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Research Paper

The Effect of Dill and Cumin Essential Oils on Physiological and Microbiological Traits of Cut Alstroemeria (*Alstroemeria hybrida*)

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Abstract

Alstroemeria (*Alstroemeria hybrida*) is one of the most important cut flowers in the world. The aim of this study is investigating on the effect of dill and cumin essential oils on physiological and microbiological traits of alstroemeria cut flowers. Hydro-distillation method and GC isolated the essential oils of seed and GC/MS examined the chemical compositions of the samples. This experiment carried out as factorial based on completely randomized design with two factors of essential oils of dill and cumin (50 and 100 ppm). The second factor was used of methods (pre-harvest, post-harvest). The results showed that the dill essential oil treatment (100 ppm) in pre-harvest was the best treatment for all known traits. 11 colonies of bacteria were identified in stem end of cut alstroemeria flowers; which are *E-coli*, *Enterobacter*, *Klebsiella*, *Proteus*, *Serratia*, *Citrobacter*, *Pseudomonas*, *Staphylococcus aureus*, *Streptococcus*, *Bacillus cereus* and *Actinomycetes*. Due to the positive impact of dill essential oil (100 ppm) in the pre-harvest method in improving traits associated with vase life of cut alstroemeria flowers, these treatments are recommended.

Keywords: Alstroemeria, Bacteria, Postharvest, Pre-harvest, Vase life.

INTRODUCTION

Alstroemeria with the scientific name of *Alstroemeria hybrida* belongs to the family of Alstromeriacea (Chanasut *et al.,* 2003). The yellowing leaves is a major problem for this plant so that in half of the varieties, leaves become yellow before falling flowers (Ferrante *et al.,* 2002). Sucrose is the basic ingredient used in the maintenance of cut flowers. Treatment with sucrose delays aging-related activities (Pun *et al.,* 2005). Antimicrobial properties of essential oils have been known for years. Recent findings have demonstrated that treatment with exogenous sugar (Mohd Rafdi *et al.,* 2018) and some herbal essential oils (Manfredini *et al.,* 2017; Razi, 2017) prevents floral senescence and loss of the decorative life of cut flowers. It has also been reported that in addition to prolonging shelf-life, use of these treatments leads to an increase in the activities of (SOD) and (CAT). At the same time, it decreases the content of (MDA), showing a significant relationship between the content of these enzymes and the vase life of cut alstroemeria flower (Alborz *et al.,* 2015).

Thus introducing effective compounds with appropriate concentrations that are relatively good in controlling microbial population as well as, being devoid of side effects and toxicity on flowers was the aim of this study in order to facilitate the absorption of the vase solution and prevent weight loss of the flowers to achieve our main goal which was to increase the vase life and quality of cut alstroemeria flowers.

MATERIALS AND METHODS

Plants and preparation of treatments

A commercial greenhouse was considered to cultivate alstroemeria located in Mahallat city in Markazi province. Plants were grown under standard greenhouse conditions at 22˚C and 16˚C day and night temperatures. The composition of the seed distilled essential oil of dill (*Anethum graveolens* L.) was investigated by GC and GC/MS, 13 components were identified of which carvone (61%), limonene (18.23%), β-myrcene (8%), trans-dihydrocarvone (5.30%) and α-phellandrene (5.1%) were the major constituents and amounted to 95% of the oil (Table 1). The composition of the seed distilled essential oil of cumin (*Cuminum cyminum*) was investigated by GC and GC/MS, 14 components were identified of which cuminaldehyde (32.08%), γ-terpinene (23.02%) and cymene-p (21.20%) were the major constituents and amounted to 95.40% of the oil (Table 2).

Table 1. Chemical composition of essential oil of dill (*Anethum graveolens*).

Compound	Retention index	Percent
α-thujene	902	0.01
- pineneα	948	0.07
Sabinene	958	8
- pineneβ	969	5.01
Myrcene	998	0.02
-terpineneα	1018	18.23
Limonene	1031	0.8
Cymene-p	1142	0.01
-phellandrene β	1179	5.30
1,8-Cineol	1190	61
γ-terpinene	1516	0.04
γ-terpinolene	1568	0.01
Cuminaldehyde	1705	0.5
Sum		95

Table 2. Chemical composition of essential oil of cumin (*Cuminum cyminum*).

Compound	Retention index	Percent
α-thujene	925	0.5
- pineneα	932	0.8
Sabinene	970	1.01
- pineneβ	975	5.80
Myrcene	990	1.60
-terpineneα	1013	0.6
Limonene	1025	0.5
Cymene-p	1027	21.20
-phellandrene β	1028	0.5
1,8-Cineol	1031	0.4
γ-terpinene	1061	23.02
γ-terpinolene	1085	0.3
Cuminaldehyde	1247	32.08
γ-terpinene-7-al	1304	7.09
Sum		95.40

Some of the plants were sprayed (approximately 500 ml per plant) with different essential oils while the control flowers were sprayed with distilled water. About three weeks later, cut flowers were harvested and transported with appropriate cover immediately to the laboratory of Horticulture Department, Islamic Azad University of Rasht Branch. The vase life of cut flowers was evaluated in a 12 h photoperiod, the light intensity of 12 µmol m^{-2} s^{-1}, relative humidity of 60 to 70%, and the temperature of 20 ± 2˚C. Treatments used in this work are dill essential oil 50 ppm (AN1), 100 ppm (AN2), cumin essential oil 50 ppm (CM1) and 100 ppm (CM2). This study was performed with three replications and 8 branches of alstroemeria flowers for each treatment as a factorial experiment based on completely randomized design. Analysis of variance of data obtained from this experiment was performed using SAS software and treatment means were compared through the LSD test.

Measurement of traits

Vase life

The vase life of the cut flower was determined with the period (day) between the start of the experiment until yellowing or fall of half of the flowers (Ferrante *et al.,* 2002).

Peroxidase enzyme (POD)

In *et al.* (2007) method was used. Changes in absorption were measured at the wavelength of 470 nm using a spectrophotometer on the 1th, 4th, 8th and 12th days. Finally, the activity of peroxidase was expressed in µmol 100 g^{-1} FW min^{-1} as follow:

$$\text{POD } (\mu\text{Mol g}^{-1}) = \frac{OD_{min^{-1}}}{13.2}$$

OD min^{-1}: Changes of the absorption per unit of time

SOD activity

SOD activity in petals of cut alstroemeria was measured at 1st, 4th, 8th, and 12th days based on the spectrophotometry by Giannopolitis and Ries (1997) method on petal tissue. The reaction solution (1 ml) contained 50 mM phosphate buffer (pH=7), 12 mM riboflavin, 13 mM methionine,

0.1 mM EDTA, 7 mM nitro blue tetrazolium (NBT) and 10 µl of extracted enzyme solution. A solution with no enzyme was used as the control. Test tubes were irradiated under fluorescent lights at 100 µmol $m^{-2}s^{-1}$ for 20 min. The absorbance of each solution was measured at 560 nm using a spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme that would inhibit 50% of NBT photo reduction.

MDA content

Changes in the malondialdehyde (MDA) content of petals were measured on days 1, 4, and 12. For this purpose, in each treatment-method combination, the MDA content of petal was measured according to the method suggested by Heath and Packer (1986).

Counting bacteria of vase solution

In order to count bacteria of vase solution, sampling was conducted before the experiment on the second and sixth days of the experiment. 0.1 ml samples diluted with normal saline was cultured on nutrient agar and Mac Conkey agar mediums in a sterile petri dish. Culturing mediums were kept for 24 h in a growth chamber at 37°C and then, the number of bacteria was determined by the page counting method. Total count of bacteria based on the colonies growth in the plate has been calculated as follows:

Total count of bacteria (CFU) = Number of colonies counted × Reverse of dilution factor

Microbe growth (MG) = Log 10 (Mt);

Mt: The bacterial measured on days (0, 2 and 6)

After counting bacteria, different diagnostic tests were used to identify the type of bacteria.

Identifying bacteria in the vase solution

In this study, nutrient agar medium, Mac Conkey agar, TSI, SIM, CS, urease, gelatinase, Broth MRVP, mannitol salt agar was prepared.

RESULTS AND DISCUSSION

Vase life

The results of analysis of variance showed that the effect of interaction of treatments (T) in different methods (M) on the vase life of alstroemeria was significant at 1% level so that there was a significant difference at 1% level between treatments at all levels and preservative methods. The maximum vase life of alstroemeria was related to the interaction effect of dill essential oil treatment with a concentration of 100 ppm (AN2) and the pre-harvest methods (B) with an average of 16 days. Furthermore, the minimum vase life of alstroemeria was related to the interaction effect of the DW treatment and the pulse method (A1) with an average of 11.33 days (Fig. 1). The use of herbal essential oils treatments increased vase life. Vase life compared to the control that these results correspond with the results of other research in this area (Lee *et al.,* 1997; Zhang *et al.,* 2003). Similar to other cut flowers, the abscission and senescence of petals are two major constraints that reduce the ornamental value of cut alstroemeria flowers. In this regard, Chanasut *et al.* (2003) and Razi (2017) suggested that ethylene susceptibility and bacterial infection are major vase-life reducing factors in this plant.

Peroxidase (POD)

The analysis of variance results showed that the effect of interaction of treatments (T) in different methods (M) on the amount of peroxidase (POD) of alstroemeria was significant at 1% level so that there was a significant difference at 1% level between treatments at all levels and in preservative methods. The maximum POD was related to the dill essential oil treatment with a

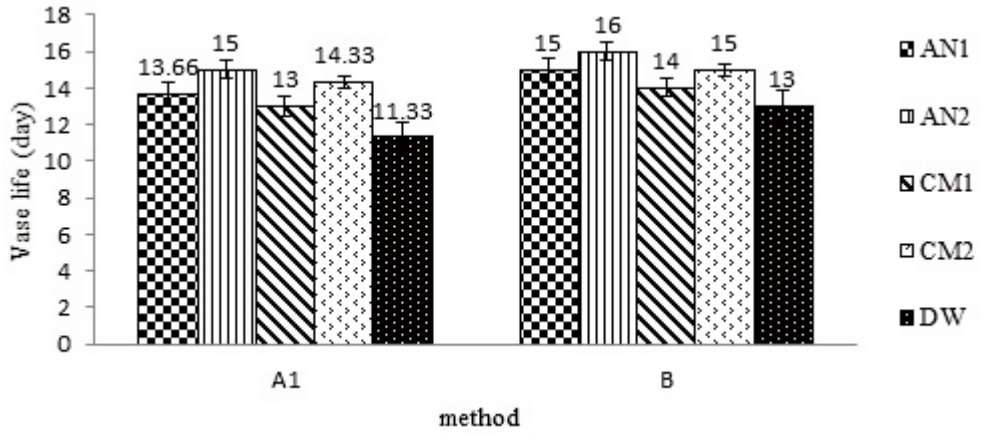

Fig. 1. Effect of different methods and treatments on vase life of *Alstroemeria hybrida*. Method: (A1: Pulsing 24 h, B: Pre-harvested); Treatment: AN1: Essential oil of dill 50 ppm, AN2: Essential oil of dill 100 ppm, CM1: Essential oil of cumin 50 ppm, CM2: Essential oil of cumin 100 ppm, DW: Distilled water).

concentration of 100 ppm (AN2) with 3% sucrose and the pre-harvest spraying method (B) on 12th day twelve with an average of 2.10 µmol 100 g^{-1} FW min^{-1} (Figs. 2 and 3). The use of treatments of natural essential oils enhances the activity of peroxidase that this increase is due to the activation of the cells through proper absorption of nutrient solution and cell turgor. The activity of cells is also due to the activity of antioxidant enzymes and stability of cell membrane (Palma *et al.,* 2002).

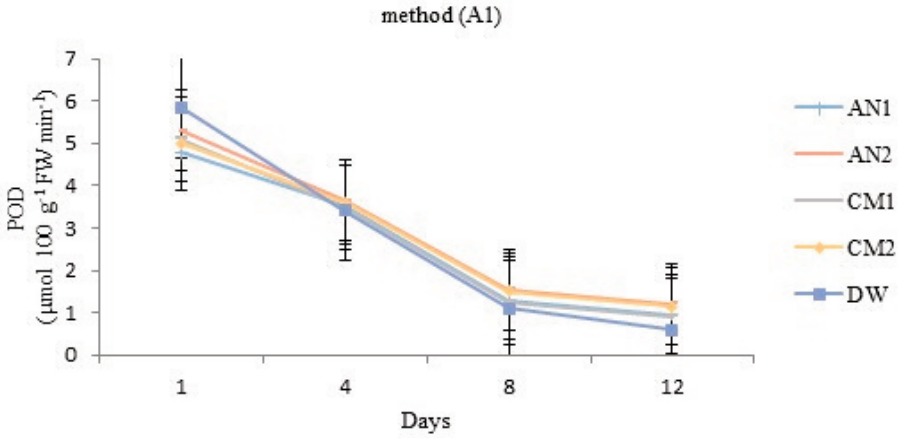

Fig. 2. Effect of different treatments on POD of *Alstroemeria hybrida* during vase life at the method of A1 (Pulsing 24 h). Treatment: (AN1: Essential oil of dill 50 ppm, AN2: Essential oil of dill 100 ppm, CM1: Essential oil of cumin 50 ppm, CM2: Essential oil of cumin 100 ppm, DW: Distilled water) + sucrose 3%.

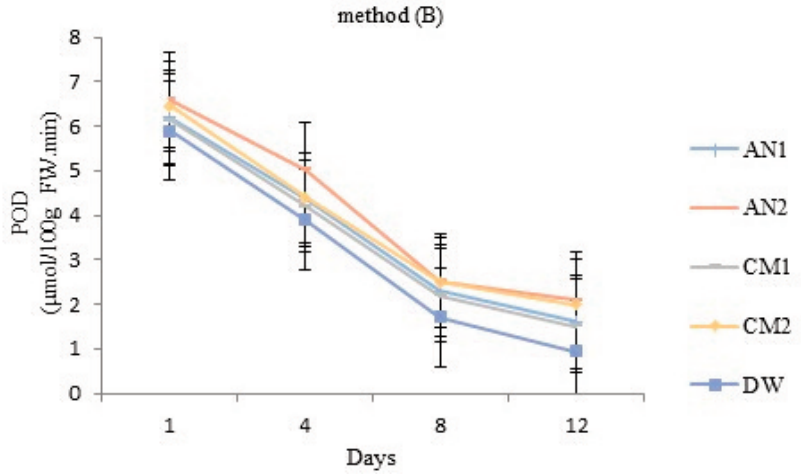

Fig. 3. Effect of different treatments on POD of *Alstroemeria hybrida* during vase life at the method of B (Pre-harvest). Treatment: (AN1: Essential oil of dill 50 ppm, AN2: Essential oil of dill 100 ppm, CM1: Essential oil of cumin 50 ppm, CM2: Essential oil of cumin 100 ppm, DW: Distilled water) + sucrose 3%.

SOD Activity

The ANOVA test showed that interaction treatments (T) in the methods (M) of the SOD enzyme was significant on cut flowers on different days. The results showed that SOD activity decreased significantly during the experiment. Among all treatments of dill essential oil, AN2 had the highest SOD activity and the lowest activity of the SOD enzyme was related to control treatment (DW) at all methods. Overall, the highest amount of SOD enzyme was the application of pre-harvest dill essential oil (100 ppm) with 3% sucrose on the 12th day with an average of 59.5 µmol g^{-1} FW and the lowest amount of SOD activity was the application of pulsing (distilled water with 3% sucrose) with an average of 22.68 µmol/g FW on the 12th day (Figs. 4 and 5). Reactive oxygen species (ROS) react with proteins, lipids, and nucleic acids and degrade them leading to senescence of cells. Antioxidant enzymes such as superoxide dismutase (SOD) have radical scavenging activity for ROS. As the first step in the biochemical defence of plants, SOD catalyses the dismutation of superoxide molecules into hydrogen peroxide and oxygen based on the chemical reaction (Dwivedi *et al.,* 2016). The activity of the SOD enzyme was found to be higher in the petals of those plants that were exposed to the treatments than in the control. Thus, the higher activity of the enzyme results in further scavenging of oxygen free radicals, culminating in less damage to cell walls and retardation of senescence. A same conclusion had previously been made by Palma *et al.* (2002).

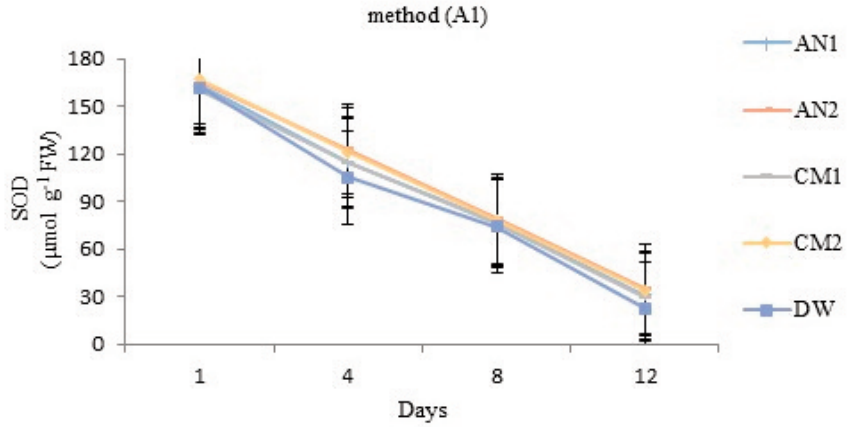

Fig. 4. Effect of different treatments on SOD of *Alstroemeria hybrida* during vase life at the method of A1 (Pulsing 24 h). Treatment: (AN1: Essential oil of dill 50 ppm, AN2: Essential oil of dill 100 ppm, CM1: Essential oil of cumin 50 ppm, CM2: Essential oil of cumin 100 ppm, DW: Distilled water) + sucrose 3%.

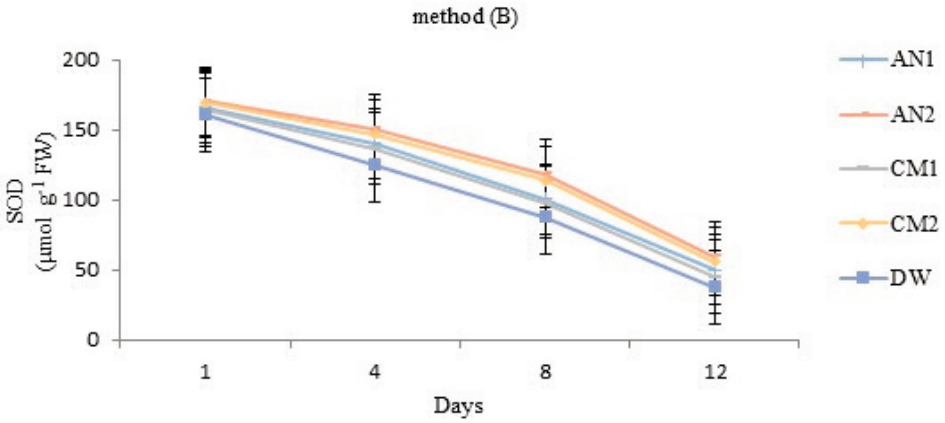

Fig. 5. Effect of different treatments on POD of *Alstroemeria hybrida* during vase life at the method of B (Pre-harvest). Treatment: (AN1: Essential oil of dill 50 ppm, AN2: Essential oil of dill 100 ppm, CM1: Essential oil of cumin 50 ppm, CM2: Essential oil of cumin 100 ppm, DW: Distilled water) + sucrose 3%.

MDA content

As proposed by Kazemi and Ameri (2012) the content of malondialdehyde is a suitable marker for lipid peroxidation and cell membrane damage. The ANOVA test results showed that the statistically significant effect of different treatments and methods on content of MDA ($P<0.05$). Information obtained by mean comparison revealed that all treatments caused a decrease in MDA accumulation than that of the control (DW). The results of this experiment showed that MDA content increased during vase period. The lowest content of MDA (77 nmol g^{-1} FW) was observed in the application of pre-harvest (B) dill essential oil treatment (100 ppm) with sucrose 3% on the 12th day. On the other hand, the highest content of MDA was observed in the 12th day of DW (distilled water and 3% sucrose) in pulsing method (A1) with an average of 120 nmol g^{-1} FW on the plant fresh weight (Figs. 6 and 7). MDA, a product of lipid peroxidation, has been proposed as a suitable marker of lipid peroxidation (Bailly *et al.,*1996). Kazemi and Ameri (2012) reported the

positive effect of herbal essential oils of thyme and lavender on the stability of the membrane and reduction of MDA in clove cut flowers, which are consistent with the results of the present research. We found that all compounds had antibacterial effects on stem ends microorganisms and could decrease these preservative compounds. Microorganisms and their decay products are a common cause of stem end blockage. Hence, essential oil treatment has antibacterial effects and can extend the quality and vase life of cut flowers (van Doorn and de Witte., 1997).

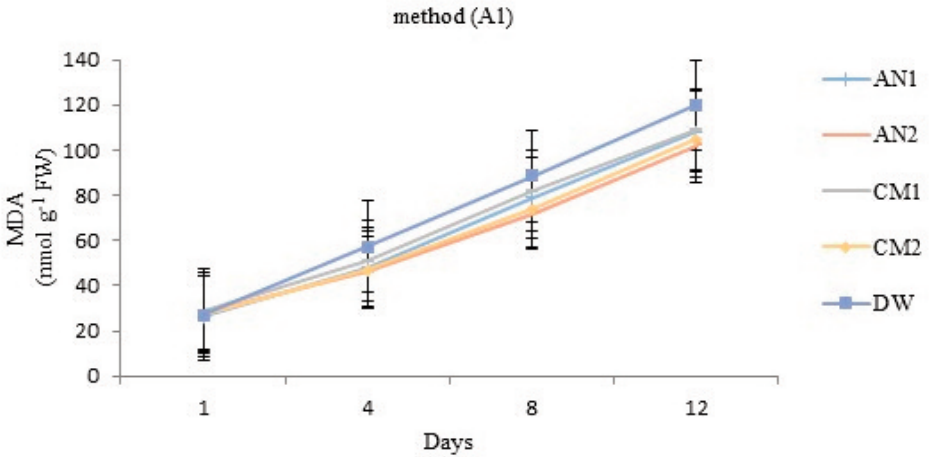

Fig. 6. Effect of different treatments on MDA of *Alstroemeria hybrida* during vase life at the method of A1 (Pulsing 24 h). Treatment: (AN1: Essential oil of dill 50 ppm, AN2: Essential oil of dill 100 ppm, CM1: Essential oil of cumin 50 ppm, CM2: Essential oil of cumin 100 ppm, DW: Distilled water) + sucrose 3%.

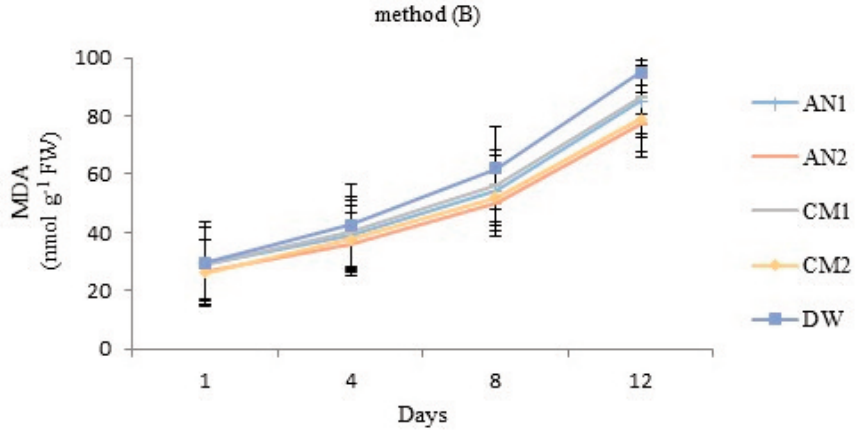

Fig. 7. Effect of different treatments on MDA of *Alstroemeria hybrida* during vase life at the method of B (Pre-harvest). Treatment: (AN1: Essential oil of dill 50 ppm, AN2: Essential oil of dill 100 ppm, CM1: Essential oil of cumin 50 ppm, CM2: Essential oil of cumin 100 ppm, DW: Distilled water) + sucrose 3%.

Counting bacteria

The analysis of variance results showed that the effect of interaction of treatments (T) in different methods (M) on the bacterial population of alstroemeria was significant, so that there was a significant difference at 1% level between chemical treatments at all levels and application

methods. In general, the minimum bacteria population was related to the dill essential oil (AN2) treatment with a concentration of 100 with 3% sucrose and the pre-harvest spraying method (B) on 6th day with an average of 6.9 Log_{10} CFU/ml. Additionally, the maximum one on 6th day was related to the DW treatment (distilled water and sucrose 20%) and the pulse method (A1) with an average of 8 Log_{10} CFU ml^{-1} (Fig. 8).

As Figs. 8 and 9 showed, treatments of cumin essential oil and dill essential oils with different concentrations had a positive impact on bacteria population and were effective in the bacteria population compared to the distilled water treatment. Antimicrobial effects of cumin essential oil and dill essential oils especially against a wide range of microorganisms such as bacteria, fungi and viruses have been reported by various researchers (Maneerung *et al.,* 2008; Navarro *et al.,* 2008).

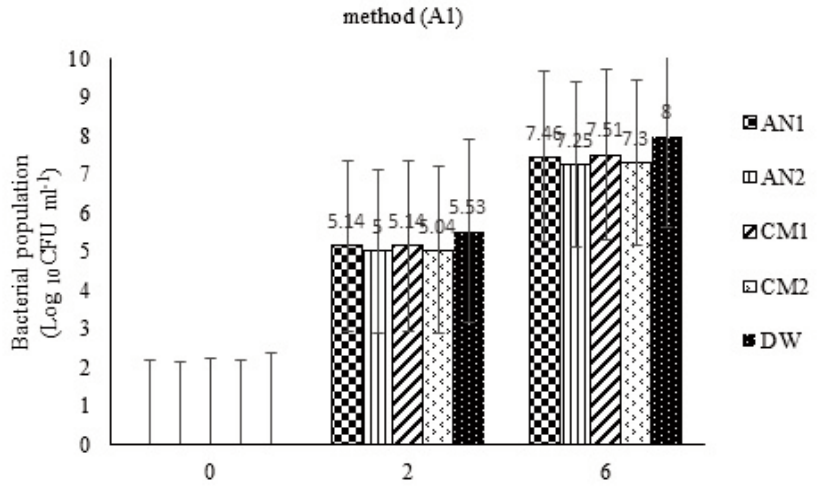

Fig. 8. Effect of different methods and treatments on bacterial population of *Alstroemeria hybrida* (Pulsing 24 h). Treatment: AN1: Essential oil of dill 50 ppm, AN2: Essential oil of dill 100 ppm, CM1: Essential oil of cumin 50,5,50 ppm, CM2: Essential oil of cumin 50,5,50 ppm, DW: Distilled water).

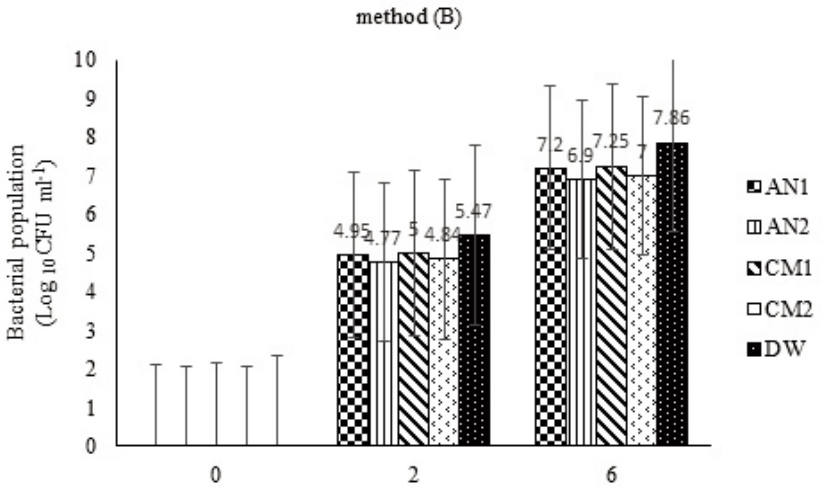

Fig. 9. Effect of different methods and treatments on bacterial population of *Alstroemeria hybrida* (Pre-harvested). Treatment: AN1: Essential oil of dill 50 ppm, AN2: Essential oil of dill 100 ppm, CM1: Essential oil of cumin 50,5,50 ppm, CM2: Essential oil of cumin 50,5,50 ppm, DW: Distilled water).

Identifying bacteria of the vase solution

The results of this study identified 11 different bacteria colonies. This fact that most of microorganisms of alstroemeria vase solution were different species of bacteria is consistent with the findings of other post-harvest studies of cut flowers (Jowkar, 2006; Put, 1990). Different kinds of Enterobacteriaceae, which are a large group of gram-negative bacilli, were identified in the vase solution and stem end of cut alstroemeria flowers, including *E. coli, Enterobacter, Klebsiella, Proteus, Serratia* and *Citrobacter*. Moreover, the non-fermentative Gram-negative bacterium *Pseudomonas* was identified. A variety of gram-positive bacteria, including *Staphylococcus aureus, Saprophyticus, Streptococcus, Bacillus cereus* and *Actinomyces* were identified after staining. In other studies, other bacteria were observed in the vase solution of rose cut flower. van Doorn *et al.* (1995) found that the dominant bacterial species in the stem of cut rose 'Sonia' flowers were *Pseudomonads* and *Enterobacteria*. The other bacteria found in the vase solution of cut narcissus flowers were *Bacillus* spp., *Staphylococcus* spp. and *Actinomycetes* spp. (Jowkar, 2006). In addition, *Acinetobacter*, *Calcoaceticus* spp., *Alcaligenes* sp., *Enterobacter agglomemn*s, *Pseudomonas alcaligenes* and *Pseudomonas fluorescens* were isolated from the stem end of clove cv. 'Scania' (van Doorn *et al.,* 1995).

CONCLUSIONS

Positive impact of dill essential oil at the concentrations of 100 ppm in the pre-harvest method in improving traits associated with vase life of cut alstroemeria flowers, these treatments are recommended. Among the treatment methods, the pre-harvest method was more effective in vase-life and some traits than the post-harvest method.

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