occur and on day 7, more than 50% of water was lost in pollinated flowers. In contrast, little water was lost in unpollinated flowers. From the 1st to the 5th day after pollination less than 5% of water was lost. On day 7, unpollinated flowers lost a total of 11% of water. Pollinated flowers therefore showed approximately five folds more water loss than unpollinated flowers.

Treatment solutions showed positive effects in reducing percentage of water loss in pollinated *D.* Pompadour blossoms. Pollinated flowers held in 0.05 mM AOA and 0.6 mM STS showed trends of water loss similar to that of unpollinated flowers. By the end of the experiment water loss in 0.05 mM AOA and 0.6 mM STS were 14% and 13% respectively. 4% glucose and 2% sucrose also had some positive effects on water loss albeit not as effective as 0.05 mM AOA and 0.6 mM STS. On day 4 after pollination, water loss in flowers held in 4% glucose was 6.3% while in 2% sucrose water loss was recorded at 5.5%. Subsequently water continued to be lost and by the end of the experiment total water loss was 30% and 34.8% in 4% glucose and 2% sucrose respectively.

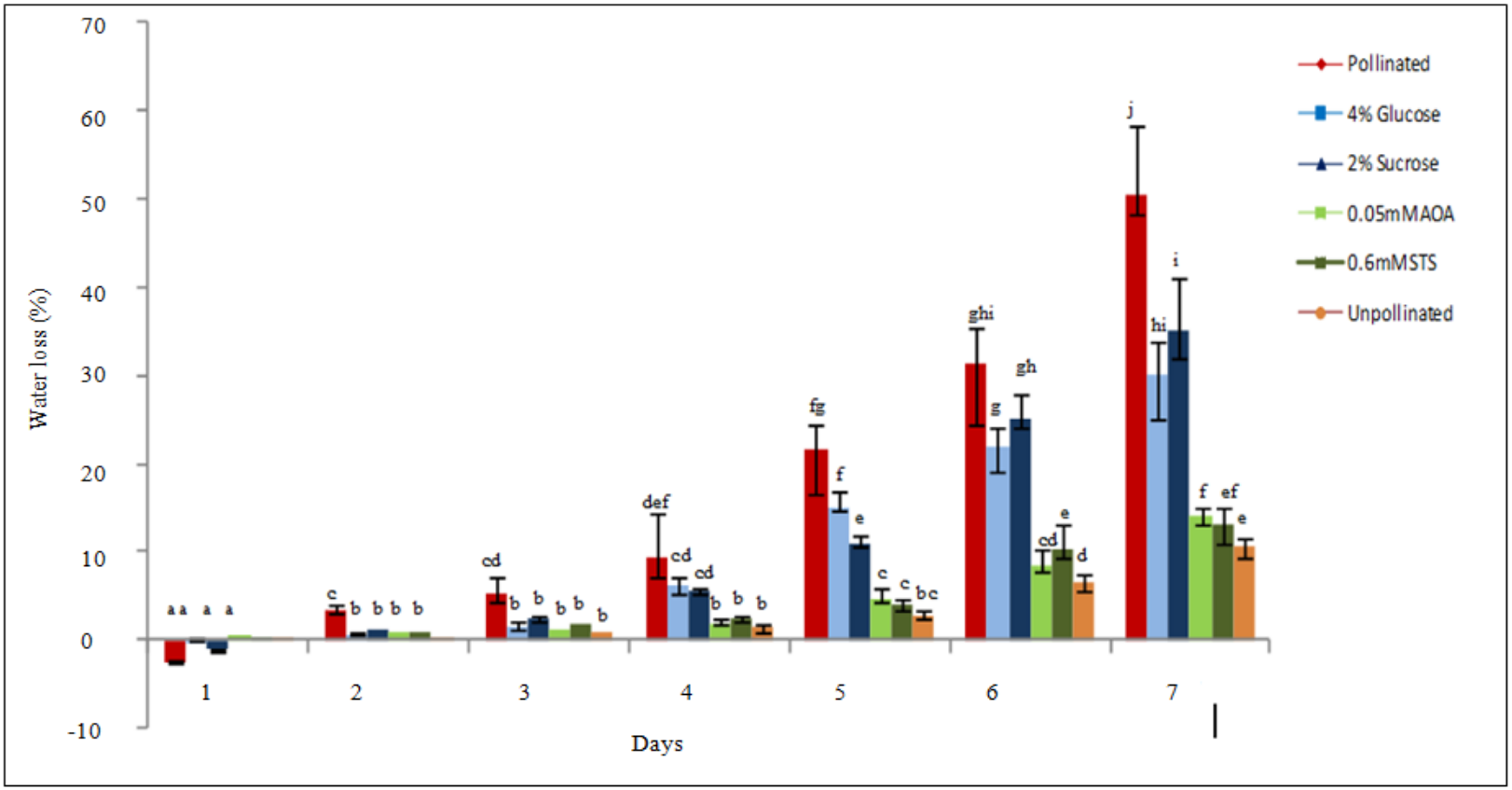

Figure 3.7: Water loss in unpollinated, pollinated and treated *D.* Pompadour flowers. Each value is the mean ± SE (n = 30) and values with different letter(s) are significantly different according to the Duncan's Multiple Range Test ($P < 0.05$).

3.4 Discussion

Maintenance of flowers is an energy dependant process. This is especially true for orchids due to the complexity of the flowers, fragrances and pigmentation. It is therefore advantageous for plants to dispose of floral segments that have fulfilled their functions as for example the perianth which serves to attract pollinators. Pollination stops fragrance production, pigment replenishment, structural maintenance (Arditti, 1992).

In this study, unpollinated *D.* Pompadour flowers remain fresh and open for more than two weeks. Pollination significantly reduces the longevity to a mere 2 days. At this point full closure of the perianth was observed. Further observation of pollinated *D.* Pompadour flowers demonstrated a rapid decline in the appearance and structure of the flower, with necrosis and browning as the end result. This pattern of morphological changes reflects the protection mechanism of flowers undergoing pollination. Flower closure is reported to be a means that protects the germinating pollen grains and developing pollen tubes (Arditti, 1992; Niu *et al.*, 2011). Furthermore, the closure of the perianth is a mechanism which conserves energy and resources, protects the pollinated stigma and renders flowers unattractive to pollinators (Avadhani *et al.*, 1994).

Similar reduction in life span has been reported for other orchid flowers including but not limited to *Phalaenopsis* and *Cymbidium.* Some orchid flowers live many weeks when unpollinated but die within a few days when pollinated. For example *Phaphiopedillum* flowers last up to 3 months, but die shortly after pollination (Avadhani *et al.*, 1994). In unpollinated *Digitalis* (Plantaginaceae), aging symptoms such as loss in corolla composition and turgor occur for leading to abscission in just 24 hours after the receptive stigma of the flower is pollinated (Stead and Moore, 1979*).*

Early investigations on pollination induced senescence in *D.* Pompadour (Nair and Tung, 1987; Ketsa and Luangsuwalai, 1996; Ketsa and Rugkong, 2000) established that like other orchid flowers those of *Dendrobium* Pompadour are ethylene sensitive and pollination hastens perianth senescence. Pollination in this experiment induced endogenous ethylene production, which brought about additional changes, including hyponasty, changes in colour, thinning of petals and loss of fresh weight. These phenomena have been observed in other orchids such as *Arachnis* (Yip and Hew, 1988) *Cattleya* (Strauss and Arditti, 1984), *Cymbidium* (Chadwick *et al.*, 1980; Goh *et al.*, 1985; Attri *et al.*, 2008), *Oncidium* (Nair, 1980), *Phalaenopsis* (Porat *et al.*, 1994) as well as other flowers such as carnations (Caryophyllaceae) (Nichols, 1971), and petunias (Solanacea) (Gillisen, 1977).

Ethylene evolution starts simultaneously with the initial upward movement of petals that subsequently results in full closure of the perianth and its senescence. The same is true for many other orchid flowers (Avadhani *et al.*, 1994). Perianth closure initiated by pollination in *Phalaenopsis* was prevented by ethylene synthesis inhibitors. This is an indication that ethylene is involved in the regulation of the upward movement of perianth segments (Porat *et al.*, 1994). In this experiment, the onset of petal closure was initiated 12 hours after pollination, coinciding with the peak of ethylene evolution. Movement of the perianth segments continues even as ethylene evolution is reduced. Thus it seems that ethylene triggers the onset of petal closure but need not be present throughout the movement process. In *Cattleya,* flowers start to produce ethylene just 4 hours after pollination (Davidson, 1970) whereas ethylene evolution by *Cymbidium* was noticeable 10-12 hours after pollination (Goh *et al.*, 1985). Arditti (1969) suggested that ethylene is effective in the regulation of these phenomena because it diffuses readily through the air and reaches all floral segments without having to be transported internally within the plant.

The ethylene evolution profile of *D.* Pompadour is that of a climacteric plant. In many flowers, the initial response to pollination is an early increase in ethylene production by the stigma that is often followed by increased ethylene production by ovaries and petals. In a study done by Burg and Djikman (1967), the gynostemiun was suggested to be the earliest and major source of ethylene. A number of studies on orchids point to the rostellum as the main production site of ethylene (Dijkman and Burg, 1970; Arditti and Flick 1974; Strauss and Arditti, 1984).

Direct measurements of ethylene production by the rostellum of *Cymbidium* proved that the organ was the major source of ethylene (Chadwick *et al.,* 1986). Furthermore, following pollination, an increase in ACC levels were observed in *Dendrobium* (Nair *et al.,* 1991) and *Phalaenopsis* (Lizada and Rimando, 1982). In both the flowers ACC level was highest in the gynostemium.

Ethylene production in pollinated *D.* Pompadour was detected as early as 6 hours after pollination, peaked 12 hours after pollination and dropped after that. The time to ethylene production differs in orchids. For example In *Cymbidium* Jacobi, ethylene was detected 2 hours after pollination (1990), while in *Phalaenopsis* hybrid Herbet Hager, ethylene was detected 10 hours following pollination (Porat *et al.,* 1994). Ethylene production is catalysed by two enzymes: 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS), which catalyses the conversion of *S*-adenosyl-l-Met into ACC, and ACC oxidase (ACO), which converts ACC into ethylene (Kende, 1993). Studies of these two enzymes in both natural and pollination- induced senescence in have shown an inter-organ translocation of the two enzymes originating from the stigma (Zhang and O'Neill, 1993; Ichimura *et al.,* 2009; Klee and Clark, 2010; Luangsuwalai *et al.,* 2011).

High concentrations of ACC content in the column have been detected compared to that of the petal or sepal of petunia flowers, suggesting that the bulk of ethylene production is in the stigma (Pech *et al.,* 1987). This pattern of tissue-specific activity is consistent with the hypothesis that in the gynoecium senses pollination of the

petunia flower and then propagates this information throughout the flower by synthesizing a translocated signal that must travel to other floral organs to induce ethylene biosynthesis in distal regions (Gilissen and Hoekstra, 1984; Hoekstra and Weges, 1986; Jones, 2010; Kovaleva *et al.*, 2011*).* In carnations following pollination, a translocation of ethylene was reported to occur in a sequential manner starting from the styles and subsequently in the ovary and petals (Jones and Woodson, 1999). The nature of the transmitted signal has yet to be confirmed and is still debated upon in literature. Although some studies have described the signals to be hormonal (Nichols 1971) others have reported that electrical signals were involved (Linskens and Spanjers, 1973; Spanjers, 1978).

The extent of senescence is often apparent as changes which include colour change, thinning of petals, fresh and dry weight changes and shift in water status. Colour change following pollination is an evolution in orchids that aims to eliminate floral attractiveness to pollinators (van Doorne, 2002). One of the earliest reports regarding ethylene production and flower senescence deals with the production of ethylene by fading flowers of the orchid *Vanda* Miss Agnes Joaquim (Akamine, 1963). In this Vanda cultivar, the natural colour fading, which is a change from pinkish purple to white during senescence, was found to be accelerated by exogenous ethylene. This led to the suggestion that endogenous ethylene triggered the colour fading. In the experiments described here, pollination caused the perianth segments of *D.* Pompadour to fade from a bright crimson purple to a lighter shade of purple. This colour change was observed visually with the naked eye and confirmed by objective

measurement using the chromameter. The loss in colour may be a result of a combination of pigment and structural change of the petals. As flowers senesce, the thinning of the petals results in a perception of fading colour, as petals become more translucent and venation becomes more evident, as observed at stage IV. The destruction of anthocyanins is reported to occur in many orchids species following pollination (for a review Avadhani *et al.,* 1994).

In studies by Gori (1983) with non *Orchidaceous* plants, colour changes following pollination were found to be regulated by endogenous ethylene. In that study, ethylene inhibitors were able to prevent colour change in pollinated flowers of several species. pH of vacuole also significantly influence the changes in the colour of senescing flowers (Stewart *et al.,* 1975). The intensity of colour is very much determined by co-pigmentation with other flavonoids and related compounds. This in turn is greatly affected by even the slightest change in pH (Asen *et al.,* 1972).

Pollination-induced senescence in *D.* Pompadour flowers also resulted in rapid thinning of petals and loss of fresh weight. Thinning of the petals causes further deterioration of the pollinated flower. Appearance of veins become more apparent and the colour of the petals are more translucent. Furthermore, thin petals would also mean that they are more susceptible to physical injury. These events are indicative of structural degradation of the flower. Fresh weight loss is due to the export of water and dry matter from the pollinated blossoms. According to previous reports substrates are exported from senescing floral segments to sites of utilization (Chapins

and Jones, 2009; Guiboileau *et al.,* 2010). In pollinated *Cymbidium*, transport of phosphate from the perianth segments to the gynostemia and ovaries was increased (Harrison and Arditti, 1976). Similarly, movement of phosphorus was also evident from the perianth to the ovary of pollinated *Arachnis* (Hew *et al.,* 1989).

Nitrogenous substances are also exported from senescing floral segments to areas of new metabolic activity. Amino acids form the bulk of the energy supply/exports during advanced stages of senescence following the depletion of carbohydrates (for a review see van Doorn, 2004). These amino acids are derived from the hydrolysis of proteins by proteases. In *Arachnis,* protease levels increased dramatically after pollination (Hew *et al.,* 1989). Furthermore, soluble amino acids were also found to increase rapidly in the ovary and gynostemium while continuously decreasing in the perianth. Similar observations have also been reported in other flowers such as *Sandersonia aurantiaca*. Increase in protease and other hydrolytic enzymes resulted in the breakdown of proteins and cell wall. The latter ultimately weakened cell structure and degraded the flowers (O'Donoghue *et al.,* 2002).

Weight loss can also result from a combination of utilization of substrate and water loss. In this study, increased water uptake by *D.* Pompadour flowers was observed to coincide the increases in fresh weight at the initial stages of the experiments. By the end of the experiment water content in and uptake by pollinated *D.* Pompadour decreased significantly. Owing to that, veins in the petals of pollinated flowers were clearly visible especially at Stage 4, where senescence was at an advanced stage. In

contrast, the increased water content and uptake exhibited by unpollinated flowers coincided with the higher fresh weight and more turgid appearance of the flower. These observations are consistent with the traditional notion that the loss of water content in petal tissues contributes to wilting (Nooden, 2004). The observations with *D.* Pompadour are in line with previous reports regarding orchids (Arditti *et al.,* 1973; Arditti and Flick, 1976; Hew *et al.,* 1989; Porat *et al.,* 1998; Attri *et al.,* 2008; Yam *et al.,* 2009; for reviews see Arditti, 1979,1992; Avadhani *et al.,* 1994) and other flowers (Nichols, 1977; Stead, 1992; Aizen, 1993; Xu and Hanson, 2000) indicating clearly that pollination initiated a number of physiological changes which lead to the death of floral segments which have completed their functions.

Further observations of *D.* Pompadour flowers showed that pollination-induced senescence can be suppressed to various extent by ethylene inhibitors, sugars, aspirin and several proprietary compounds (for review see Avadhani *et al.,* 1994). In this study, the role of AOA and STS in extending vase life of pollinated flowers was more pronounced compared to sucrose and glucose. As already mentioned, pollination in *D.* Pompadour flowers is followed by production of ethylene, and accelerated senescence. Blocking ethylene production using AOA proved to be an effective method of delaying senescence. Longevity of pollinated flowers was extended more than 6 folds.

Similar results were reported for *Dendrobium* Khao Sanan (Lerslerwong and Ketsa, 2008), *Oncidium* Gower Ramsey (Chandran *et al.,* 2005) and *Dendrobium* Jew Yuay Tew (Rattanawisalanon *et al.,* 2002). In a study with carnation (Caryophylaceae) flowers carried out by Wawrzynczak and Goszcynska (2003), AOA was found to be most active in inhibiting ethylene production in "Dolca Vita" carnations. Applications of AVG (another inhibitor of ethylene production) prior to pollination effectively blocked ethylene production and eliminated pollination induced symptoms (Woltering *et al.,* 1993). AVG also contributed to the maintenance of turgidity of flower petals as it improves water uptake, which is similarly observed following AOA treatments. In fruits, such as peach, inhibition of ethylene production by the application of AVG also resulted in increased firmness and delayed flesh softening (Bregoli *et al.,* 2002). Although AOA is effective in prolonging vase life of pollinated flowers, it cannot protect flowers from the action of exogenous ethylene.

Another approach to circumvent the effects of ethylene is interference with the sensitivity to ethylene by flowers. Silver competitively blocks the binding of ethylene to receptors and reduces sensitivity, attenuating the climacteric rise of ethylene and ethylene-induced responses (Goren *et al.,* 1984). The action of STS may be the result of the exchange between silver ions and copper in the receptor protein ETR1, which plays a role in the binding of ethylene (Rodriquez *et al.,* 1999; McDanial and Binder, 2012). *D. Pompadour* flowers treated with 0.6 mM STS resulted in a 5 folds increase in longevity.

Similarly, *Dendrobium* Jaquelyn Thomas flowers that were pulsed in STS for 24 hours were found to stay alive for 15 days compared to 5 days when untreated (Nowak and Vacharotayan, 1980). Pulsing *Dendrobium* Heang Beauty flowers with 0.5 mM STS and a solution which combined STS and sucrose resulted in more than 5-fold increase in longevity compared to untreated flowers (Abdullah, 2005). However, competitive binding means that suppression of sensitivity to ethylene perception is not permanent and depends on the concentration of STS and ethylene. Once the ethylene concentration exceeds that of STS, the onset of senescence will proceed (Sisler *et al.,* 1986; Serek and Reid, 1993). Pulsing with STS has been shown to successfully extend vase life of *Alstromeria* (Alstroemeriaceae), *Anthirrhinum majus,* (Veronicaceae) and petunia (Solanacease) flowers. Aside from STS, silver nitrate has also been widely used as holding solutions for orchids and has similar effects to that of STS. For example, pulsing flowers with silver nitrate successfully extended the vase life of *Phalaenopis* (Aharoni and Halevy, 1977) and *Dendrobium* Youppadeewan (Ketsa and Boonrote, 1990).

Sugars are commonly used in flower preservatives to extend longevity. They are applied either as vase solutions, or as a pulse treatment prior to shipping. The concentration and the type of sugars are species and cultivar specific. The reason for the difference in preference is still unclear. Our studies showed that both glucose and sucrose extended the vase life of pollinated *D.* Pompadour for a similar period.

The use of sugars as preservatives to prolong vase life and quality of cut flowers is based on the notion that cut flowers detached from the mother plant lose their source of energy and hence turns to alternative sources i.e., cell wall, proteins etc. (van Doorn, 2004; Trivellanni *et al.,* 2011). When uncut, flowers are sink organs receiving sugars from the mother plant. However, once detached, the flowers are transformed into a source organ with negligible or no production of photosynthates by the remaining green parts and dependant only on the limited pool of respirable substrates available within the flower. Loss of sugars can result in loss of the semi-permeability of the cell membrane and eventually lead to an irreversible loss of membranes and structures. The inclusion of sugars acts as a continuous supply of energy for the cut flowers, thus delaying the breakdown of structural components. In many plants, it was observed that sugars decline after harvest (King and Morris, 1994; Davies *et al.,* 1996) and sugar application extended the plant life (Ichimura *et al.,* 1999; Coupe *et al.,* 2003; Verlinden and Garcia, 2004; Gholami *et al.,* 2011). In flowers like carnation and freesias (Iridaceae) sucrose seem to be the sugar of choice (Podd *et al.,* 2002; Pun *et al.,* 2003; Verlinden and Garcia, 2004; Weerts, 2008). This may be due to the fact that sugars are transported to sink tissues as sucrose and it would be expected that the other sugars were hardly transported into the cells

A study carried out by Nair and Tung (1980) showed that inclusion of sucrose into holding solutions managed to extend the shelf life of *Oncidium* Goldiana orchids by a week. Sucrose treatment also extended vase life of *Aranthera* James Storie (Nowak and Vacharotayan, 1980) and *Dendrobium* Jaquelyn Thomas (Suisuwan, 1986) by ca.

5 days. Studies which involved roses (Rosaceae) (Liao *et al.,* 2000) and carnations (Verlinden and Garcia, 2004) have also shown similar results. In contrast the inclusion of sucrose did not seem to have any positive effect on *Arachnis* Maggie Oei and *Vandopsis* Christine (Nowak and Vacharotayan, 1980). Our data showed that 4% glucose and 2 % sucrose increased the vase life of pollinated *D.* Pompadour flowers by 2 fold. The inclusion of 0.1 mg l^{-1} of chloramphenicol into holding solutions containing sugars act as antimicrobial growth inhibitor to prevent the growth of bacteria which may cause blockage of stem and disallow uptake of water and sugar (Inoue and Takata, 2001; Asil and Roein, 2012). Our findings are not in agreement with a report by Rattanawisalanon *et al.,* (2003), who demonstrated that *Dendrobium* flowers held in 4% glucose had a shorter vase life compared to that of control. This could be due to the fact that bacterial growth occurred because no anti-microbial chemical was added into the holding solutions used in that particular study.

In flowers where senescence is regulated by ethylene, exogenous sugars considerably delay the large increase in production and the time to visible senescence. The relationship between sugars and ethylene production and sensitivity has also been reported by a number of investigators. Leon and Sheen (2003) envisaged a model which showed that sucrose inhibits the ethylene signal pathway through the production of low levels of ABA. In broccoli florets, an adverse relationship was observed where decline in sugars resulted in increased sensitivity to ethylene and accelerated senescence. Exogenous sugars were also found to suppress the production of ethylene and delay the climacteric rise of ethylene in flowers (Dilley and Carpenter

1975; Sacalis and Lee 1996; Sriyong *et al.,* 2009;). In sweet pea flowers, sucrose did not alter the concentration of the ethylene precursor, ACC. In fact, accumulation of ACC was apparent. This led to the suggestion that the production of ethylene is blocked by decreased ACCO activity which results in the inability of ACC to be oxidized into ethylene (Ichimura and Suto, 1999).

From the results of this study, it is obvious that the treatment solutions not only extend shelf life, but they are also effective in maintaining flower quality. Flowers held in 0.05 mM AOA, 0.6 mM STS, 2% sucrose and 4% glucose exhibited better visual appearance compared to those held in water. By the end of the experiment (7 days), flowers held in water had already reached the 4^{th} stage in which the fully closed perianth showed apparent venation, shrivelling and discoloration. On the other hand, flowers held in AOA and STS solution remained fresh, fully opened and without discoloration. A study carried out by Kusuhara *et al.,* (1996) attributed improved colour of *Eustoma* (Gentianaceae) flowers held in sucrose to the increased expression of anthocyanin biosynthesis genes. Sucrose may even be supplied as glycoside residues for anthocyanins as anthocyanins are usually present with glycosides.

Maintenance of water relations is pivotal in ensuring that flowers remain fresh. Both water uptake and water content in flowers held in the treatment solutions were improved in comparison to the control where reduced water uptake was apparent as early as 2 days after pollination. Water relations are adversely affected by a number of factors, among which is osmotic concentration, vascular occlusion in the stem of

flowers due to breakdown of vascular structure or clogging of stem by bacteria. For AOA, low pH value of the solution may be a factor for improved water relations as low pH inhibits blockage and allows water uptake (Loubaud and van Doorn, 2004). Acidic solutions have been reported to travel more readily through the stems of flowers compared to solutions that are neutral or alkaline (Reid and Koffranek, 1981). Hydration of flowers can therefore be considerably improved by using acidic solutions of pH 2-3 (van Doorn and Perik, 1990). The pH of solutions containing AOA which were used to treat cut *Oncidium* Gower Ramsey (Chandran *et al.,* 2005) and *O.* Goldiana (Chandran *et al.*, 2007) flowers maintained a pH between 3-3.5 for more than 2 weeks. Those flowers also showed improved water uptake compared to non-treated flowers and flowers treated with sugars. In *Gerberra Jamesonni*, it is reasonable to assume that the anti-senescence affect of AOA is related to improved water status as significant water content was observed in the flowers after AOA application. This is further corroborated by the fact that 80-90% of the flower is made up of water (Emongor, 2004).

Pre-treatment with AOA was found to effectively mitigate the adverse effects of water stress on carnation flowers and delayed wilting. Unlike untreated flowers in which reduction in membrane phospholipids and permeability were observed, the conditions in AOA pre-treated carnations were found to be similar to the non-stressed control (Mayak *et al.,* 1985). Ohkawa *et al.*, (1999) reported that STS had an anti-bacterial property evident by the significant reduction in total bacterial counts on the surface of rose stem treated with STS. Similarly, anti-bacterial effect was also observed in the

basal parts of gladiolus and stems of roses treated with STS (Al Humaid, 2004). Accumulation of solutes would improve the ability of petals to take up water from holding solutions and thus provide a better water balance for flowers. Sugars have been reported to increase vase life of flowers by way of increasing osmotic concentration which in turn improves the ability of flowers to absorb water and maintain turgidity (Halevy and Mayak, 1979; Ali *et al.,* 2009). Flowers held in 2% sucrose and 4% glucose solutions maintained consistent water uptake for 3-4 days before the onset of senescence took place and petals started to move upwards and eventually reach full closure. Nevertheless, although treatment the sugars managed to improve the appearance of pollinated flowers compared to control, it was inferior when compared to the ability of the ethylene antagonists to extend shelf life. From these results, it is possible to deduce that increase in osmotic concentrations may not be the major contributor to improved water relations.

Pollinated flowers held in water exhibited rapid thinning of petals and loss of fresh weight. In contrast, treatment solutions retained petal thickness and fresh weight better than water. Kaltaler and Steponkus (1976) reported that exogenous sugars may be maintaining the structural integrity of the cell membranes of rose flowers. It has been reported that sugar also prevents the up-regulation of senescence-associated genes, many of which are related to cell wall hydrolysis (Hoeberichts *et al.,* 2007). Microscopic studies on *Coleus* explants (petioles /leaves) reported that exposure to ethylene resulted in disruption of plasma membrane and cell separation in the middle

lamella. Pre-treatment with STS prevented ethylene induced plasma membrane disruption and middle lamella dissolution (Baird *et al.,* 1984).

In conclusion the available evidence suggests that pollination accelerates physiological changes in *D.* Pompadour flowers which cause rapid deterioration in flower quality and ultimately terminates the vase life of the pollinated flowers. The results indicate that treatment of pollinated flowers with ethylene inhibitors and sugars have senescence-inhibiting effects albeit at varying degrees on pollinated flowers. 0.05 mM AOA and 0.6 mM STS were most effective in extending vase life and improving flower quality of pollinated *D.* Pompadour. The addition of 4% glucose and 2% sucrose in holding solutions were not as effective when compared to the ethylene inhibitors in extending vase life and improving flower quality.