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Abstract

Studies were conducted to evaluate the effects of different relative humidity levels (60, 75, and 90%) and recutting (0, 1, 2, 3, 4, and 5 cm recutting end of flower stem) treatments on vase life of cut rose flower. Two separate experiments (bucket and vase experiments) were conducted based on completely randomized design with factorial arrangement with 8 replications in bucket experiment and 5 replications in vase experiment. Analysis of variance revealed that two ways effects of various RH and recuts did not significantly ($P \leq 0.05$) affected flower vase life, relative fresh weight, solution uptake, and bacterial populations. Cut rose flower stored in chamber with 90% relative humidity had the longest vase life, while those one kept in 60% showed the shortest longevity. The result of mean comparisons revealed with increasing relative humidity from 60% to 90%, bacterial populations was increased too.

Keywords: bacterial count, flower diameter, relative fresh weight, solution uptake

Introduction

Control the uptake of CO_2 for photosynthesis and prevention of water vapor is done via stomata functioning, which are on the leaf surface. Guard cells bounded to the stomata and equipped for autonomous ABA synthesis to control stomata opening and closure (Bauer *et al.*, 2013). Growing conditions largely effect on stomata responsiveness to closing during postharvest life of flowers (Fanourakis *et al.*, 2016). Flowers longevity is limited at an early stage of postharvest due to a range of factors mainly happen during preharvest. The shortened longevity

of cut roses is primarily related to water loss for their large leaf area and unfavorable growth conditions affect the stomatal response (In *et al.,* 2016b) and phenotype, which is determined by genotype (In *et al.*, 2016a). Further, it's also reported that the variation in rose flower longevity has been associated mainly with vascular obstruction through the stem, which affect water status (van Doorn, 1997). In addition, some other factors such as limitation by fungi infection, vascular occlusion and pedicel bending, can be resulted to early flower senescence in cut roses. To increase vase life of cut rose flowers, it's necessary to prevent or retard flower wilting (Rasouli *et al.*, 2015) .

Water deficit stress during postharvest handling is one of the most important factors to determine cut rose flower longevity. Abnormal flower opening, flower wilting, bent neck, and failure to open are the results of water deficit stress (Jin *et al.*, 2006). Hence, correct postharvest handling and preventing dehydration as well as controlling temperature and relative humidity during postharvest storage is essential to maximize flower vase life and quality (Reid, 2001). Among the postharvest factors effecting on flowers longevity, RH is strongly correlated with cut rose's longevity (In *et al.*, 2016a). Carvalho *et al.* (2015) reported that preharvest high RH (≥ 85%), hampered stomata functioning and adversely affected cut rose longevity. Arve *et al.* (2017) reported that plants developed at high RH during leaf development, when exposed to daily change in RH and temperature, showed improved stomata functioning. Moreover, different cut rose cultivars showed different patterns of reaction during postharvest, where in cultivar 'Akito' decreased stomata functioning adversely affected the vase life. However, 'Grand Prix' cultivar did not significantly affect by stomata functioning (Woltering & Paillart, 2018).

Some physical treatments such as splitting or crushing stems and also removing bark at the base of the stem increased water uptake and 25% increase in fresh weight which resulted in enhancing flower longevity compared to control (Milner, 2009). Similarly, it's reported that bark removal and stem-end splitting when applied after short term storage for 24 h at 4°C, increased the vase life of cut rose and acacia. However, crushing stems had no effect on the vase life of fresh-cut rose (Ahmad *et al.*, 2011). It's reported to improve water uptake and maintain water balance for cut flowers and foliage, water loss should be decreased by reduction in leaf area and store them in low temperature and high RH and using some pulse treatments such as sucrose in vases to improve their vase life (Ahmad *et al.*, 2011). In the ambient air, relative humidity was

affected water loss in harvested crops. With consideration that various crops need different relative humidity, however, harvested crops keep their nutritional quality, appearance and weight at very high RH (Hong *et al.*, 1999).

Influence on overseas shipping on flowers longevity has not been well understood. Furthermore, Ahmad *et al.* (2011) reported that high RH decreases water loss and maintain flower longevity. However, In *et al.* (2016 b) reported that high RH lead to the attenuated stomatal responsiveness and increase water loss. Due to study the effect of shipping influence and some contradictory results on effect of RH on flowers longevity, the aim of present experiments was to examine the relationship between different relative humidity, various recuts and bacterial population's effects on cut rose flowers longevity. Here, we have shown that various postharvest conditions can significantly affect cut roses longevity.

MATERIAL AND METHODS

Plant material

Cut H3O rose flowers produced in Ethiopia greenhouses were obtained at the commercial stage of bud opening (petals starting to reflex) from MM Flower Factory in Cambridge and transferred to the Reading University and were held in a cold room (4°C) and then transported within 3 days to the experiment chambers (phytotrons). No symptoms of botrytis were observed in flowers. Two separate experiments (bucket and vase experiments) were conducted based on completely randomized design by factorial arrangement with 8 replications in bucket experiment and 5 replications in vase experiment .

Bucket experiments

Cut rose flowers were recut under the tap water to 1, 2, 3, 4 and 5 cm and placed in various relative humidity (RH) at rates 60, 75, and 90%. No recut flowers were used as a control. Cut rose flowers place in buckets. Three buckets were used for each recut and each bucket was containing 8 cut rose flowers (exactly 24 flowers were used for each recut). Experiment replicated to confirm the results of first experiment at room condition [Temp: 22±2°C and light intensity:10 µmol. m^{-2}. s^{-1}].

Vase experiment

In vase experiment, 10 cut rose flowers were used for each RH. Half of them 5 cm recut and half of them without recut placed in various RHs at rates 60, 75, and 90%. Each cut rose flowers place in a vase. Experiment replicated to confirm the results of first experiment at room condition [Temp: 22±2°C and light intensity:10 µmol. m^{-2}. s^{-1}].

Assessments

Vase life

Vase life was recorded as the time in days after treatment (day 0) that flowers reached the end of their longevity due to bent neck or advanced signs of fading on all petals (Liao *et al.*, 2000; Chamani *et al.*, 2006).

Relative fresh weight

Following formula was used for calculation of relative fresh weight of stems: RFW (%) = (Wt/Wt=0) × 100; where Wt = weight of stems (g) at t = days 0, 2, 4, 6, etc. and Wt=0 = weight of the same stem (g) on day 0 .

Solution Uptake

Vase solution uptake was determined by using the formula: Solution uptake (ml day-1 g-1 fresh weight) = (St-1-St)/Wt=0; where, St = solution weight (g) at t = days 1, 2, 3, etc. St-1 = solution weight (g) on the preceding day, and Wt=0 = fresh weight of the stem (g) on day 0.

Microbial count analysis

Preparation of nutrient agar and Maximum recovery diluent (MRD) for total plate count was done as described previously by Chamani and Wagstaff, (2018). However, For the bacterial count, 5'cm from the basal end of flower stems removed and then sterilized by careful blotting with ethanol (98% v/v). After that, further cut the stem into 2 mm segments and weighted and then were added to 90 ml of MRD in a stomacher bag and shaken for 60 seconds with 230 rpm which this will create 10-1 dilution (w/v). 1 ml of the homogenized/inoculum was sampled from the bag and it was serially diluted in 9 ml MRD to obtain 10-2, 10-3, 10-4 until 10-7. Then 1 ml of the respective solution was placed on 15 ml nutrient agar temperature between 45 °C to 50 °C on petri dish using pour plate technique and the plates were swirled to mix evenly. The inoculated plates were allowed to cool at room temperature until the liquid solidified. The plates

then were incubated at 30 °C in inverted condition. After 24 ± 1 h of incubation, number of colonies per plate was counted using a colony counter. Plates with colonies more than 300 colonies are labelled with TNTC (too numerous to count) and plates with colonies less than 30 colonies were discarded .

Statistical analysis

All experiments were done in completely randomized design based on factorial arrangement. Bucket experiments were done by 8 replications. Vase experiments were done by 5 replications in each trial for morphological traits and 3 replications for microbial count. Data were subjected to analysis of variance (ANOVA) using Statistical Analysis System Ver. 9.2. (SAS Institute, Cary, NC, USA). Mean differences between treatment were compared using Duncan's Multiple Range Test at $P<0.05$. Graphs were then plotted using Excel spread sheet .

Results

Bucket experiment

Vase life

Analysis of variance revealed that various RH and recuts significantly ($P \leq 0.05$) affected flower vase life, relative fresh weight, and solution uptake in both of the experiments. However, no significant ($P \leq 0.05$) difference was found in interaction effects of RH and recuts. Mean comparison of results showed that different recut significantly ($P \leq 0.05$) affected flower vase life. Cut flowers with No recut significantly ($P \leq 0.05$) had the lowest vase life. However, cut flowers which recut 3, 4, and 5 cm had the highest vase life compared to no recut and 1 cm recuts. No significant difference was found between 1 and 2 cm recuts. It's found that at least 2 cm recut is necessary to get the highest vase life (Figure 1A & Figure 4). The result also revealed that different relative humidity levels significantly ($P \leq 0.05$) affected flower vase life. The highest and significant vase life was found in flowers placed in 90 % RH compared to 60 and 75% RH. However, 75% Relative humidity significantly increased flower vase life compared to 60% RH (Figure 1B & Figure 4).

Solution uptake

The result of experiment revealed that cut flowers placed in 60% and 90% relative humidity had the highest and lowest solution uptake during whole experiment time, respectively. However, 75 % relative humidity made intermediate effects on cut flowers (Figure 2A). Mean comparison revealed that recut flowers with 2, 3, 4, and 5 cm significantly ($P \leq 0.05$) had the highest solution uptake during whole experiment time. Moreover, cut flowers with No recut (C0) had the lowest solution uptake (Figure 2B).

Relative fresh weight

Fresh weight of cut rose flowers decreased from the second day of vase life, indicating a deterioration of their water status. The result of experiment also showed that cut flowers placed in 90% and 60% relative humidity had the highest and lowest relative fresh weight during whole experiment time, respectively. However, 75 % relative humidity made intermediate effects on cut flowers (Figure 3A). Mean comparison revealed that recut flowers with 2, 3, 4 and 5 cm, significantly ($P \leq 0.05$) had the highest relative fresh weight during whole experiment. However, cut flowers with no recut had the lowest relative fresh weight during experiment (Figure 3B).

Vase experiment

Vase life

Analysis of variance revealed that various RH and recuts significantly ($P \leq 0.05$) affected flower vase life, relative fresh weight and solution uptake. But, no significant ($P \leq 0.05$) difference was found in interaction effects of RH and recuts. The result revealed that different relative humidity significantly ($P \leq 0.05$) affected flower vase life. The highest and significant vase life was found in flowers placed in 90 % RH compared to 60 and 75% RH. However, 75 % Relative humidity significantly increased flower vase life compared to 60% RH (Figure 5A & Figure 8). In fact, with increasing relative humidity, flower vase life increased too .

Cut flowers with no recut significantly ($P \leq 0.05$) had the lowest vase life. However, cut flowers which recut 5 cm had the highest vase life compared to no recut. It's concluded that recut the flowers increased its vase life two times compared to No recut (Figure 5B and Figure 8).

Relative fresh weight

The result of experiment revealed that cut flowers placed in 90% and 60% relative humidity had the highest and lowest relative fresh weight during whole experiment time respectively and 75 % relative humidity made intermediate effects on cut flowers (Figure 6 A). Mean comparison revealed that recut flowers with 5 cm significantly ($P \leq 0.05$) had the highest relative fresh weight during whole experiment time compared to no recut (Figure 6 B) .

Solution uptake

The result of experiment revealed that cut flowers placed in 60% and 90% relative humidity significantly had the highest and lowest solution uptake till days 7 respectively. After days 7, solution uptake in flowers placed in 90% RH remained constant (Figure 7A). Whereas, solution uptake in flowers placed in 60 and 75% RH decreased because of flowers drying. It's also revealed that recut flowers significantly had the highest and significant solution uptake during experiment after days 5 compared to No recuts (Figure 7B).

Bacteria count

The result of mean comparisons revealed with increasing relative humidity from 60% to 90%, bacterial populations was increased too. But, no significant ($P \leq 0.05$) differences were found among them. However, with recutting the stem ends in cut rose flowers which held in vase solution containing Crysal, bacterial populations significantly ($P \leq 0.05$) decreased in stem ends. It seems Crysal, did not deleted bacterial population. However, it's inhibited bacterial multiplications. In fact, bacterial count in the 5 cm of stem end showed that, bacterial populations were significantly decreased after 4 cm recut compared to recut less than 3 cm recut (Figure 9A). However, in vase experiment, there were significant ($P \leq 0.05$) differences between recut and non-recuts flowers. However, it was found much more bacterial populations in stem ends on non-recut flowers compared to recut flowers (Figure 9B).

Discussion

Range of factors including preharvest growth conditions, shipping overseas, proper harvest time, and appropriate storage can affect flowers longevity (Fanourakis *et al.*, 2015; In *et al.*, 2016, Baker, 2018). To maintain the natural appearance of flowers, quality deterioration should be delayed. All consumers prefer cut flowers with high longevity (Asghari *et al.*, 2014).

The results of our experiments showed that various RH and recut treatments significantly affected flower vase life, relative fresh weight, solution uptake, and bacterial populations in both of the experiments. The highest vase life and relative fresh weight was observed in flowers treated with 90% RH. Flowers stored in 60% RH had the highest solution uptake in bucket experiment but in vase experiment in flower stored in 90% RH the amount of solution uptake did not change after day 7 while decreased in 60 and 75%. It can be deduced that 90% RH is ideal condition for prolonged vase life of rose flowers. However, it's reported that low temperature and high RH decreases water loss and maintain flowers quality (Ahmad *et al.*, 2011). The difference between saturation vapor pressure and actual air vapor pressure defines evapotranspiration of leaf and petals and is playing key role in water uptake. Hence, if it happens with high difference, evapotranspiration will be increased too. However, high air relative humidity reduced evaporation of water from the flower petals and leaves, resulted in to high fresh weight and long longevity (Siddiquei, 2015). Our finding is in line with the study that reported the solution uptake was affected strongly by RH compared to sucrose concentration, and greater solution uptake was happened in lower relative humidity condition (Shimizu & Ichimura, 2007).

Moreover, Faragher et al (1986) reported that although keeping of cut rose flowers (*Rosa hybrid* L. cv. Mercedes) at 65% relative humidity (RH) decreased petal water content by 20% compared to flowers stored at 95% RH, it did not shorten vase life. However, it can be because of cultivar types and some other unknown factors. For reduction of disease development low temperature and higher relative humidity have also been suggested (Harkema *et al.*, 2013). 90% relative humidity has been preferred for keeping of *Anthurium andraeanum* lindl, Strelitzea reginae (Vieira *et al.*, 2014).

Results of both bucket and vase experiments also showed that recutting rose flower stem could extent it's vase life, maintain higher fresh weight and uptake more solution in comparison to no recut. Experiment with various recut showed that recutting flowers with 1, 2, 3, 4, and 5 cm significantly increased vase life during whole experiment time compared to control. In fact, to obtain the best results, it's necessary to recut flower stem ends at least 2 cm. Our results are in consistent with an earlier study, where difference in vase life of cut carnation flowers was due to flower stalks height (Chandra *et al.*, 2013). All done experiments to evaluate flower vase life, were conducted by using of various stems lengths which sometimes influenced flowers vase life.

In fact, cut flowers have been tested either at the stem length of harvest as little as 12 cm to as much as 75 cm depending upon tested cultivars (Fanourakis *et al.*, 2013). It's found that there was significant negative correlation between vase life and stem length (Mortensen & Fjeld, 1998). Actually, Short stems (i.e. short water transport path) and/or less leaves (i.e. lower water loss in cut flower basis) would reduce loss of water balance resulted in longer vase life. Additionally, some physical treatments such as splitting or crushing stems and also removing bark at the base of the stem increased water uptake and 25% increase in fresh weight which resulted in enhancing flower longevity compared to control (Milner, 2009). It's reported that bark removal and stem-end splitting increased the vase life of rose and acacia (Ahmad *et al.*, 2011).

Our finding also revealed that with increasing relative humidity from 60% to 90%, bacterial populations were increased too. However, with recutting the stem ends in cut rose flowers which held in vase solution containing Crysal, bacterial populations significantly ($P \leq 0.05$) decreased in stem ends. Vase life of cut rose flowers is short which could be related to excessive water loss from the rose leaves, resulting in leaf desiccation and the development of bent necks (Mortensen & Fjeld, 1998). Actually, short vase life in cut flowers is often the results of vascular occlusions that restrict vase solution supply. Water absorption in stem is typically caused by blockage of cut stem ends by microbes and physiological plugging which inhibits water uptake by flower stalk (Hussen & Yasin, 2013). The accumulation of bacteria, in the stem ends may play an important role in reduction of vase life, as a result of decreasing water uptake (van Doorn, 1997).

Conclusions

Cut roses longevity closely depends on preharvest growth conditions, genetic background, and cut flowers storage. According to results of present study, high RH (90%) with recutting cut H3O rose flower stems at least 2 cm, extended the cut rose longevity via maintaining the proper stomatal functioning, reduction in bacterial population, developing a normal water balance, and maintaining relative fresh weight Moreover, by increasing recut the stem end from 0 to 5 cm, bacterial population in the stem was decreased too.

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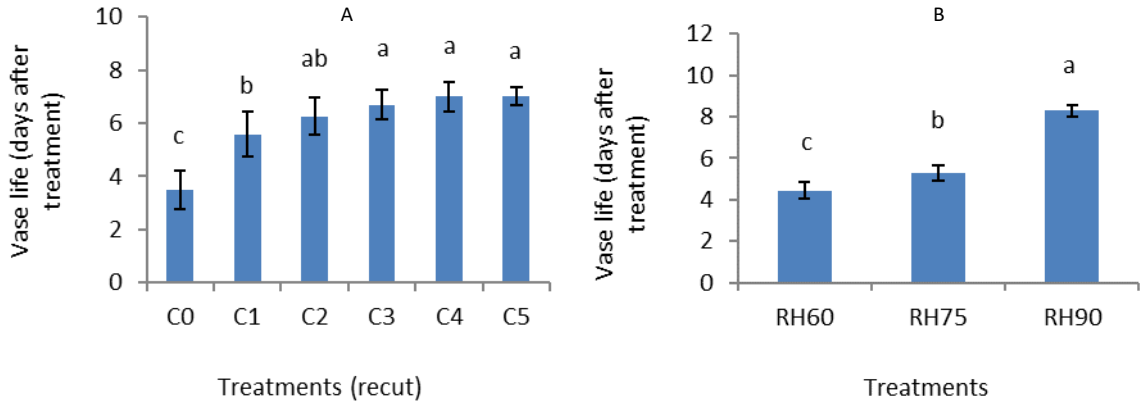

Figure 1. Vase life of rose flowers in different RH (A) and various recutting (B)

Different letters indicate significant differences determine using a Duncan's multiple range test ($P<0.05$). Error bars= SE (n=8)

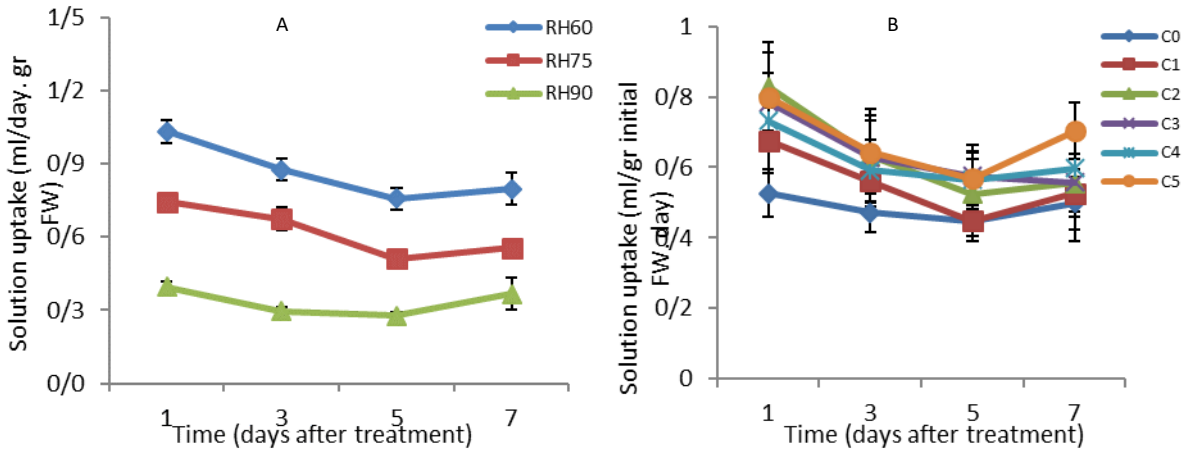

Figure 2. Solution uptake of rose flowers in different RH (A) and various recutting (B)

Different letters indicate significant differences determine using a Duncan's multiple range test ($P<0.05$). Error bars= SE (n=8)

B

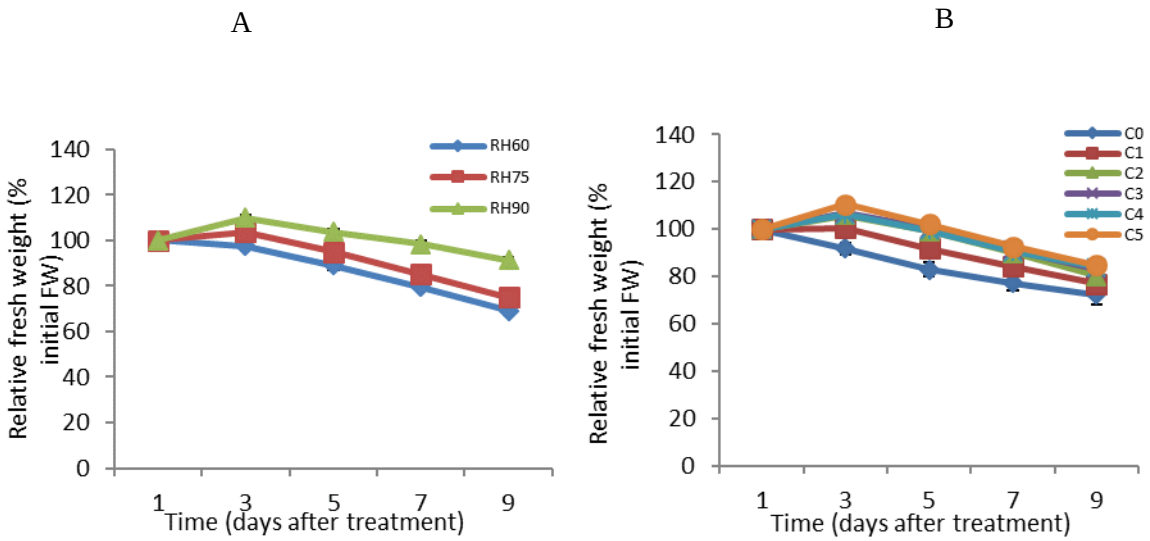

Figure 3. Relative Fresh weight of rose flowers in different RH (A) and various recutting (B)

Different letters indicate significant differences determine using a Duncan's multiple range test ($P<0.05$). Error bars= SE (n=8)

Figure 4. Effects of various RH (A=60%, B=75% and C=90%) with different recuts on cut rose flower vase life

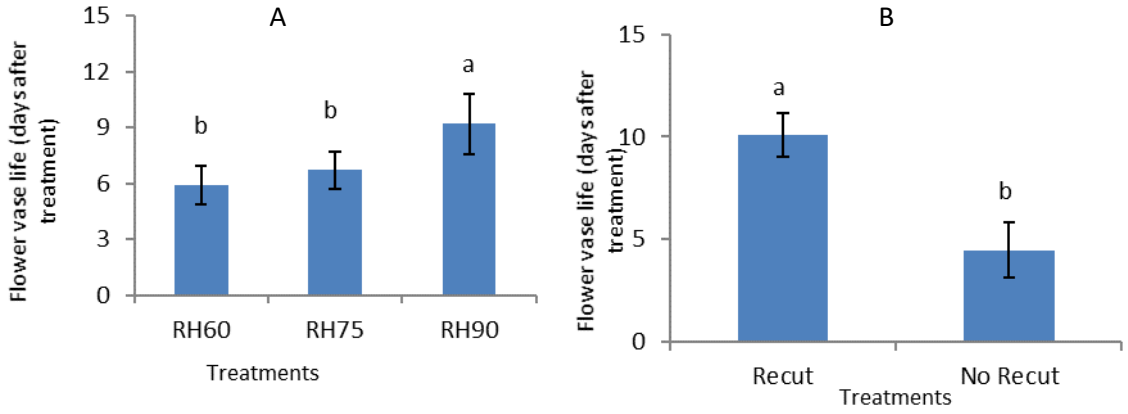

Figure 5. Vase life in different RH (A) and different recutting (B) conditions

Different letters indicate significant differences determine using a Duncan's multiple range test ($P<0.05$). Error bars= SE (n=5)

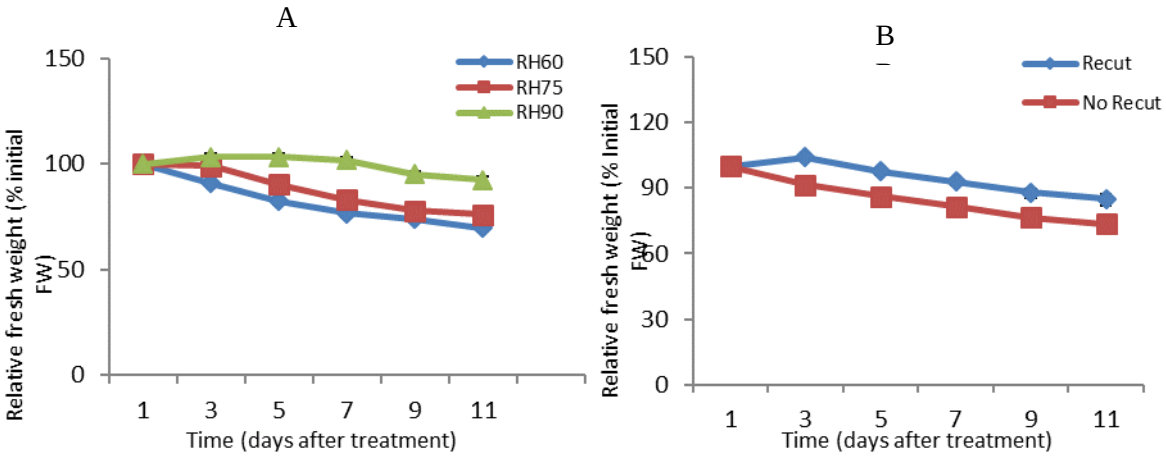

Figure 6. Relative Fresh weight in different RH (A) and different recutting (B) conditions

Different letters indicate significant differences determine using a Duncan's multiple range test ($P<0.05$). Error bars= SE (n=5)

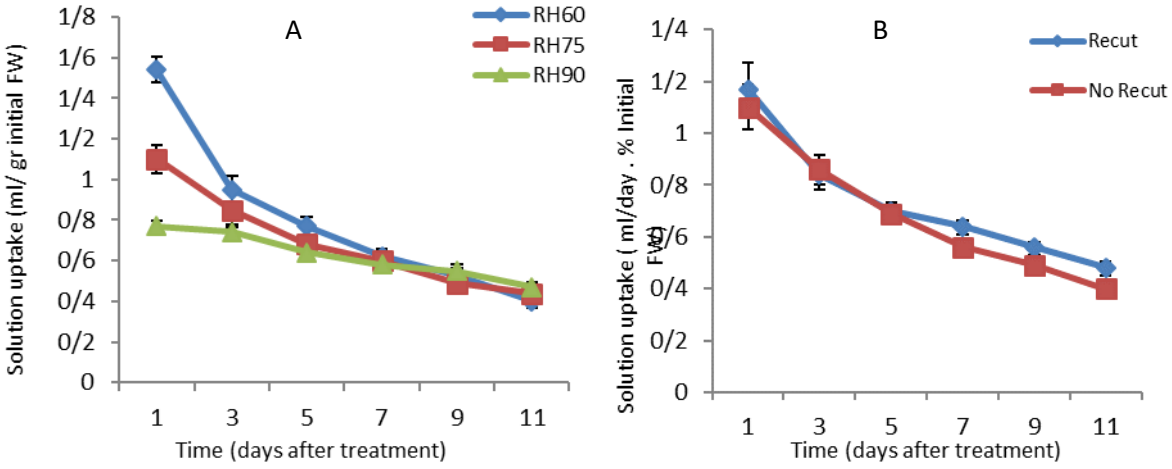

Figure 7. Solution uptake in different RH (A) and different recutting (B) conditions

Different letters indicate significant differences determine using a Duncan's multiple range test ($P<0.05$). Error bars= SE (n=5)

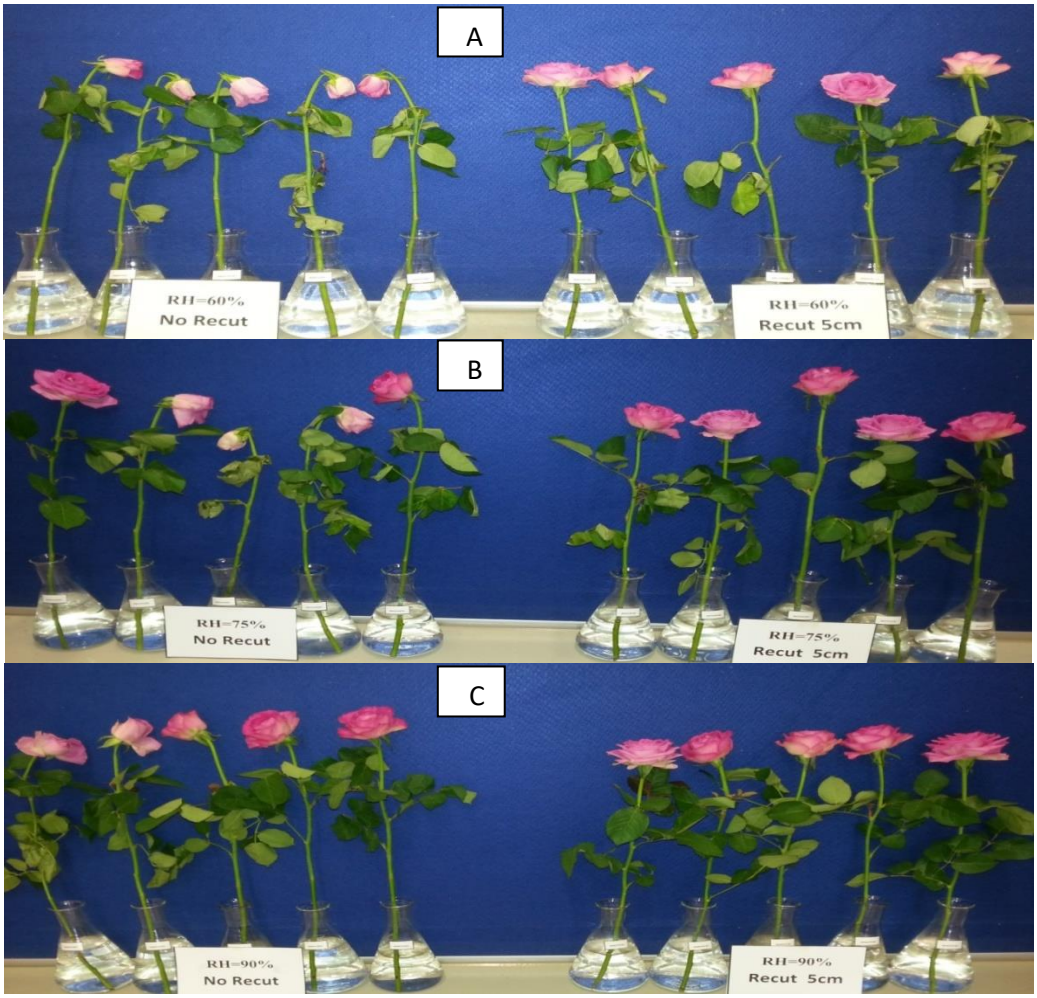

Figure 8. Effects of different RH (A=60%, B=75% and C=90%) and recut on flower vase life

Different letters indicate significant differences determine using a Duncan's multiple range test ($P<0.05$). Error bars= SE (n=5)

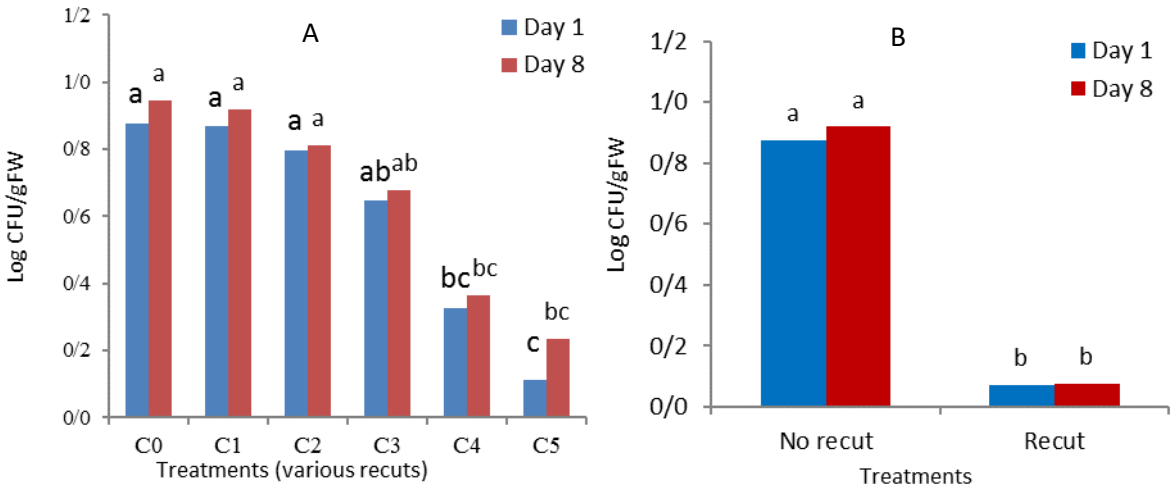

Figure 9. Bacterial population in different re-cutting stem ends in cut rose flowers in bucket experiment (A) and vase experiment (B)

Different letters indicate significant differences determine using a Duncan's multiple range test ($P<0.05$). Error bars= SE (n=5)