Effects of postharvest relative humidity and various re-cutting on vase life of cut rose flowers

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Abstract: Studies were conducted to evaluate the effects of different relative humidity levels (60%, 75%, and 90%) and re-cutting (0 cm, 1 cm, 2 cm, 3 cm, 4 cm, and 5 cm re-cutting end of flower stem) treatments on vase life of cut rose flower. Two separate experiments (bucket and vase experiments) were conducted based on completely randomised design with factorial arrangement with eight replications in bucket experiment and five replications in vase experiment. Analysis of variance revealed that two ways effect of various RH and re-cuts 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.

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1 Introduction

Control the uptake of CO_2 for photosynthesis and prevention of water vapour 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 unfavourable growth conditions affect the stomatal response (In et al., 2016b) and phenotype, which is determined by genotype (In et al., 2016a). Further, it is 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 is 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 (RH) during postharvest storage is essential to maximise 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 and 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 is 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 is 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, RH was affected water loss in harvested crops. With consideration that various crops need different RH, however, harvested crops keep their nutritional quality, appearance and weight at very high RH.

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. (2016b) 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 RH, various re-cuts and bacterial population's effects on cut rose flowers longevity. Here, we have shown that various postharvest conditions can significantly affect cut roses longevity.

2 Material and methods

2.1 Plant material

Cut H_3O 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 three 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 randomised design by factorial arrangement with eight replications in bucket experiment and five replications in vase experiment.

2.2 Bucket experiments

Cut rose flowers were re-cut under the tap water to 1, 2, 3, 4 and 5 cm and placed in various RH at rates 60, 75, and 90%. No re-cut flowers were used as a control. Cut rose flowers place in buckets. Three buckets were used for each re-cut and each bucket was containing eight cut rose flowers (exactly 24 flowers were used for each re-cut). 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}].

2.3 Vase experiment

In vase experiment, ten cut rose flowers were used for each RH. Half of them 5 cm re-cut and half of them without re-cut 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}).

3 Assessments

3.1 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 (Chamani et al., 2006).

3.2 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.

3.3 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.

4 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 sterilised 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 homogenised/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 too numerous to count (TNTC) and plates with colonies less than 30 colonies were discarded.

4.1 Statistical analysis

All experiments were done in completely randomised design based on factorial arrangement. Bucket experiments were done by eight replications. Vase experiments were done by five replications in each trial for morphological traits and three 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.

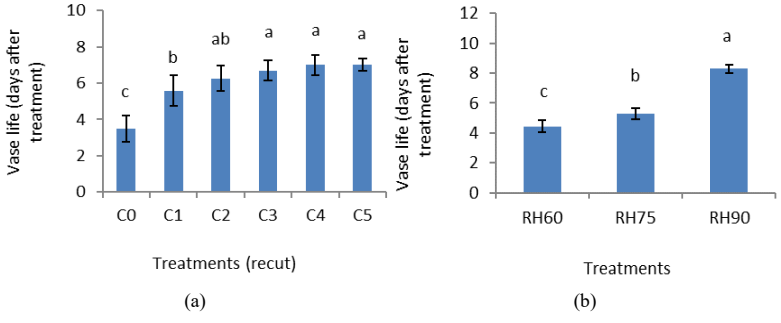
5 Results

5.1 Bucket experiment

5.1.1 Vase life

ANOVA revealed that various RH and re-cuts 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 re-cuts. Mean comparison of results showed that different re-cut significantly ($P \leq 0.05$) affected flower vase life. Cut flowers with no re-cut significantly ($P \leq 0.05$) had the lowest vase life. However, cut flowers which re-cut 3, 4, and 5 cm had the highest vase life compared to no re-cut and 1 cm re-cuts. No significant difference was found between 1 and 2 cm re-cuts. It is found that at least 2 cm re-cut is necessary to get the highest vase life [Figures 1(a) and 4]. The result also revealed that different RH 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% RH significantly increased flower vase life compared to 60% RH [Figure 1(b) and 4].

Figure 1 Vase life of rose flowers in, (a) different RH (b) various re-cutting (see online version for colours)

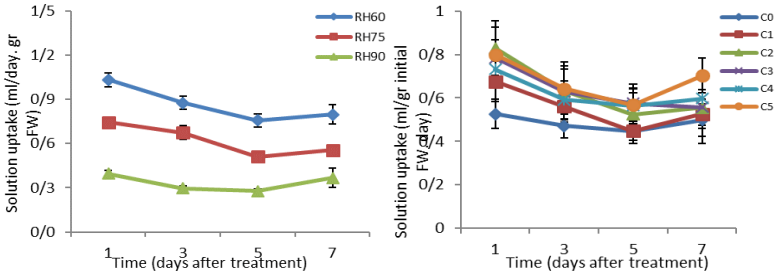


Notes: Different letters indicate significant differences determine using a Duncan's multiple range test ($P < 0.05$). Error bars = SE ($n = 8$).

5.1.2 Solution uptake

The result of experiment revealed that cut flowers placed in 60% and 90% RH had the highest and lowest solution uptake during whole experiment time, respectively. However, 75% RH made intermediate effects on cut flowers [Figure 2(a)]. Mean comparison revealed that re-cut 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 re-cut (C0) had the lowest solution uptake [Figure 2(b)].

Figure 2 Solution uptake of rose flowers in, (a) different RH (b) various re-cutting (see online version for colours)

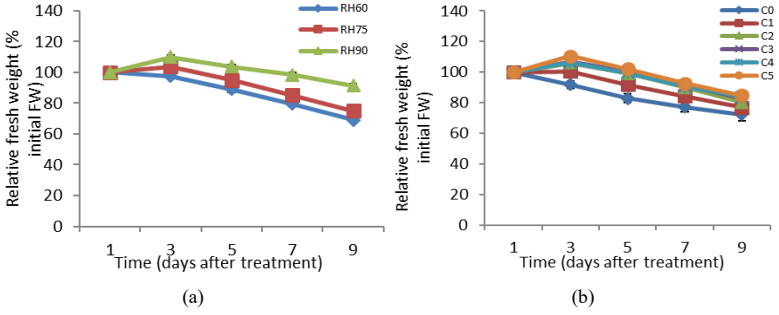


Notes: Different letters indicate significant differences determine using a Duncan's multiple range test ($P < 0.05$). Error bars = SE ($n = 8$).

5.1.3 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% RH had the highest and lowest relative fresh weight during whole experiment time, respectively. However, 75% RH made intermediate effects on cut flowers [Figure 3(b)]. Mean comparison revealed that re-cut 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 re-cut had the lowest relative fresh weight during experiment [Figure 3(b)].

Figure 3 Relative fresh weight of rose flowers in, (a) different RH (b) various re-cutting (see online version for colours)



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

5.2 Vase experiment

5.2.1 Vase life

ANOVA revealed that various RH and re-cuts 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 re-cuts. The result revealed that different RH 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% RH significantly increased flower vase life compared to 60% RH [Figure 5(a) and 8]. In fact, with increasing RH, flower vase life increased too.

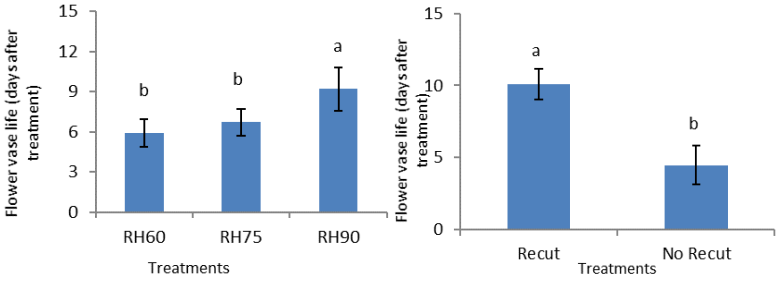
Figure 4 Effects of various RH, (a) 60% (b) 75% (c) 90% with different re-cuts on cut rose flower vase life (see online version for colours)

(a)

(b)

(c)

Figure 5 Vase life in, (a) different RH (b) different re-cutting conditions (see online version for colours)



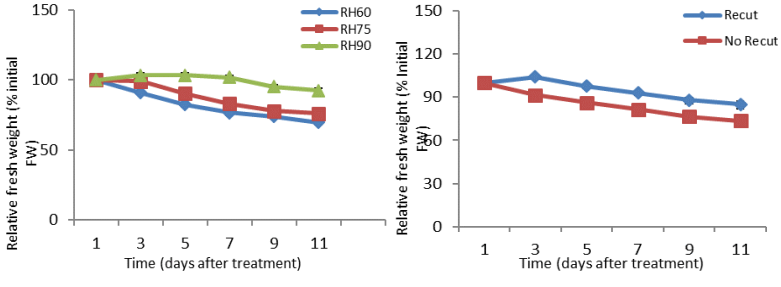
Notes: Different letters indicate significant differences determine using a Duncan's multiple range test ($P < 0.05$). Error bars = SE ($n = 5$).

Cut flowers with no re-cut significantly ($P \leq 0.05$) had the lowest vase life. However, cut flowers which re-cut 5 cm had the highest vase life compared to no re-cut. It is concluded that re-cut the flowers increased its vase life two times compared to no re-cut [Figures 5(b) and 8].

5.2.2 Relative fresh weight

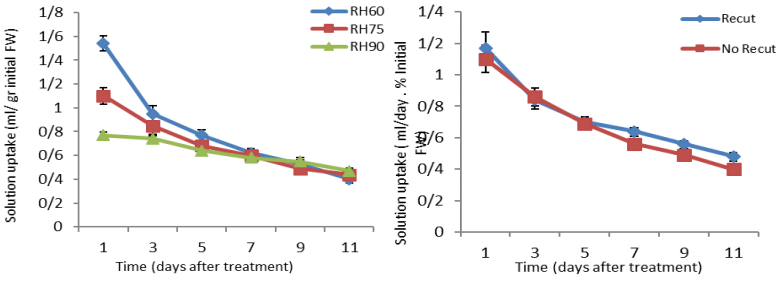
The result of experiment revealed that cut flowers placed in 90% and 60% RH had the highest and lowest relative fresh weight during whole experiment time respectively and 75% RH made intermediate effects on cut flowers [Figure 6(b)]. Mean comparison revealed that re-cut flowers with 5 cm significantly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

Figure 6 Relative fresh weight in, (a) different RH (b) different re-cutting conditions (see online version for colours)



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

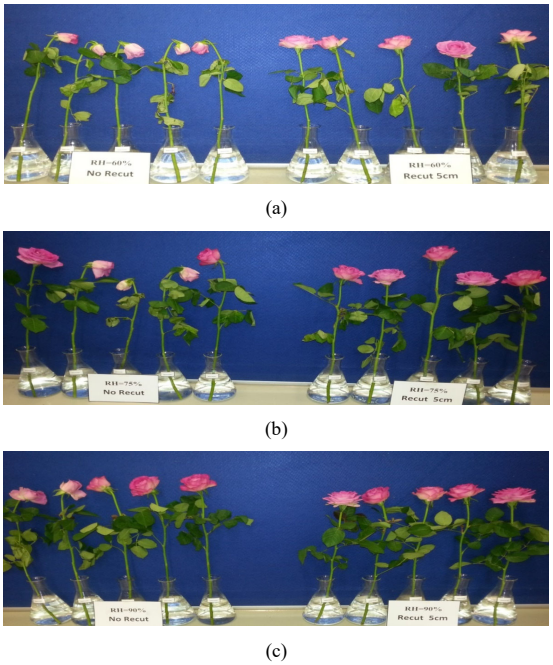
Figure 7 Solution uptake in, (a) different RH (b) different re-cutting conditions (see online version for colours)



(a) (b)

Notes: 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% (c) 90% and re-cut on flower vase life (see online version for colours)



(a)

(b)

(c)

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

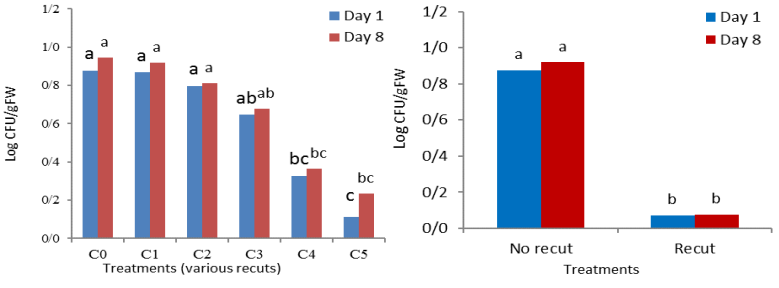
5.2.3 Solution uptake

The result of experiment revealed that cut flowers placed in 60% and 90% RH significantly had the highest and lowest solution uptake till days seven respectively. After days seven, solution uptake in flowers placed in 90% RH remained constant [Figure 7(a)]. Whereas, solution uptake in flowers placed in 60 and 75% RH decreased because of flowers drying. It is also revealed that re-cut flowers significantly had the highest and significant solution uptake during experiment after days five compared to no re-cuts [Figure 7(b)].

5.2.4 Bacteria count

The result of mean comparisons revealed with increasing RH from 60% to 90%, bacterial populations was increased too. But, no significant ($P \leq 0.05$) differences were found among them. However, with re-cutting 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 is inhibited bacterial multiplications. In fact, bacterial count in the 5 cm of stem end showed that, bacterial populations were significantly decreased after 4 cm re-cut compared to re-cut less than 3 cm re-cut [Figure 9(a)]. However, in vase experiment, there were significant ($P \leq 0.05$) differences between re-cut and non-re-cuts flowers. However, it was found much more bacterial populations in stem ends on non-re-cut flowers compared to re-cut flowers [Figure 9(b)].

Figure 9 Bacterial population in different re-cutting stem ends in cut rose flowers in, (a) bucket experiment (b) vase experiment (see online version for colours)



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

6 Discussion

Range of factors including preharvest growth conditions, shipping overseas, proper harvest time, and appropriate storage can affect flowers longevity (In et al., 2016a, 2016b, 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 re-cut 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 seven 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 is reported that low temperature and high RH decreases water loss and maintain flowers quality (Ahmad et al., 2011). The difference between saturation vapour pressure and actual air vapour 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 RH 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 RH condition (Shimizu-Yumoto and Ichimura, 2007).

Moreover, Faragher et al. (1986) reported that although keeping of cut rose flowers (*Rosa hybrid L*. cv. Mercedes) at 65% 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 RH have also been suggested (Harkema et al., 2013). 90% RH has been preferred for keeping of *Anthurium andraeanum lindl*, *Strelitzea reginae* (Vieria et al., 2014).

Results of both bucket and vase experiments also showed that re-cutting rose flower stem could extent it is vase life, maintain higher fresh weight and uptake more solution in comparison to no re-cut. Experiment with various re-cut showed that re-cutting 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 is necessary to re-cut 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 is found that there was significant negative correlation between vase life and stem length (Mortensen and 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 is 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 RH from 60% to 90%, bacterial populations were increased too. However, with re-cutting 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 and 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 (Hussein and Yassin, 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).

7 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 re-cutting cut H_3O 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 re-cut the stem end from 0 to 5 cm, bacterial population in the stem was decreased too.

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