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Effect of Absorbent Granules Coated by Potassium Permanganate on Postharvest Quality of Rose (*Rosa hybrida*) Cultivars

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Abstract
Enrichment of zeolite and sponge as ethylene absorbent with potassium permanganate was the idea of this study to provide an efficient way to scavenge ethylene during storage period of three *Rosa hybrida* cv. 'Shiraz', 'Avalanche', and 'After-party' cut flowers. A preliminary experiment revealed that two mL of potassium permanganate solution (1 mM) was enough to enrich two grams of zeolite and two cm^3 of sponge. Additionally, one µL L^{-1} of external ethylene also found to be effective on induction of quality damage to certain cut flowers of rose cultivars, which were selected for injection in each isolated container for further evaluations. The treatments used in this study included control (without absorbent), enriched zeolite or sponge, enriched zeolite or sponge + ethylene (one µL L^{-1}). The quality of flowers, weight, number of dropped petals, electrical conductivity of vase solution, ethylene concentration in the container and petal anthocyanins evaluated through/the end of experiment. Results revealed that change or reduction in the quality parameters were minor (more maintenance or stability of quality was achieved) with enriched zeolite and then enriched sponge compared to the control. Enriched zeolite could even maintain the quality of cut flowers at the level of control under external ethylene injection superior to enriched sponge treatment. 'Avalanche' and 'Shiraz' cultivars figured as the most sensitive and resistant cultivars to the evaluated level of external ethylene concentration, respectively.

Keywords: Ethylene Sensitivity, Cut flower Transportation, Postharvest Quality, Superabsorbent, Vase life.

Introduction

Losses of cut flowers such as roses (*Rosa hybrida*) during displacement are significant and will often increase the final price of the product. There are many factors influencing crop loss during handling and transport, which reduces their quality and vase life (van Meeteren and Aliniaeifard, 2016) including senescence as key factor mainly influenced by the hormonal regulation e.g. the presence of external ethylene or ethylene produced by the plant itself, the negative water balance during postharvest handling caused by physiological blockage of vascular, vascular obstruction by microbes (Kamiab et al., 2017) and air bubbles, Botrytis infection and water loss (Chamani and Wagstaff, 2018), activation of enzymes involved in

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discolouring and yellowing of leaves, environmental factors and reduction in the level of energy supply (Halevy and Mayak, 1981). Among these factors, external ethylene existence and its autocatalytic production in many plants have a high prominence and detrimental effect on the quality and longevity of many horticultural products especially roses cut flowers (Reid and Wu, 1992; Macnish et al., 2010). It has been reported that various cut rose cultivars respond differently to ethylene treatment as inhibition/acceleration of opening, abnormal opening, petal and leaf abscission, and loss of petal glossiness (Reid et al., 1989). For this reason, there has long been interested in removing ethylene from the floral crop environments and in suppressing its effects particularly during product transportation.

Observation of leaf senescence in a crop at exposure to exogenous ethylene of 0.5-1 µL L^{-1} (ppm) indicates its sensitivity to the ethylene (Ferrante and Francini, 2006). On the other hand, the sensitive plants could reflect their higher and fast reaction to the external ethylene of 1-10 µL L^{-1} (Abeles et al., 1992). Therefore, short-term exposure of a product to the external ethylene level of 0-10 µL L^{-1} would be sufficient for evaluation of its sensitivity and reaction level to the ethylene, since further concentration rarely observed in the closed or even in the open atmosphere (Ferrante and Francini, 2006). Marked differences in ethylene sensitivity exist between plant species and even within its varieties e.g. *Rosa hybrida* cultivars (Reid et al., 1989). Exogenous or endogenous ethylene exerts similar effects for sensitive roses and induces petal or flower abscission (Scariot et al., 2014). To avoid such detrimental effects on rose cut flowers quality during transport and storage, detection and removal of ethylene is more advisable. Three main approaches of removal, oxidation, absorption, or in combination are available to reduce ethylene levels (Scariot et al., 2014).

The main compounds used as ethylene absorbers are activated carbon and zeolites. The commercial application of activated carbon in the adsorption of gases and vapor started in the 1930s, although the specific use for ethylene was in the late 1950s (Martínez-Romero et al., 2007). Zeolites have great potential in the agro-chemical industry to remove ethylene and are applied as important catalysts for many industrial processes, due to their shape, specific surface area, cation exchange capacity, molecular sieving and adsorption. Concerning this fact, many have investigated adsorption of hydrocarbons and ethylene on this material, experimentally or theoretically (Limtrakul et al., 2001). There are several films filled produced with zeolites that are now used in storage and transport industry of horticultural crops as some mentioned in a review by Martínez-Romero et al. (2007).

The absorbers efficiency could even be greater when used in combination with oxidizers or catalysts (chemi-adsorption) for absorbing or oxidizing the residual ethylene. Potassium permanganate ($KMnO_4$) could oxidize ethylene to the ethylene glycol (Ozdemir and Floros, 2004). However, it must be taken into account that potassium permanganate cannot be used in contact with food products due to its high toxicity; therefore, some devices similar to sachets, films, or filters have been supplied to the markets for this purpose (Martínez-Romero et al., 2007). The scavengers adsorb ethylene and potassium permanganate oxidizes it to the ethylene glycol or acetic acid and finally to the water and CO_2 (Ozdemir and Floros, 2004). The specific surface area and the amount of potassium permanganate affect the performance of these systems. In fact, absorbers other than zeolites such as silica gel, activated carbon, perlite, sponge or alumina might also be enriched by potassium permanganate to achieve higher absorbing or oxidizing capacity. The potential for the formulated 1-MCP-loaded β-cyclodextrin-based nanosponges (β-CD-

NSs) suspension demonstrated to extend the post-harvest longevity of carnation cut flowers (Segli et al., 2011).

Therefore, the present study aimed to investigate effects of zeolite and sponge coated by potassium permanganate on the quality of cut flowers of some likely ethylene sensitive rose cultivars during postharvest stage.

Material and methods

Experimental set up and treatment applications

In this study, two preliminary experiments were conducted. At the first experiment, enough amount of potassium permanganate solution (1 mM) sufficient for enrichment of certain amount of zeolite (PERMUTIT, Zeolite, BDH England) or sponge (industrial grade; Polyester resin based) and to avoid saturation of the existing porosity of materials was used under normal lab conditions. For this, 1 mM of potassium permanganate was added drop by drop to different density or volume of zeolite and sponge until just moistening the materials and not saturating. This trail revealed that 2 mL of potassium permanganate (1 mM) solution is enough to enrich or coat two grams of zeolite and/or two cm^3 of sponge. At the second experiment, the effectiveness of external ethylene concentration was evaluated for visible damages on three different cultivars of *Rosa hybrida* (‘Shiraz’, ‘After-Party’ and ‘Avalanche’) cut flowers quality. To reach this objective, different external concentrations of ethylene were evaluated on different cultivars of roses in isolated containers each containing one cut flower from each cultivar.

The isolated transparent container prepared using two different cut part of 1.5 L mineral water bottles (a thicker lower part from one bottle and a thinner over part from another), putting one (thinner neck) inside the other (thicker neck). Each cut flower (40±1 cm of length) of different cultivars put inside the prepared container that is isolated by transparent tape. Different concentrations of ethylene (0, 0.5, 1 and 2 $\mu L\ L^{-1}$) were injected, and immediately after injection the pores closed by the same transparent tape. A complete randomized design with three replications considered for both trials. These experiments run under normal lab conditions.

Since reported that the most ethylene sensitive cut flowers may lose their longevity in short-term exposure to the external ethylene level of 1 $\mu L\ L^{-1}$ (Reid and Wu, 1992), so ethylene concentrations of 0, 0.5, 1 and 2 $\mu L\ L^{-1}$ applied as evaluation treatments to find efficacy of detrimental ethylene concentration level on rose cut flowers quality. The quality parameters evaluated before ethylene injection using Likert scales of 1-5 as very bad to very good, respectively (Likert, 1932). Treatment containers kept in two different storage rooms, one in normal room temperature of 25±2 °C and supplementary irradiance of 50±5 $\mu mol\ m^{-2}\ s^{-1}$ and the second in cold room temperature of 5±1 °C and the same supplementary irradiance as the first room. Containers opened after 7 days, some traits such as visible quality, number of dropped leaves and number of dropped petals counted, and corresponding effective ethylene concentration (1 $\mu L\ L^{-1}$) taken for the main experiment application.

According to the results of preliminary experiments, the main experiment designed. The running procedure was the same as the second preliminary experiment. Two gram of zeolite and/or two cm^3 of sponge enriched by two mL of potassium permanganate (1 mM) solution according to the first preliminary experiment results. Containers for cut flowers and absorbers prepared as the second preliminary experiment. Treatments included of enriched zeolite absorber (without external ethylene injection), enriched zeolite absorber (with external ethylene injected, 1 $\mu L\ L^{-1}$), enriched sponge absorber (without external ethylene injection), enriched sponge absorber (with external ethylene injected, 1 µL L^{-1}) and control (without absorber and external ethylene).

Likert scale for quality parameters also used in this step before ethylene injection, as well. Treatment containers kept at the same conditions as the second preliminary experiment and containers opened after 7 days as suggested as the longest transport time of cut flowers in the traditional transport system (Leonard et al., 2011). Number of dropped leaves and number of dropped petals counted at this time. After opening of containers, the collected cut flowers put in an open 1.5 L vase container, containing 500 mL of tap water by EC of 750 µmhos cm^{-1} and kept at room temperature for extra 7 days in order to evaluate their vase life and other related parameters. Therefore, traits evaluations performed at three distinct points of first at the beginning (day zero, before closing of the containers), second at the opening of the containers (7 days later) and third at the end (14 days after). Change of traits between two distinct evaluations considered for well assessment.

Measurements

Change of fresh weight, number of dropped petals, visual quality (longevity) and ethylene concentration assessed by considering of the data collected from the first and the second trials (change between 0 and 7th day), while electrical conductivity (EC) of vase water and petal anthocyanins assessed by considering of the data collected from the first and the third trial (change between 0 and 14th day). Visual quality evaluated by using Likert scale of 1-5 for very bad, bad, moderate, good and very good, respectively (Likert, 1932).

Ethylene determination

To measure the amount of ethylene, the air samples were taken by direct discharge to a 6 mL volume vacuumed Venoject tubes (EDTA K3) from each container at the second trail. Then, one mL of each samples injected into the gas chromatography (GC) and the amount of ethylene recorded (before sampling available ethylene in Venoject tubes considered in the calculation).

Anthocyanin determination

Anthocyanin contents of petals assessed by the method of Wagner (1979). Fresh petal samples of 0.1 g were soaked in 10 ml acidified methanol [methanol: HCl 99:1 v/v], crushed and kept at 25 °C for 24 h in the dark. The extracts then centrifuged at 4,000g for 5 min at room temperature. The absorption rate of the supernatant read by spectrophotometer (4802, Unico, NJ, United states) at 550 nm. To calculate the amount of petal anthocyanins, the Beer equation of $A=\varepsilon bc$ in which A, is the absorbance at a given wave length, ε is the extinction coefficient equal to 33,000 (L mol^{-1} cm^{-1}), b is the cuvette width (cm); c is the solution concentration (mol L^{-1}) was used and anthocyanins content were expressed as µmol g^{-1} FW.

Statistical analysis

Statistical analysis performed using MATLAB software and means of comparisons done by Duncan multiple range tests with 95% confidence ($p \leq 0.05$).

Results

Determination of effective ethylene concentration

Results of statistical analysis revealed that visual quality declined and the number of dropped or damaged petals increased by ethylene exposure (Fig. 1 and 2). Although, decline in cut flower quality was observed at 0.5 µL L^{-1} of injected ethylene mainly for 'Avalanche' and 'After-Party' cultivars, however the effective concentration of ethylene for a considerable detrimental impact on visual quality and petal dropping was one µL L^{-1} of injected external ethylene. 'Shiraz' cultivar showed some degrees of ethylene tolerance than the two others at lower concentrations of external ethylene, but not at the higher concentrations.

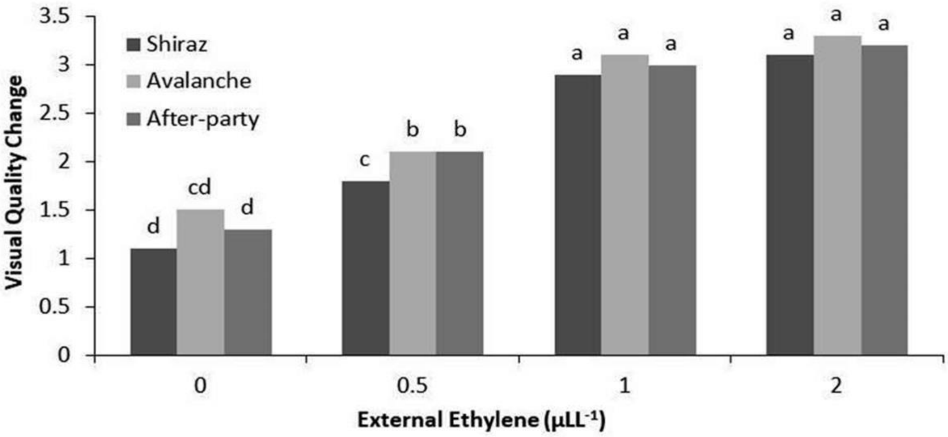

Fig. 1. Effect of external ethylene concentrations on the visual quality change of three *Rosa hybrida* cultivars cut flowers based on Likert scale. Different letters show significant difference among treatments (P≤0.01).

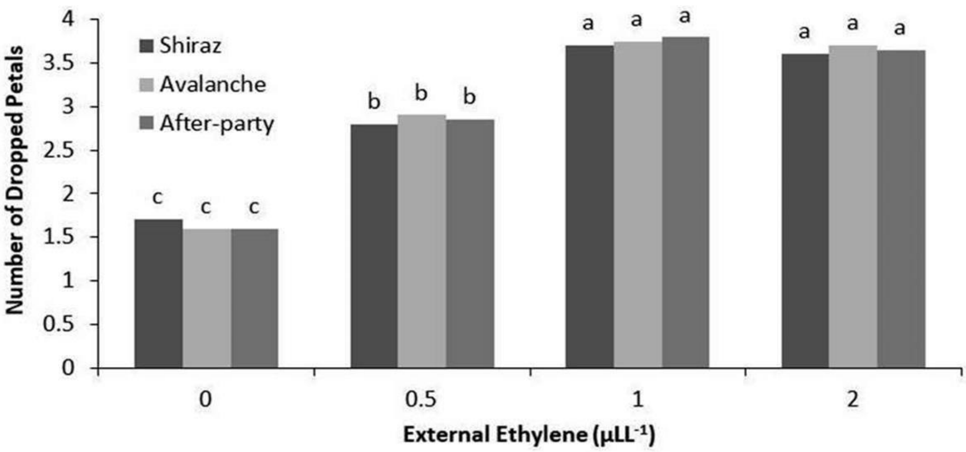

Fig. 2. Effect of external ethylene concentrations on the number of dropped petals of three *Rosa hybrida* cultivars cut flowers. Different letters show significant difference among treatments (P≤0.01).

Determination of ethylene absorbers influence on product maintenance quality

Ethylene absorbers influenced product maintenance quality evaluated in two different storage rooms of normal room temperatures and in cold room. However, since the results obtained from both conditions followed a similar pattern, only the results of room temperature storage condition reported here to avoid repetition.

Results revealed that the largest decline in the quality of cut flowers (visual quality) after two weeks, found in the enriched sponge with potassium permanganate with external ethylene exposure and this decrease was even higher than the control (Fig. 3). In contrast, enriched zeolite with potassium permanganate could maintain the visual quality of cut flowers in all three cultivars in control treatment level (without absorber and external ethylene exposure) even with external ethylene exposure despite the fact that higher decline expected in exposure to the external ethylene. Overall, both enriched sponge and zeolite absorbers had positive impact on keeping the visual quality of examined rose cultivars than the control when lacking external ethylene exposure. Enriched zeolite was able to act as an effective absorber of internal and external ethylene at the level of 1 µL L^{-1}. The impact of enriched zeolite was considerable for 'Shiraz' and 'After-Party' cultivars than the 'Avalanche' and effective than enriched sponge in this aspect.

Mean comparison of the number of

dropped petals showed that enriched zeolite and sponge prevented dropping of petals in ‘After-Party’ and ‘Avalanche’ cultivars compared to the control, but this impact was not significant for ‘Shiraz’ cultivar (Fig. 4). Enriched zeolite absorber could prevent petal dropping of cut flowers for all three cultivars that exposed to the external ethylene, despite the fact that a large number of petal dropping expected in this regard. However, enriched sponge absorber could not prevent dropping of petal in examined roses cut flowers under external ethylene as it observed for enriched zeolite.

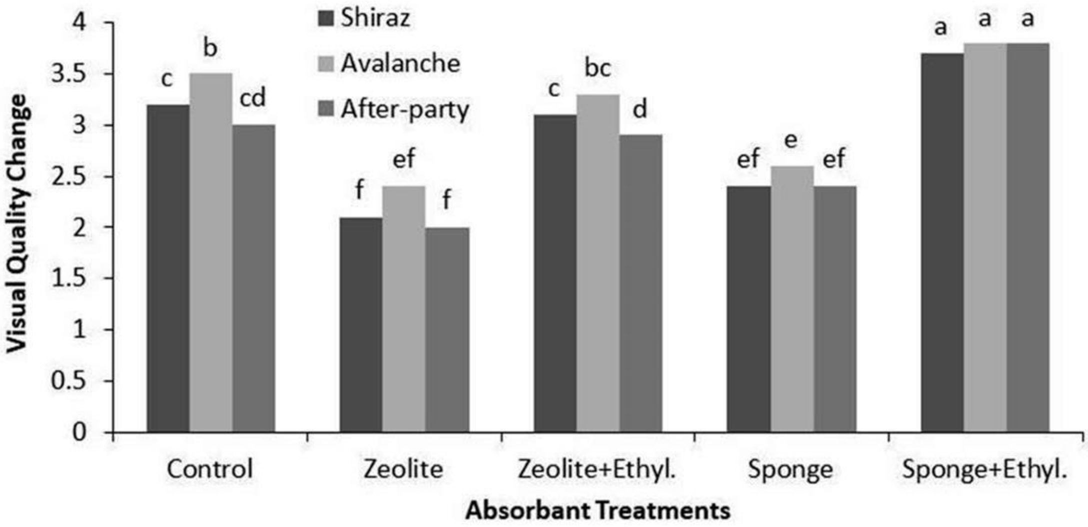

Fig. 3. Effect of different absorbers enriched/non-enriched by potassium permanganate on the visual quality change of three *Rosa hybrida* cultivars cut flowers with/without external ethylene concentration of 1 μL L^{-1} in room temperature of 25 °C based on Likert scale. Different letters show significant difference among treatments (P≤0.01).

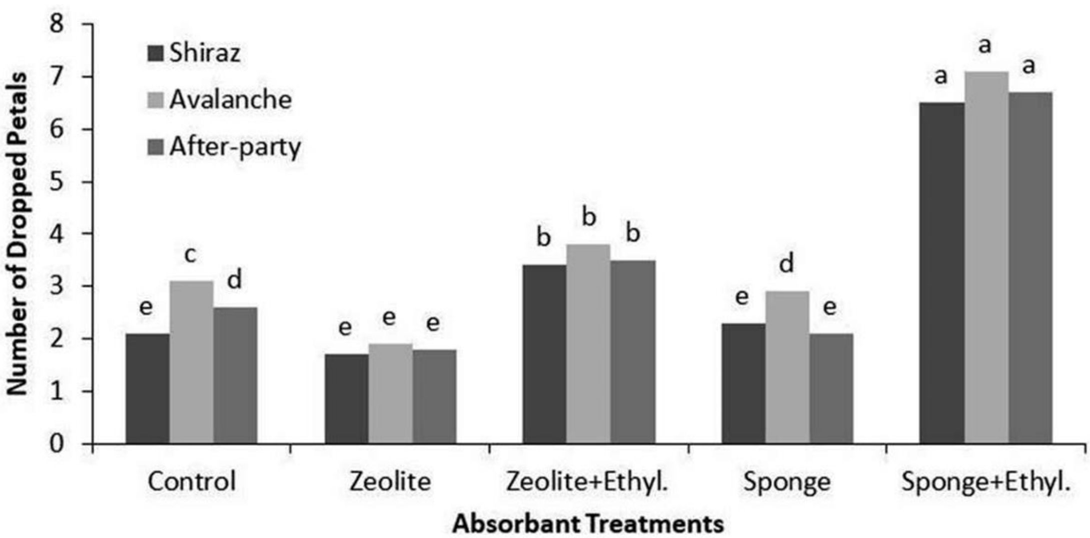

Fig. 4. Effect of different absorbers enriched/non-enriched by potassium permanganate on the number of dropped petals of three *Rosa hybrida* cultivars cut flowers with/without external ethylene concentration of 1 μL L^{-1} in room temperature of 25 °C. Different letters shows significant difference among treatments (P≤0.01).

Results indicated that cut flower fresh weight decreased during the assessment for all cultivars, but the amount of weight decrease was higher for ‘Avalanche’ than the others with control treatment. Both enriched zeolite and sponge absorbers could protect weight loses of all cultivars, similarly (Fig. 5). Cut flower weight loses pronounced by exposure to the external ethylene. However, under such situation, enriched zeolite could significantly prevent weight loses of cut flowers of all cultivars at the level of control or even more protecting for ‘Avalanche’ cultivar compared to the enriched sponge where a clear weight loses observed and even more weight loses observed for ‘Avalanche’ cultivar than the control.

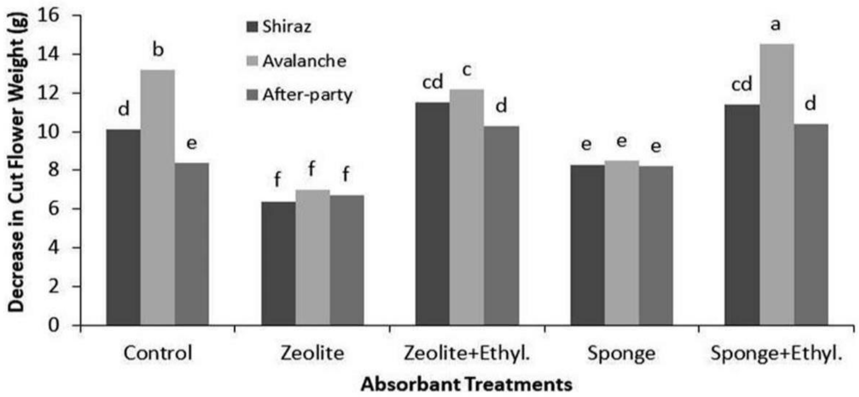

Fig. 5. Effect of different absorbers enriched/non-enriched by potassium permanganate on decrease in cut flower weight of three *Rosa hybrida* cultivars with/without external ethylene concentration of 1 µL L^{-1} in room temperature of 25 °C. Different letters shows significant difference among treatments (P≤0.01).

Ethylene concentration change after 7 days inside the treatments containers compared to the initial level at the beginning revealed that enriched zeolite could adsorb ethylene more efficiently than the enriched sponge where the change was not significant (Fig. 6). The concentration of inside container ethylene lowered to around 100-200 nL L^{-1} with enriched zeolite. The enriched zeolite had also the efficiency to control ethylene concentration even under further injection of 1 µL L^{-1} (1000 nL L^{-1}) external ethylene, but enriched sponge could not influence ethylene as the level shown under zeolite treatment. The ethylene level for ‘Shiraz’ cultivar was lower than for ‘Avalanche’ and ‘After-Party’ cultivars under both enriched zeolite and sponge treatments.

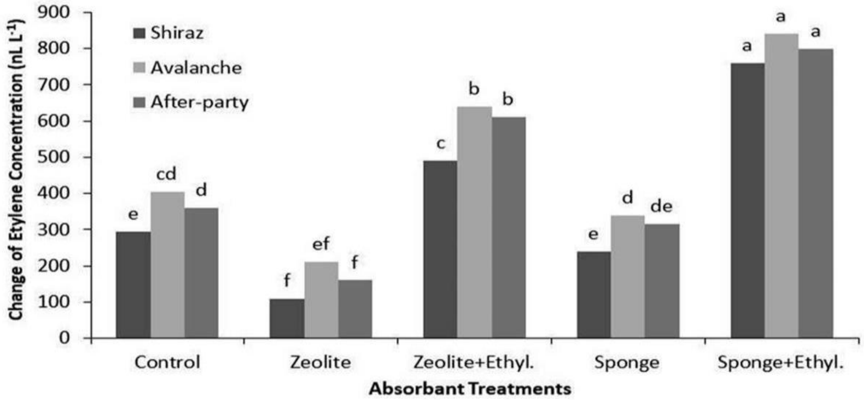

Fig. 6. Effect of different absorbers enriched/non-enriched by potassium permanganate on the level of ethylene change inside the container for three *Rosa hybrida* cultivars cut flowers compared to the initial level for treatments with/without external ethylene concentration of 1 µL L^{-1}. Different letters show significant difference among treatments (P≤0.01).

Increase in EC of vase water between initial (at the opening of the containers and putting the cut flowers in the vase water; 7 days after treatment) and final (14 days after treatment) evaluations inhibited by application of enriched zeolite (the change level was lower) (Fig. 7). However, enriched sponge was not able to prevent the change of EC between two evaluations and EC increased at the end (14 days after) dramatically as it detected for the control, as well.

Change of EC was the highest for ‘Avalanche’ cultivar by external ethylene injection under enriched sponge, but for two other cultivars, the response was almost similar.

The change of anthocyanins content of petals declined by application of enriched zeolite and sponge compared to the initial content, which is indicative of inhibition of petal loss of this pigment by these treatments and mostly via enriched zeolite. This influence was more distinctive for ‘Shiraz’ cultivar than ‘Avalanche’ and ‘After-party’ in case of zeolite but for sponge, the response of ‘Shiraz’ and ‘After-party’ was almost similar (Fig. 8). The release of anthocyanins out of petals between two trials prevented via enriched zeolite when external ethylene of 1 µL L^{-1} injected; whereas this was not observed with enriched sponge at the same conditions compared to the enriched zeolite. This is clearly indicative of the role of ethylene in effecting membrane degradation, which result in loss of membrane integrity during senescence and the influence of enriched zeolite in preventing the release of anthocyanin.

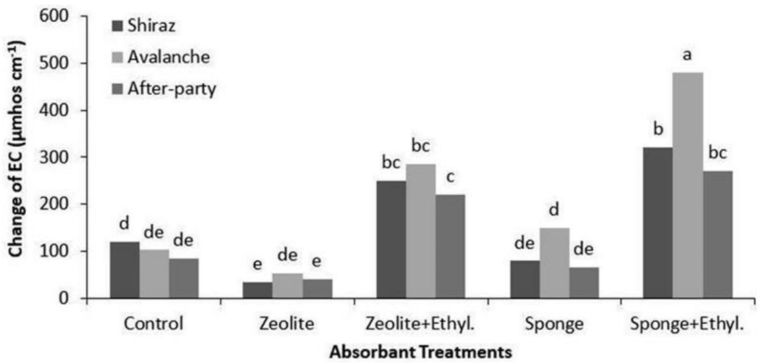

Fig. 7. Effect of different absorbers enriched/non-enriched by potassium permanganate on the change of vase water electrical conductivity (EC) of three *Rosa hybrida* cultivars cut flowers with/without external ethylene concentrations of 1 µL L^{-1} in room temperature of 25 °C. Different letters shows significant difference among treatments (P≤0.01).

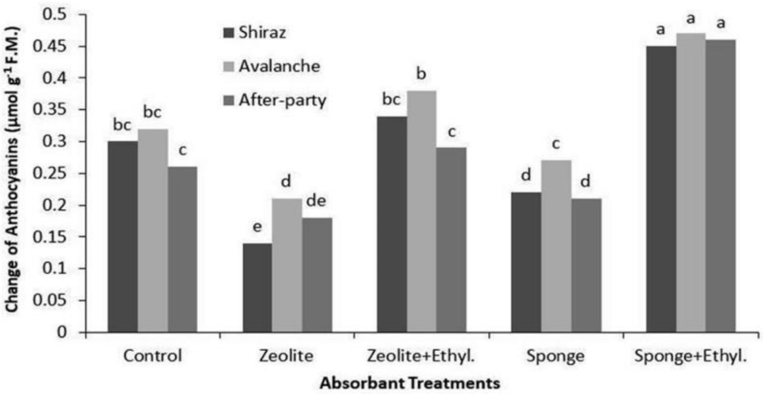

Fig. 8. Effect of different absorbers enriched/non-enriched by potassium permanganate on the change of petals anthocyanins of three *Rosa hybrida* cultivars cut flowers with/without external ethylene concentration of 1 µL L^{-1}. Different letters show significant difference among treatments (P≤0.01).

Discussion

It has been reported that most of ethylene sensitive plants such as carnation will considerably lose their quality when exposed to the external ethylene concentration of 1 μL L^{-1} (Reid & Wu, 1992), and ethylene concentrations higher than 0.1 μL L^{-1} can trigger and accelerate the senescence processes in these plants (Wills and Warton, 2000). However, ethylene concentrations around 20 μL L^{-1} in the fruit storage chambers would be high enough to promote ripening in climacteric fruits (Ivanov et al., 2005). Therefore, ethylene concentrations at lower than 0.1 μL L^{-1} is highly recommended for processing and storage rooms of sensitive horticultural crops (Scariot et al., 2014; Wills and Warton, 2000). Furthermore, the tolerance and/or sensitivity of crop cultivars to ethylene should be considered as partial ethylene sensitivity has been reported for some rose cultivars such as 'Amber', 'Amorous' and 'Big Fun' (Macnish et al., 2010).

Since the main objective of the current research was evaluation of enriched absorbents benefits under effective ethylene concentration to assess their capability to maintain quality of different cut flowers of rose cultivars or inhibit the quality loses, therefore, 1 μL L^{-1} of injected external ethylene found to be corresponding and practical. Therefore, treatments used in this study included control (without absorbent), enriched zeolite or sponge, enriched zeolite or sponge + ethylene (1 μL L^{-1}).

More maintenance or stability in the visual quality of *Rosa hybrida* cut flowers, more reduction in the ethylene concentration of container, lower number of dropped petals, less weight loses, little change in the electrical conductivity of vase solution, and lower out of petal release of anthocyanins obtained with enriched zeolite and then enriched sponge compared to control. Enriched zeolite could even maintain the quality of cut flowers at the level of control under external ethylene injection superior to enriched sponge treatment. 'Avalanche' figured as the most sensitive and 'Shiraz' as the most resistant cultivars to the evaluated level of external ethylene concentration.

Some reports indicated that the vase life of most of the cut flowers decline markedly when they are placed in the vicinity of each other, which shows that the produced ethylene might be responsible for such reaction (Van Doorn, 1998; Van Doorn et al., 2004). The capability of enriched zeolite in absorbing and oxidizing of ethylene was due to its shape, specific surface area, cation exchange capacity and molecular sieving (Limtrakul et al., 2001), which elevated by combination with potassium permanganate as ethylene oxidizer (Scariot et al., 2014; Ozdemir and Floros, 2004). The positive effect of zeolite has been reported on improving of shelf life of some crops (Smith et al., 2009). This positive evidence resulted for a single cut flower of sensitive rose cultivars in the current study might be recommended to be evaluated for bulk transportation of these cultivars cut flowers.

Result indicated that enriched zeolite was effective than enriched sponge concerning ethylene removing or oxidizing and preventing its detrimental effect especially under effective level of external ethylene (1 μL L^{-1}). Moreover, comparison of the number of dropped petals between enriched zeolite (absent of external ethylene treatment) and control pointed out that no prevention concerning the petal dropping occurred for 'Shiraz' cultivar, but significant prevention happened for two other cultivars of 'Avalanche' and 'After-Party'. This might be related to its less internal ethylene production or to its less ethylene sensitivity.

Lower weight loses of cut flowers during transportation and postharvest is ideal and considered as a quality trait. The effect of enriched zeolite on maintaining of cut flower weight was more prominent than the enriched sponge. It was clearly shown that ‘Avalanche’ cultivar had a higher weight loses than the two other cultivars, therefore; its lower longevity was due to its higher sensitivity to the ethylene or some other possible physiological factors.

Due to isolated environment for a period of 7 days, higher ethylene sensitivity is highly expected than the other factors. Ethylene synthesis by the plant during postharvest could have direct unfavourable effect on the longevity of some horticultural products (Abeles et al., 2002). Some reports indicated that different cultivars may have different level of sensitivity to the ethylene (Macnish et al, 2010). Although ethylene level for ‘Avalanche’ and ‘After-Party’ cultivars did not differ significantly, but regarding to the previous results as shown in Figures 3, 4 and 5 the quality parameters of ‘Avalanche’ were highly affected by ethylene than the two other cultivars; indicating its more sensitivity to the ethylene. Different sensitivity of various plant species or even cultivars to ethylene has been widely reported (Reid and Wu, 1992; Serek et al., 1995; Macnish et al., 2000). Lower sensitivity of some rose cultivars such as ‘Amber’, ‘Amorous’ and ‘Big Fun’ to ethylene levels has been also previously reported (Macnish et al., 2010). In another study, ‘Rubor’ and ‘Carrousel’ are reported as sensitive and resistance rose cultivars to ethylene, respectively (Ranwala, 2006).

Application of enriched zeolite prevented vase water EC change between initial and final evaluations. Change of EC was the highest for ‘Avalanche’ by external ethylene injection under enriched sponge. Increase in the value of EC during the vase life reflects permeability of the membrane. Increased membrane permeability and consequently enhancement of cellular components efflux such as electrolytes and pigments are the main and typical attribute of changes in the membrane integrity that occur during senescence of plant tissues (van Meeteren and Aliniaeifard, 2016; Suttle and Kende, 1980). Consequently, wilting and desiccation of leaves and petals suffer the cellular integrity and subsequent ionic leakage could change the EC of vase water during the postharvest handling of fruits and cut flowers (Antunes and Sfakiotakis, 2008). The release of anthocyanin from isolated petals of *Tradescantia* has been reported during senescence process and presented that the onset of this process hastened by pre-treatment of the petals with 10 µL L^{-1} of ethylene (Suttle and Kende, 1978).

Finally, enriched sponge and zeolite absorbers had a positive impact on keeping the quality of all examined rose cultivars rather than the control when lacking external ethylene exposure. This might suggest that these treatments were able to control the detrimental effect of existing ethylene, but only enriched zeolite was able to act as an effective absorber of internal and external ethylene at the level of 1 µL L^{-1}. The impact of enriched zeolite was considerable for ‘Shiraz’ and ‘After-party’ cultivars than ‘Avalanche’ and effective than enriched sponge in this respect.

Conclusion

Results revealed that enrichment of zeolite and to a lesser extent sponge with potassium permanganate as ethylene adsorbents in the isolated container, storage room and transportation cabinets of cut flowers could be useful to maintain the quality of rose cut flowers. Evaluation of different levels of the materials used in the present study for large transportation systems and evaluation of other species and cultivars can be interesting issues for further investigation.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest for this study.

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