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Response of Cut Rose Flowers to Relative Humidity and Recut During Postharvest Life

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Abstract

Experiments were conducted to evaluate the response of different originated (the same mother plants with different growers from different cities in Ethiopia) cut rose flowers to various relative humidity (RH) and recut (recut and non-recut). Three different experiments (E1, E2 and E3) using three RH (60, 75 and 90%) and three Ethiopian growing areas (Fleur, Liki and Longonot) with 10 replications were designed. Cut rose flowers from three growing area were recut to 5 cm and placed in 60, 75 and 90% RH as a commercial supply chain. Rose flowers without any recut were selected as control. Mean comparison in three experiments revealed that by increasing storage time in cold room, flower vase life significantly decreased. 75% RH produced the highest flower vase life compared to 60 and 90% RHs. However, mean comparisons showed that cut rose flowers originated from Liki growers significantly had the highest vase life compared to others which followed by Longonot and Fleur ones. The results revealed that 5 cm cut the end of flower stem significantly increased flower vase life compared to control. Mean comparison of bacterial populations in commercial supplies of the three experiments, declared the highest amount of bacteria in E1 than the other experiments. Cut rose flowers originated from Liki growers had the highest bacterial populations at the bottom part of the stem compared to the other growers. The results also showed that the end part of stems contain more bacterial agent compared to upper parts.

Keywords: Bacteria, Cold room, Growers, Vase life.

Introduction

Roses (*Rosa hybrida* L.) are the most important ornamental plants around the world which belong to Rosaceae family (Butt, 2003). Roses called "The Queen of Flowers" (Mohy Eldeen, 2011) are important ornamental crops because of theirs high commercial value and widespread cultivation. Cut roses account for about 31% at the European auctions (Heinrichs, 2008; Korban et al., 2007). Cut rose cultivars varied considerably in vase life from 4.5 to 18.8 days at 21 °C depends on genotypes (Macnish, 2010). Depends on cultivars, cut rose flowers are sensitive to improper storage and shipping temperatures, microbes in hydration and vase solutions, and other handling practices that damage flower quality (Silvanda et al., 2011; Lobe et al., 2004; Van doorn, et al., 1997; Van Meetern, 2001; Bleeksma and van Doorn, 2003). The short vase life of cut rose flowers could be related to excessive water loss from the rose

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leaves, resulting in leaf desiccation and development of bent necks (Mortensen and Fjeld, 1998). Furthermore, it has been also reported that the variation in rose flower longevity has been associated mainly with vascular obstruction that affects water conduction through the stem (Torre and Fjeld, 2001; van Doorn, 1997). Three main parameters affect cut rose flower senescence including: water balance, supply of carbohydrates and susceptibility to ethylene (Paulin, 1997). Keeping cut flowers in water frequently developed water deficiency that is caused by xylem occlusion at the basal part of the stem (Dixon & Peterson, 1989). Several parts of cut flowers compete for water when the transpiration rate is more than water uptake, due to vascular occlusion, cavitation or air embolism, (Zieslin et al., 1978). However, it was found that various cut rose cultivars showed a range of responses to ethylene treatment, such as inhibition of opening, acceleration of opening, abnormal opening, petal and leaf abscission, and loss of petal glossiness (Reid et al., 1989). Wilting of rose petals is caused by either excessive water loss or lack of absorption and vascular blockage. In the process of respiration, when flowers are placed in warm environments with low humidity, water loss is often increased. However, stem blockage was the most common cause of poor absorption. The xylem becomes clogged and no water movement can occur in the stem (Reid et al., 1989). In some cut rose cultivars, wilting the flower stem and bent neck can occur due to vascular occlusion which inhibits water transduction. In fact, loss of cell turgor at the unlignified pedicel will occur and cause "bent neck" (Burdett, 1970; Zieslin et al., 1989). Bent neck syndrome is a widespread problem damaging millions of cut rose flowers before the end of inherent vase life. Moreover water status, structure and function of peduncles, exactly, tissue strength, may determine bent-neck susceptibility (Uzaki, et al., 2012). However, it has been reported that bent neck in roses occurred with high RH exposure (Tore et al., 2000). Commercially, cut rose flowers are usually harvested at the open bud stage and are extremely susceptible to water deficit stress during postharvest handling. Abnormal flower opening, flower wilting, wilting of the pedicel (bent neck), and failure to open are the results of water deficit stress (Jin et al., 2006; Xue et al., 2008).

Antimicrobial compounds in the vase water applied at adequate concentrations inhibited bacterial growth and delayed both a bacterial blockage in the xylem and flower wilting (van Doorn, 1997 & 2012).

The aim of the present experiment was to investigate the effects of various RH, growing areas and recut on vase life of cut H_3O rose flowers.

Materials and Methods

Plant material

Cut H3O rose flowers at the commercial stage of bud opening (petals starting to reflex) were obtained from MM Flower Factory (flowers imported from Ethiopia according to standard condition for flowers transport by airplane) in Cambridge and transferred to Reading University and were held in a cold room (4°C) and then transported to the experiment chambers (phytotrons). Three different experiments (experiment one=E1, experiment two=E2 and experiment three=E3) based on Completely Randomized Designs (CRD) with factorial arrangement (three RH, recut and non-recut and three growing areas) with 10 replications were conducted. Cut H3O rose flowers obtained from MM Flower Factory in Cambridge which imported from three Ethiopian growing areas (Fleur, Liki and Longonot areas) were used. All obtained cut H3O flowers (180 stems) cut to 40 cm height and stripped leaves, then divided to three groups (each group included 60 stems) according to growing areas (Fleur, Liki and Longonot areas), placed in bucket and kept in phytotron (The phytotrons condition was 22±2°C, 60% RH, 10 µmol. $m^{-2}.s^{-1}$ irradiance with 12 h photoperiod.) as a

commercial supply chain for four days. Half of each group recut to 5 cm and half of them as non-recut flowers for control. After four days keeping flowers in phytotrons based on commercial supply chain, each group divided to three sections. Each section included 20 stems (10 stems recut and 10 stems non-recut) and transferred to bottles containing 200 ml distilled water and Crysal (each stem in one bottle).

After that, all bottles contain cut H3O rose flowers from each section transferred to phytotrons with different RHs (60, 75 and 90%). The phytotrones condition was 22±2°C with 10 µmol. $m^{-2}.s^{-1}$ irradiance from cool white florescent lamps and 12 h photoperiod).

Assessments

Vase life

Vase life was recorded as the numbers of 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 (Mayak and Halevy, 1974; Liao et al., 2000).

Relative fresh weight (RFW)

Relative fresh weight of stems was calculated every two days using the formula: RFW (%) = $(Wt/W_{t=0}) \times 100$; where Wt = weight of stems (g) at t = days 0, 2, 4, 6, etc. and $W_{t=0}$ = weight of the same stem (g) on day 0.

Solution Uptake

Vase solution usage was determined every two days after transferring from bucket to vases using the formula: Solution uptake (ml day^{-1} g^{-1} fresh weight) = $(St_1\text{-}St)/W_{t=0}$; where, St = solution weight (g) at t = days 1, 2, 3, etc. S_{t-1} = solution weight (g) on the preceding day, and $Wt_{=0}$ = fresh weight of the stem (g) on day 0.

Bacterial count:

Preparation of nutrient agar for total plate count

standard plate count agar (APHA) (11.75 g) (Oxoid Ltd, Basingstoke, Hampshire, UK) was diluted in 500 ml of distilled water in 500 ml Scott Duran bottle and was stirred on a hot plate using a magnetic stirrer until boiling giving the concentration of 2.4% (w/v). Then, it was sterilized for 15 min at 121 °C. The media were kept in 50 °C water bath (Grant Operation, Cambridge, UK) to maintain the liquid state. The media were transferred to falcon tube prior of usage and the temperature was measured by using sterile thermometer to confirm the temperature did not exceed 50 °C.

Preparation of maximum recovery diluent for sampling preparation

9.5 g of maximum recovery diluent (MRD) (Sigma Aldrich, Missouri, USA) was diluted in 1 L of distilled water (0.95%) (w/v) in 1 L Scott Duran bottle and stirred using magnetic stirrer until completely dissolved. MRD diluent was poured in 100 ml Scott Duran bottle with 90 ml in each bottle. Then, it was sterilized for 15 minutes at 121^0 C. The mixture was cooled down in laminar flow hood before being used or was kept in 4^0 C cold room for longer term storage.

Bacterial count:

To count bacterial population, 5 cm of stem end and 5 cm from upper part of stem were cut. The selected parts of stems were sterilized by careful blotting with ethanol (98% v/v). After that, further cut the stem into segments of 2 mm and weighted individually. The prepared segments transferred to falcon tubes and added 9 ml of maximum recovery diluent (MRD) (Sigma Aldrich, Missouri, USA) and vortexed for 1 min. Homogenized/inoculum (1 ml) was sampled from the falcon tube and it was serially diluted in 9 ml MRD to obtain 10^{-2}, 10^{-3}, 10^{-4} until 10^{-5}. Then 1 ml of the respective solution was placed on 15 ml nutrient agar with 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^{o}C in inverted condition. After 24 ± 1h of incubation, numbers of colonies per plate were counted using a colony counter. Plates with colonies more than 300 colonies are labeled with TNTC (too numerous to count) and plates with colonies less than 30 colonies were discarded. Three stems of each treatment were used for bacterial count.

Data Analysis

All obtained data were subjected to analysis of variance (ANOVA) using Statistical Analysis System Ver. 9.2. (SAS Institute, Cary, NC, USA). All data was analyzed based on Completely Randomized Designs (CRD) with factorial arrangement (factors included three rages of RH, recut and non-recut and three growing areas) with 10 replications. T-test was used to compare the differences among measured traits of three different experiments (E1, E2 and E3). Mean differences between treatments were compared using Duncan's Multiple Range Test at P≤ 0.05. Graphs were then plotted using Excel spread sheet .

Results

In all figures and tables:

RH60= 60% Relative humidity, RH75= 75% Relative humidity, RH90= 90% Relative humidity.

Sol1= Solution uptake at day 1,…Sol 9= Solution uptake at days 9.

FW1= Relative fresh weight at day 1…FW9= Relative fresh weight at days 9.

P1= Fleur grower, P2= Liki growers & P3= Longonot growers.

e= end part & t= top part of stem.

E1, E2 &E3= Experiment 1, Experiment 2 & Experiment 3.

Vase life

Analysis of variance revealed that various RH significantly (P≤0.05) affected flower vase life in various storage times. In first experiment, the highest and significant vase life was found in flowers placed in 75 % RH compared to 60% RH. However, 75% relative humidity had no significant differences with flowers placed in 90% RH (Fig. 1A).

In second and third experiments, there were significant (P≤0.05) differences among all relative humidities. In fact, in two last experiments, 75% and 90% RH produced the highest flower vase life compared to 60% RH conditions (Fig. 9A). However there was a significant (P≤0.05) difference between 90 and 60% RH. Flower vase life in 90% RH, was higher than vase life of flowers in 60% RH. Mean comparison in three experiments revealed that with increase in storage time in cold room, flower vase life was decreased. Exactly, the highest reduction was happened in 60% RH and no significant (P≤0.05) difference was found between 75% and 90% RH (Fig. 1A).

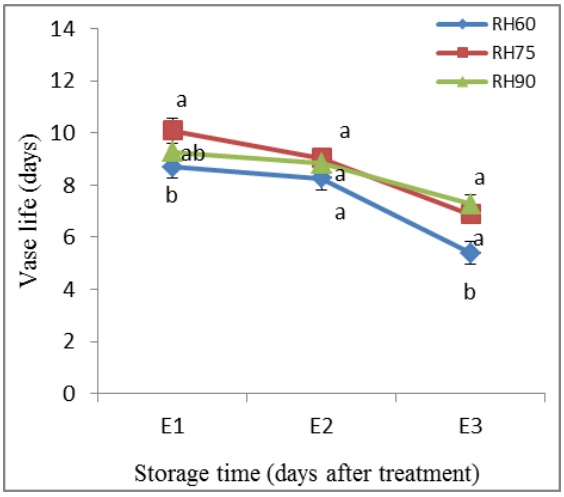
The results revealed flowers originated from various growers form Ethiopia significantly (P≤0.05) affected flower vase life. However, mean comparisons showed that cut rose flowers originated from Liki growers significantly had the highest vase life compared to others which followed by Longonot and Fleur growers (Fig. 9 B). Moreover, there were significant (P≤0.05) differences among all other origins (Fig. 1B).

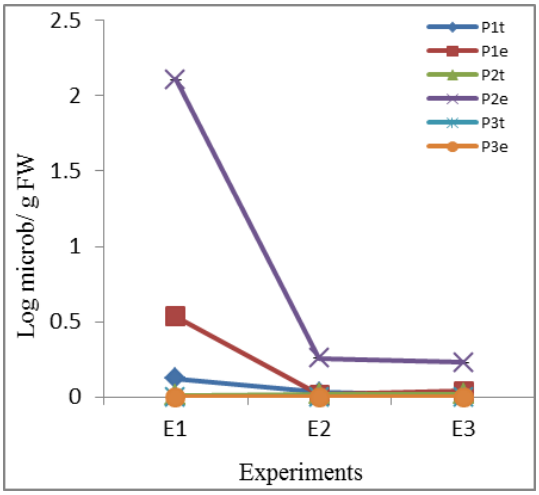
In case of recut, the result revealed that 5 cm cut the end of flower stem significantly increased flower vase life compared to no cut ones (Fig. 1C).

Relative fresh weight

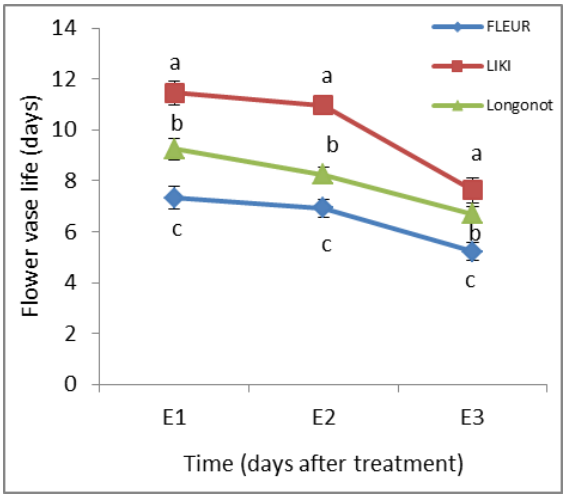
The result of experiment revealed that different relative humidities significantly affected relative fresh weight during experiment except for day 1 and day 13. In other days, cut flowers placed in 75% relative humidity significantly (P≤0.05) maintained higher fresh weight compared to 60% relative humidity. However, cut flowers placed in 90% relative humidity had no significant differences (P≤0.05) with ones that placed in75% relative humidity.

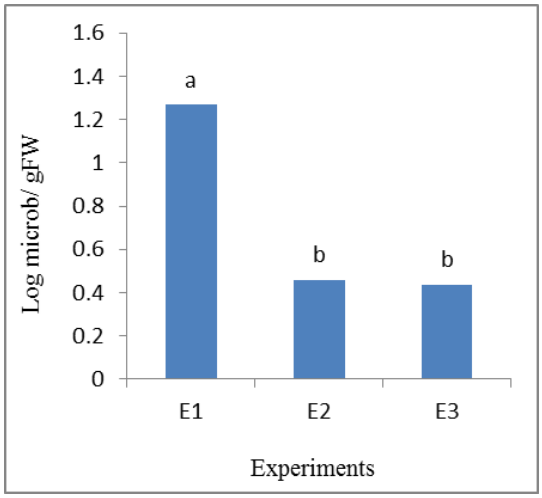
A



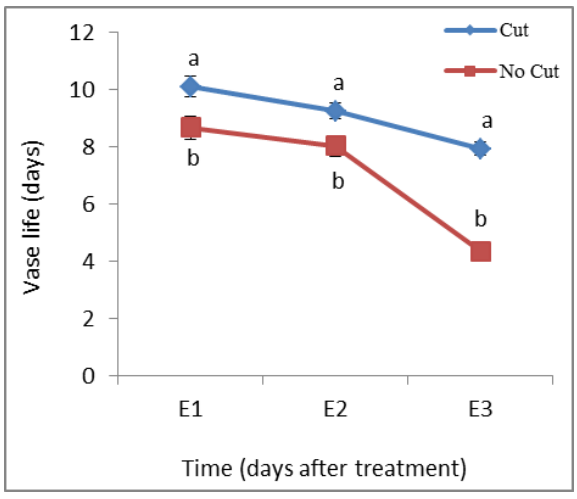


B





C



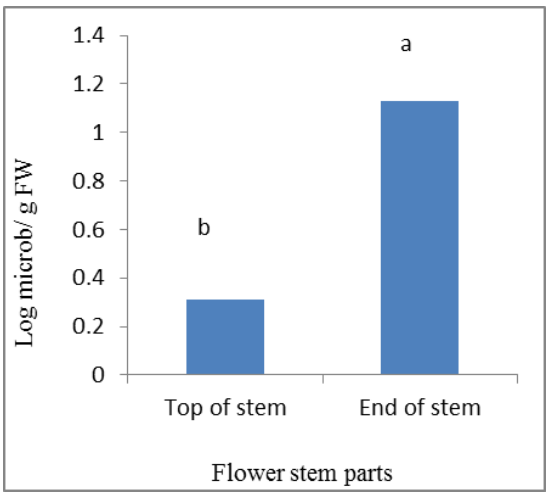
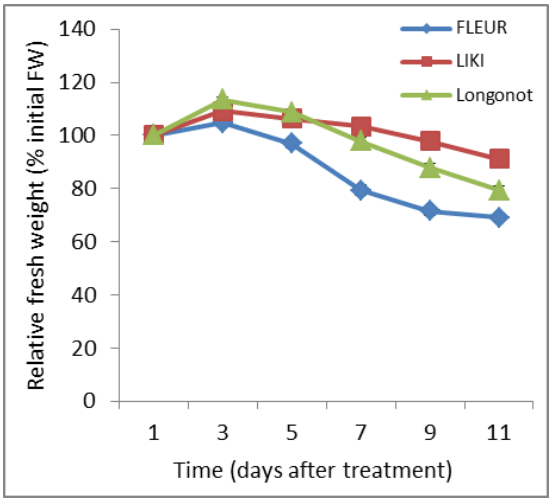
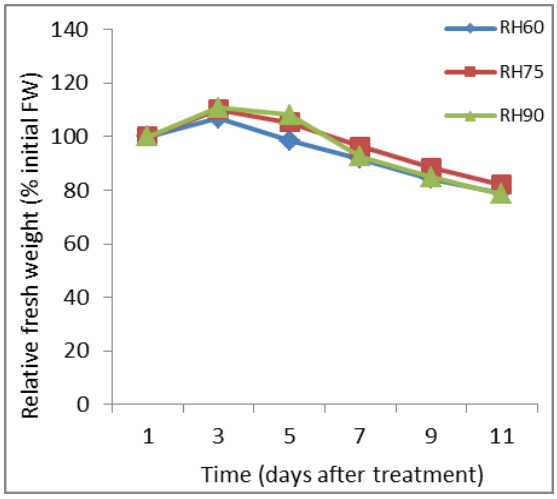


Fig. 1. Effects of different RH (A), different growing area (B) and cut and recut (C) on vase life of cut rose flowers in different experiment.

Fig. 2. Effects of different growing area (P1, P2 & P3) and stem part (e= end part & t= top part of stem) interactionS in different experiment (B) and cut and recut (C) on bacterial count of cut rose flowers.

E1





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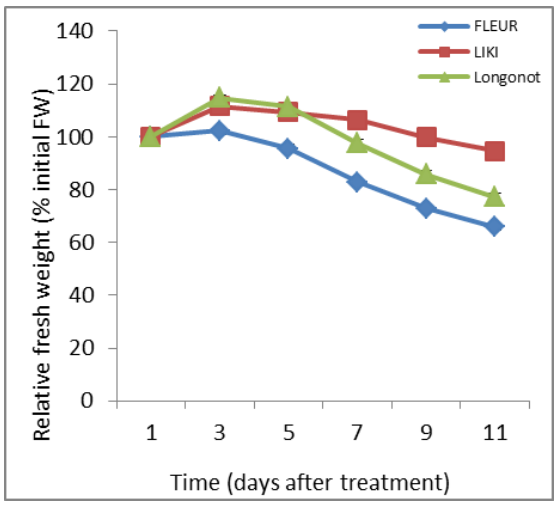
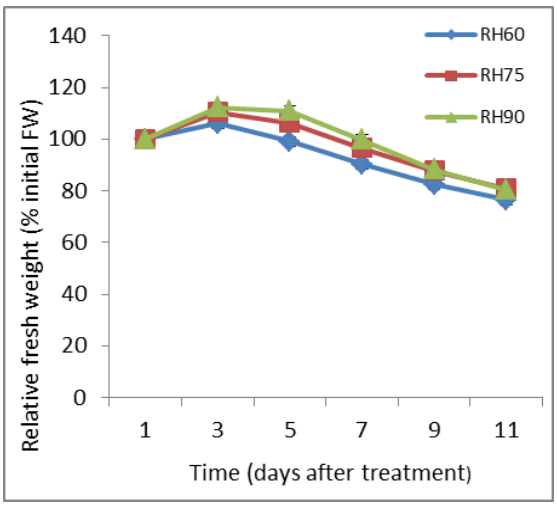
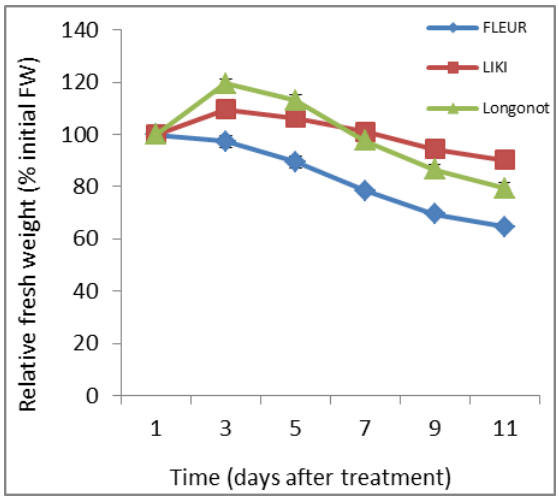




E3



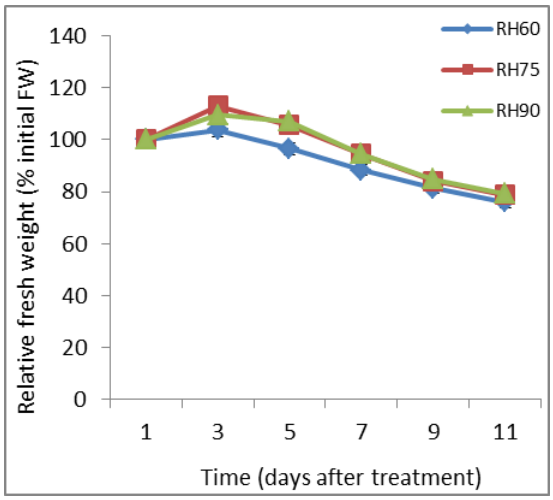
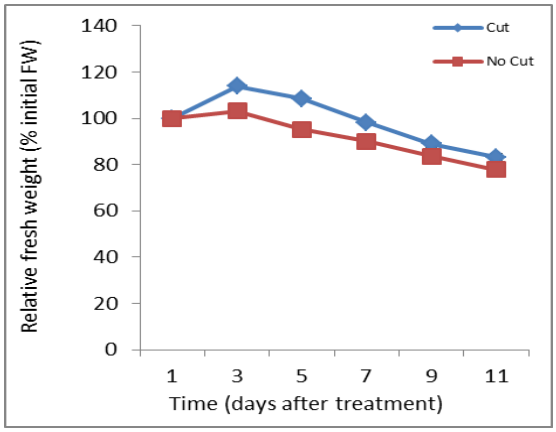
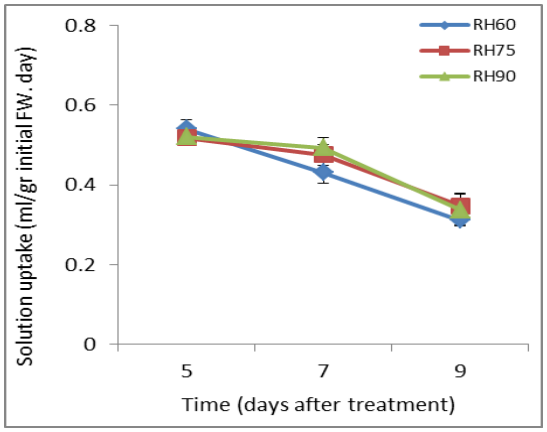


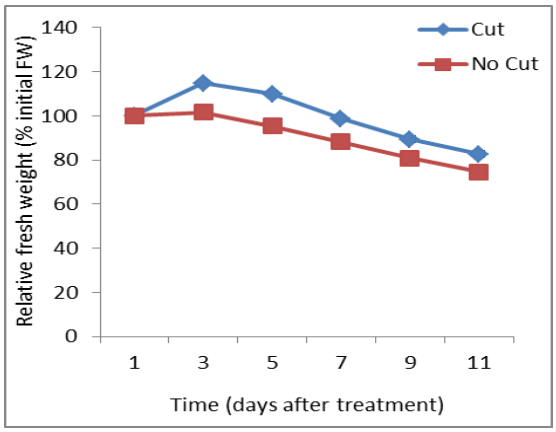
Fig. 3. Effects of different RH on relative fresh weight of cut rose flowers in different experiment (E1, E2 &E3).

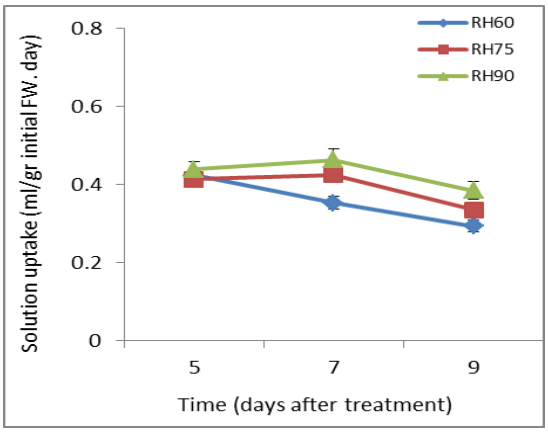
Fig. 4. Effects of different growing area on RFW of cut rose flowers in different experiments (E1, E2 &E3).



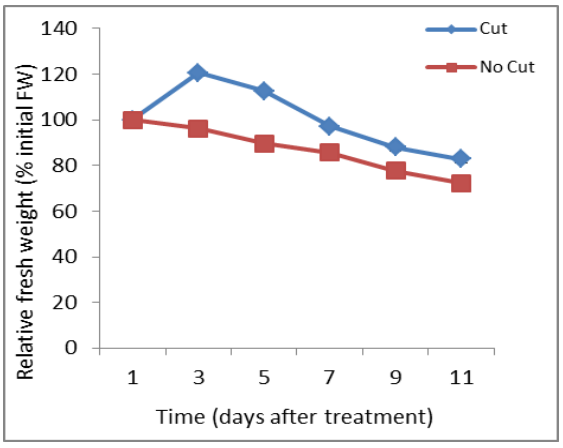


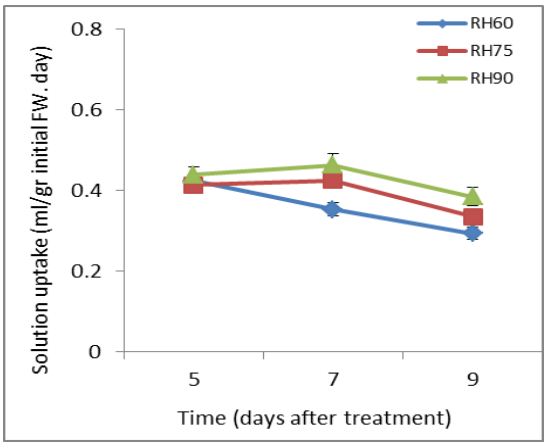
E1





E2





E3

Fig. 5. Effects of recut and non-recut on relative fresh weight of cut rose flowers in different experiment (E1, E2 &E3).

Fig. 6. Effects of different RH on solution uptake of cut rose flowers in different experiments (E1, E2 &E3).

The result of experiment revealed that different storage time affected relative fresh weight. In fact, with increasing storage time in cold room, relative fresh weight of flowers decreased. Highest decrease in relative fresh weigh was happened in last storage time (Fig. 3).

Mean comparisons revealed that relative fresh weight of flowers originated from various growers from Ethiopia significantly ($P \leq 0.05$) differed. However, in all three experiments, cut rose flowers originated from Fleur growers, significantly had the lowest RFW during experiment time compared to the others. In fact, cut H3O rose flowers harvested from Liki greenhouse, after days 7 had the highest and significant RFW during experiment time which significantly followed

(except days 5) by Longonot ones during the rest of experiment (Fig. 4). The results of experiment revealed that recut flowers significantly ($P \leq 0.05$) had higher RFW compared to non-recut flowers during whole experiments. However, the results also showed that with increasing storage time in cold room, RFW of both recut and non-recut flowers decreased (Fig. 5).

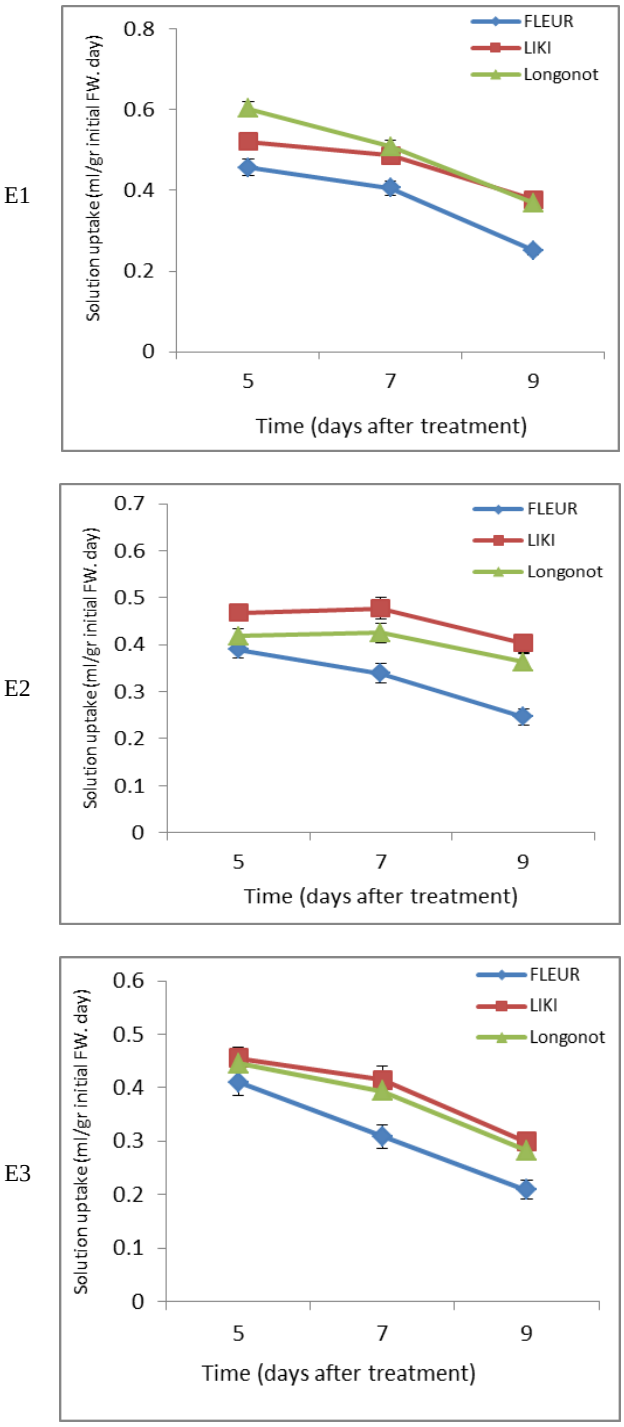


Fig. 7. Effects of different growing area on solution uptake of cut rose flowers in different experiment (E1, E2 &E3).

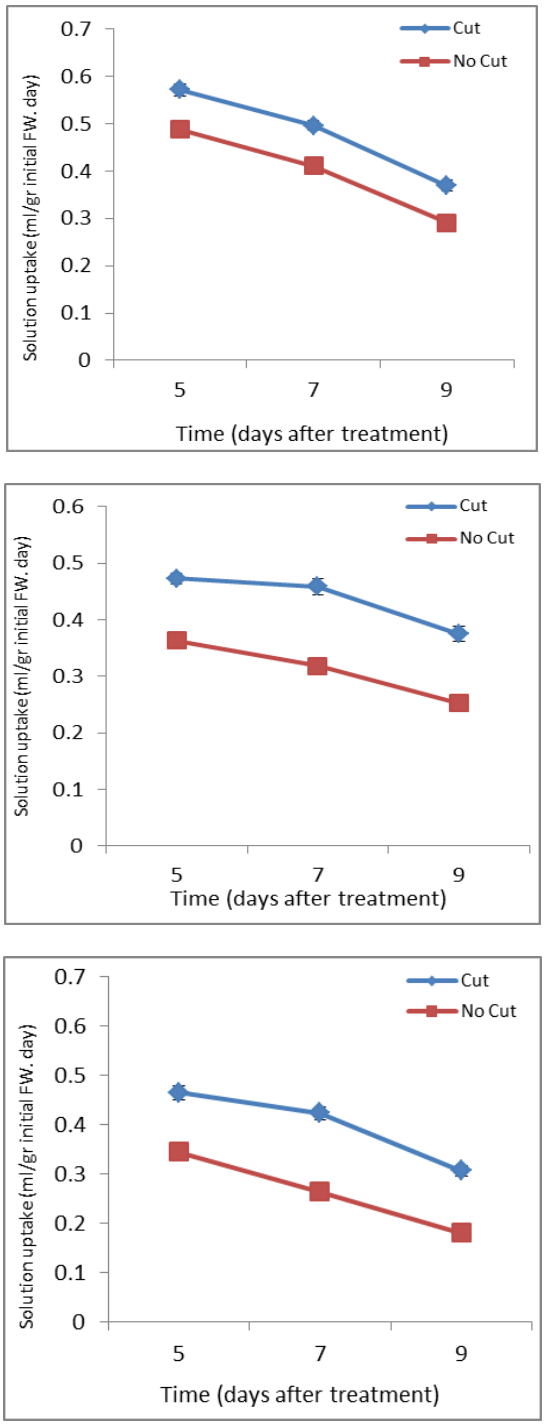


Fig. 8. Effects of recut and non-recut on solution uptake of cut rose flowers in different experiments (E1, E2 &E3).

Solution uptake

The result of experiment revealed that cut rose flowers placed in 90% and 60% relative humidity significantly (P≤0.05) had the highest and lowest solution uptake during whole experiment time respectively. However, in first experiment after obtaining flower from MM flowers Company, cut rose flowers placed in 75 % relative humidity had the highest solution uptake compared to the others. However, no significant difference was found between 75 and 90% RH. In second and third experiments after storage in cold room, mean comparison showed that cut rose flowers placed in 90% relative humidity considerably and significantly (P≤0.05) maintained solution uptake compared to the other RHs (Fig. 6). However, the results also revealed that with increasing storage time in cold room, solution uptake of flowers in all RH decreased (Fig. 6).

Mean comparisons revealed that solution uptake of flowers originated from various growers from Ethiopia significantly (P≤0.05) differed. However, in all three experiments, cut rose flowers originated from Liki and Fleur growers, significantly (P≤0.05) had the highest and lowest solution uptake in all experiments respectively (Fig. 7).

The results of experiment revealed that recut flowers significantly (P≤0.05) had higher solution uptake compared to non-recut flowers during whole three experiments. However, the results also showed that with increasing storage time in cold room, solution uptake of both recut and non-recut flowers decreased (Fig. 8).

Fig. 9. Effects of different relative humidity (A) and growing area (B) on cut rose flowers. (P1= Fleur grower, P2= Liki growers & P3= Longonot growers; RH60= 60% Relative humidity, RH75= 75% Relative humidity, RH90= 90% Relative humidity).

Bacterial count

The results of experiment revealed that cut rose flowers originated from various growers from Ethiopia significantly (P≤0.05) differed in bacterial count. Mean comparison showed that E1 (first experiment) significantly had highest bacterial population compared to E2 (second experiment) and E3 (third experiment). However, there was no significant (P≤0.05) difference between E2 and E3. It means that keeping flowers in cold room significantly decreased bacterial population on the stem (Fig. 2B). The result showed that cut rose flowers originated from various growers from Ethiopia significantly (P≤0.05) differed in microbe population. Cut rose flowers originated from Liki growers significantly (P≤0.05) had the highest microbe population in the bottom part of stem compered to others. However, cut rose flowers from Fleur significantly differed from Liki and Longonot originated flowers (Fig. 2A). The results revealed that cut H_3O rose flowers obtained from Longonot growers did not included more bacteria in the stem (30 to 300 per stem was included in calculation). Mean comparison showed that the end of stems contain lots of bacteria compared to top of stem (Fig. 2C).

Discussion

The result revealed that by increasing RH from 60% to 90%, flower longevity significantly (P≤0.05) increased. The highest vase life was found in flowers placed in 90% RH compared to 60 and 75% RH. In fact, in the present study high RH significantly improved all measured traits. In fact, there was no dried leaf in 90% RH (data not shown). Doi and colleagues (2000) reported that for prolonging vase life of cut flowers and maintaining its turgidity, it should be kept in 90-92% relative humidity. Vieira and colleagues (2014) reported that ideal RH for Strelitzia flowers was 85-90% which caused an increase in flower vase life. Paull (1987) reported that high RH (90-95%) during postharvest storage increased cut anthorium flower vase life. Our experiments revealed that by increasing flower vase life relative fresh weight, solution uptake and flower diameter and chlorophyll content were improved. Evaluation of *Heliconia psittacorum* L. over a 10 day storage period at various RHs showed that those flowers stored at 90% RH had the lowest fresh weight loss (Bañuelos-ernándeza et al., 2016). It has been reported that reduction of vase life may be due to reduced water uptake and early senescence which resulted in vascular blockage (Song et al., 1996; Ahmad et al., 2014). However, to improve water uptake in cut flowers and foliage reduction in leaf area, keep them in an environment conducive to less water loss (viz. low temperature and high RH was needed (Jones et al., 1993; Aliniaeifard and van Meeteren, 2016). In cut roses, water stress is a main proof of vase life reduction due to lack of proper flower opening, wilting of foliage and/or bent neck (van Doorn and Perik, 1990; Knee, 2000). Water loss or insufficient uptake due to adverse postharvest condition (water vapor pressure deficit or low humidity and high temperature) is one of the most important reasons for reduction of flower longevity (van Meeteren and Aliniaeifard, 2016). So, lack of sufficient RH for cut produces, will accelerate shortening of cut produce display life. In fact, occlusions due to microbial, physiological and physical plugging of xylem vessels affected water uptake by cut stem (e.g. Nijsse et al., 2000; van Doorn and Cruz, 2000). It has been reported that low RH condition caused greater absorption of pulsing solution (Shimizu-Yumoto and Ichimura, 2007) which confirmed our finding results. However, our results was not confirmed by results of Faragher et al., 1986 which reported that although storage of cut rose flowers (*Rosa* hybridaL. cv. Mercedes) at a RH of 65% reduced petal water content by

20% in comparison with flowers stored at 95% RH, it did not shortened the vase life, which is in accordance with the obtained results for RFW. However, in case of flower vase life it did not confirm our results, which can be due to variation in preharvest condition. Natural senescence was due to vascular occlusion, which inhibits water supply to the flowers (Van Doorn, 1997) because of bacteria (Durkin,1980), air emboli and physiological responses of stems to cutting (Van Doorn, 1990). However, in our experiment, there was no relationship between bacteria and reduction of vase life. It should be because of recut 5 cm of the stem end. In fact, most of bacterial population was seen in the end of stem. Therefore, with recut the end of stem as much as 5 cm, most of them was discarded. In recut flowers, bacteria did not affected flower vase life. However, in non-recut rose flowers obtained from Fleur, the top part of stem contained considerable bacterial colonies. Low vase life confirmed that bacteria affected flower vase life. It was reported that bacterial accumulation in the end of cut stem flowers or solution uptake significantly decreased flower vase lives, which confirm our research results in case of non-recut flowers (Durkin, 1980, Zagory and reid, 1986, W.G. van Doorn and Y. de Witte, 1997).

Our results revealed that in three relevant experiments with increasing storage time in cold room, all measured traits especially vase life of flowers significantly ($P \leq 0.05$) decreased. Ahmad and colleagues (2014) reported that inflorescences of both cultivars of 'Line Dance' and 'Tap Dance' eremurus stored for 1 week had similar vase life as of un-stored stems irrespective of dry or wet storages. Storage longer than 1 week greatly reduced the vase life of both cultivars. Cut stems of several species such as dahlia (*Dahlia* × *hybrida* Cav.), trachelium (*Trachelium caeruleum* L.), or zinnias are susceptible to damage by extended storage at low temperatures and have resulted in shorter vase life following storage (Dole et al., 2009; Ahmad et al., 2012).

It can be concluded that cut flowers that recut for 5 cm had the highest vase life, relative fresh weight, and solution uptake, flower diameter and chlorophyll content compared to non-recut ones. It was found that bark removal at the base of cut rose stems increased water uptake and a 25% increase in FW compared to control (de Stigter and Broekhuysen, 1986). Florists sometimes advocate splitting or crushing stems and also removing bark at the base of the stem to increase water uptake and extend vase life (Jones, 2001; Milner, 2009). Bark removal increased vase life of fresh-cut rose with better maintenance of RFW and sustained vase solution usage (Ahmad, 2011).

The result of experiment revealed that flowers originated from various growers from Ethiopia significantly ($P \leq 0.05$) affected flower qualities. However, cut rose flowers originated from Liki growers significantly had the highest vase life compared to the other growers which followed by Longonot and Fleur ones. Our finding confirmed by study that growing environment can affect cut rose flower vase life. This effect is mainly related to changes in stomata responsiveness, regulating water loss, whereas cut flower carbohydrate status appears less critical (Fanourakis et al., 2013).

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