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Effects of 1-MCP and ethylene on antioxidant enzyme activity and postharvest physio-biochemical characteristics of cut carnation flower cv. Fortune

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Abstract: Carnation (*Dianthus caryophyllus* L.) flowers are one of the most important cut flowers in the world. The majority of carnation cultivars are sensitive to ethylene which affects the physiological and biochemical postharvest characteristics of these flowers. Applying inhibitors of biosynthesis and action of ethylene is important factor to protect the display quality and extend postharvest life. In order to evaluate the effects of 1-methylcyclopropene (1-MCP) and ethylene on the antioxidant enzyme activity of cut carnation cv. Fortune and subsequently on the extension of vase-life, this experiment was designed as a Completely Randomized Design (CRD) with three replications. Carnation cut flowers were treated with 1-MCP at concentrations of 0, 0.5, 1 and 1.5 µl/l for 24 h and subsequently exposed to ethylene (1 µl/l) for 16 h. Data were analyzed using MSTAT-C statistical software and means were compared based on Least Significant Differences (LSD) test ($p< 0.01$). Our results showed that 1-MCP treatment had significant effects on vase life and biochemical characteristics like contents of leaf chlorophyll, petal anthocyanin, petal cell membrane stability and antioxidant enzymes activity such as catalase, peroxidase, and superoxide dismutase. The highest vase life and cell membrane stability were appeared in samples treated with 1.5 µl/l 1-MCP which was significantly higher than 0 and 0.5 treatments, although there was no significant difference with 1 µl treatment. The highest chlorophyll and anthocyanin contents were also measured under 1.5 µl/l 1-MCP which was significantly higher than other treatments. The highest and lowest catalase and peroxidase activity were related to 1 and 0 µl/l 1-MCP, respectively. The highest and lowest superoxide dismutase activity was observed in 1.5 and 0 µl/l 1-MCP. In conclusion, application of 1-MCP improved and delayed the onset of senescence symptoms resulted in extending the vase life of cut carnation cv. Fortune.

1. Introduction

Carnation (*Dianthus caryophyllus* L.) flowers are one of the most important cut flowers cultivated commercially in the world and they are also used as ornamental plants for decorative purposes in orchards and landscapes (Singh *et al*., 2005). Vase life of cut flowers is an important characteristics in crop quality evaluation and it depends on genetic and environmental factors (Seglie *et al*., 2011). Ethylene, as a gaseous plant hormone, plays a prominent role in accelerating senescence phenomena of most plant organs such as fruits, flowers, and floral buds (Yang and Hoffman, 1984). When plants produce ethylene, or are exposed to an external source of ethylene, receptors perceive its presence and the signal is transferred by downstream active genes. Ethylene, by inducing expression of senescence-related genes, affects the physiological characteristics vegetative and reproductive organs resulting in organ abscission and yellowing (Ahmadi *et al*., 2008). It has been found that exogenous ethylene in some plants like miniature roses, geranium, and begonia has undesirable effects on flower quality and results in accelerating senescence and decreasing flower life (Ahmadi *et al*., 2009; Seglie *et al*., 2011). Application of external ethylene in rose petals causes a considerable increase in ethylene production and activity of ACC-synthase and ACC-oxidase genes (Ma *et al*., 2006; Ahmadi *et al*., 2009), although ethylene receptor genes are not affected by external ethylene (Ahmadi *et al*., 2009). Senescence is an oxidative process in which reactive oxygen species and antioxidants play an important role. In general, reactive oxygen species accelerate flower senescence by increasing cell membrane permeability due to decreasing proteins and nucleic acids resulting from different protease and nuclease enzyme activities (Barth *et al*., 2006). Decreasing antioxidant enzyme activity and increasing peroxidation of cell membrane lipids have been indicated as possible reasons of senescence in different plant species (Buchanan-Wollaston, 1997). In addition, cell death is accelerated in this phase because of a boost in ethylene production during the senescence period (Ebeles *et al*., 1992). Plants gain from antioxidant mechanisms to

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alleviate the effects of free radicals. These mechanisms include changes in content of defense-related enzymes such as peroxidase, catalase, polyphenol oxidase and other compounds like phenols (Staskawicz *et al*., 1995). Since postharvest senescence is an important restricting factor in crop presentation and marketability of many cut-flowers, using high confidence methods to delay crop senescence is of great importance. 1-MCP as an anti-ethylene compound has been proved to be effective in inhibiting ethylene response by competing with ethylene for placing on the site of ethylene receptors (Seglie *et al*., 2011; Daneshi Nergi and Ahmadi, 2014). Studies have shown that 1-MCP has inhibited the phenomena of petal fall in geranium, considering that its effectiveness depends on transport conditions, storage temperature, and application times (Cameron and Reid, 2001). Studies on cut carnation showed that all concentrations of 1-MCP decreased ethylene production and chlorophyll destruction was delayed in comparison with control plants (Asil *et al*., 2013). Black tulip flowers treated with 1-MCP for 8 h showed maximum anthocyanin till the twelfth day of vase life (Chutichudet *et al*., 2010). Application of 1-MCP on soya plants decreased hydrogen peroxide in comparison with untreated plants as well as production of ethylene and free radicals but increased the activity of antioxidant enzymes (Djanaguiraman *et al*., 2011).

Considering the role of 1-MCP as an ethylene inhibitor, the purpose of the current study was to evaluate 1-MCP efficacy in extending display-quality 'Fortune' carnation flowers. To gain a deep understanding of biochemical characteristics of cut-flowers, enzyme assays were evaluated in this experiment.

2. Materials and Methods

Carnation cut-flowers of 'Fortune' cultivar were harvested from commercial greenhouses in Pakdasht (Iran) according to standard indexes. Flowers were immediately transferred to the laboratory of post harvest physiology of the Horticulture Department, Faculty of Agriculture, Tarbiat Modares University. Healthy and uniform flowers were selected for the considered treatments. Cut-flowers were placed in the vase solution and treated with 1-MCP (0, 0.5, 1 and 1.5 µl/l) for 24 h in 200 L glass aquarium chambers. After 1 h of ventilation, the aquarium lids were re-sealed and ethylene was injected inside each chamber using a Hamilton syringe to expose cut flowers to 1 µl/l ethylene for 16 h (Daneshi Nergi and Ahmadi, 2014). After termination of the ethylene treatment, the lids of the glass chambers were removed and the vases were placed on the lab bench. Experiments were run under the following conditions: temperature 20±2°C, relative humidity of 60-65%, light intensity of 15 µmol/m^2s^{-1} with 12 h light and 12 h darkness (Daneshi Nergi and Ahmadi, 2014). Experimentation was conducted in a completely randomized design with three replications and four treatments. Sampling was carried out to evaluate physiological and biochemical characteristics at desired times and data were analysed using MSTAT-C statistical software; means were compared according to the Least Significant Differences (LSD) test ($P<0.01$) and graphs were designed using Excel software.

Vase life

In this study, vase life was considered the time during which cut-flowers can keep their market quality and before senescence symptoms, including bending of petal margins and wilting, appear (Singh, 1994). Cut-flower durability was based on day distance after the end of cut-flower treatment till their ornamental value disappeared.

Leaf chlorophyll analysis

To measure chlorophyll, leaf samples of 0.5 g were ground using a mortar and pestle with liquid nitrogen, dipped in 15 ml 80% acetone in test tubes and centrifuged at 6000 rpm and 4°C. The absorbance of the solutions was read against blank (solvent) at 663 and 646 nm using a spectrophotometer (BIO-RAD) (Richardson *et al*., 2002). Chlorophyll content was calculated as follows:

Chlorophyll a (µg.ml) = $12/5A_{663}$ -$2/79A_{646}$

Chlorophyll b (µg.ml) = $21/51A_{646}$ -$5/1A_{663}$

Total Chlorophyll (µg.ml) = Chlorophyll a + Chlorophyll b

Petal anthocyanin

Petal samples of 200 mg were pulverized in 3 ml 99:1 (v/v) methanol and hydrochloric acid and the obtained extracts were centrifuged at 12000 rpm for 20 min at 4°C. Supernatants were kept at 4°C under darkness for 24 h. Light absorption was then estimated by spectrophotometer at 550 nm wavelength and using silence coefficient (=33000 mol^2 cm^{-1}) (Krizek *et al*., 1993).

Petal membrane stability index

To determine petal membrane stability, two petal samples, each including 200 mg of each replication, were weighted and dipped in 10 ml double distilled water. One sample was placed in 40°C Benmary for 30 min and the other 100°C Benmary for 15 min. After cooling the sample of 40°C Benmary and reaching room temperature, electrical conductivity of the solutions was measured with a conductivity meter and the stability percent of the membrane was determined according Ezhilmathi *et al*. (2007) as follows:

$$\text{Membrane stability index (percent)} = [1-(C1/C2)] \times 100$$

Enzyme assays

Peroxidase (POD) enzyme. Peroxidase (POD) was extracted from 200 mg homogenized samples in 25 mM Na-phosphate buffer (pH 6.8) followed by centrifugation at 12000 rpm for 30 min at 4°C. For assay, a mixture consisting of 25 mM Na-phosphate buffer (pH 6.1), 28 mM Guaiacol, 5 mM hydrogen peroxide and crude extract was prepared and its absorbance at 470 nm was detected for 1 min, using spectrophotometer (BIO-RAD). Enzyme activ-

ity was expressed as absorption delta of 470 nm per mg protein (Chance and Maehly, 1955).

Catalase (CAT) enzyme. Catalase (CAT) was extracted from 200 mg samples homogenized in 25 mM Na-phosphate buffer (pH 6.8) followed by centrifugation at 12000 rpm for 30 min at 4°C. The supernatant was transferred to 15 ml tubes and referred to enzyme extract. For assay, a mixture consisting of 25 mM Na-phosphate buffer (pH 6.1), 10 mM hydrogen peroxide and crude extract was prepared and its absorbance at 240 nm was detected using a spectrophotometer (BIO-RAD). Enzyme activity was described by measuring the conversion rate of hydrogen peroxide to water and oxygen molecules, as the decrease of absorbance per time per mg of protein (Cakmak and Horst, 1991). Enzyme activity was expressed as absorption delta of 240 nm per mg protein. All steps of enzyme extraction were performed on ice.

Superoxide dismutase (SOD) enzyme. 200 mg of plant tissues were extracted in 50 mM HEPES-KOH buffer (pH 7.8) containing 0.1 mM EDTA. The homogenate was transferred to centrifuge tubes and was centrifuged at 12000 rpm for 30 min at 4°C. The supernatant was transferred to 15 ml tubes and referred to enzyme extract. For assay, a mixture consisting of 50 mM HEPES-KOH (PH 7.8) containing 0.1 mM Na-EDTA, 50 mM Na_2CO_3 (PH 10.2), 12 mM L-methionine, 75 µM Nitro Blue Tetrazolium (NBT), 1µM Riboflavin and crude extract was prepared and enzymatic extract as a unit of SOD activity was considered as enzymatic amount which resulted in 50% inhibition of NBT in 560 nm (Chance and Maehly, 1955). Reaction mix absorption was measured by spectrophotometer.

Total soluble protein was measured using the Bradford (1976) method. Absorption of 1 ml Bradford reagent along with 100 µl enzymatic extract were mixed completely and registered in 595 nm. Protein content was estimated using calibration curve of cow albumin serum (BSA) (Bradford, 1976).

3. Results and Discussion

Vase life

Vase life is one of the important post harvest characteristics of ornamental plants especially for cut carnation, which is highly affected by ethylene. Reduction of unfavorable effects of ethylene is an appropriate method for enhancing postharvest durability of plants or plant organs and compounds like 1-MCP are used extensively in order to alleviate the undesired effects of ethylene in horticultural crops. Results showed that the effect of 1-MCP on characteristics of cut carnation cv. Fortune was significant (P<0.01). Increasing 1-MCP concentration enhanced vase life of cut flowers so that the longest vase life (11.8 days) was related to 1.5 µl/l 1-MCP which was significantly (P<0.01) higher than control and 0.5 µl/l 1-MCP, while there was no significant (P<0.01) difference with 1 µl/l 1-MCP. By preventing external ethylene action, treatment with 1-MCP increased vase life (Fig. 1). In accordance with our results, Yamane *et al*. (2004) showed that 1-MCP increased vase life of cattleya flowers. It seems that 1-MCP prevents ACC-oxidase and ACC synthase expression, subsequently decreasing ethylene production in primary days of treatment with 1-MCP, resulting in increased vase life (Yamane *et al*., 2004; In *et al.,* 2013; Yang *et al*., 2013). This is also in agreement with the results of Chutichudet *et al*. (2010) who reported that 1-MCP protects tulip cut-flower quality by preventing ethylene production (Chutichudet *et al*., 2010). Hence, increasing vase life of cut-flowers treated with 1-MCP is related to inhibiting ethylene action and ethylene biosynthesis (Serek *et al*., 1994; Serek and Sisler, 2001).

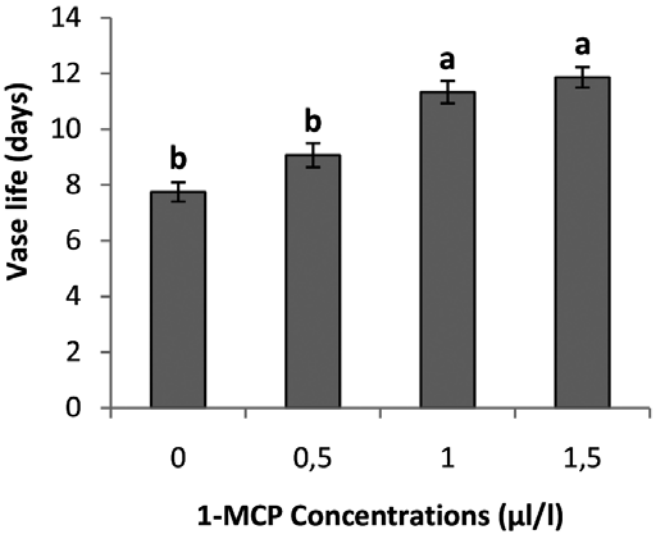

Fig. 1 - Effect of 1-MCP on vase life of carnation cv. Fortune. Data are means with standard errors (n=3).

Membrane stability index

1-MCP treatment preserved membrane stability in carnation cut flowers. The greatest membrane stability on the seventh day after treatment was related to 1.5 µl/l 1-MCP with no significant (P<0.01) difference toward 1 µl/l, but it was significantly (P<0.01) higher than control and 0.5 µl/l 1-MCP. 1-MCP can prevent membrane degradation by decreasing the lipid peroxidation which is regulated by ethylene (Yuan *et al*., 2010) (Fig. 2). Since ethylene is the main factor for increasing respiration rate in climacteric crops and causes an acceleration of physical characteristic changes and cell membrane phospholipids degradation, it seems that 1-MCP treatment protects membrane stability by preventing ethylene action.

Chlorophyll content

Based on the results, the highest chlorophyll content on the seventh day after treatment measured in 1.5 µl/l 1-MCP showed a significant (P<0.01) difference with the other treatments. The effect of 1-MCP on protecting chlorophyll content is a result of ethylene action and consequently inhibition of ethylene biosynthesis, which is considered the

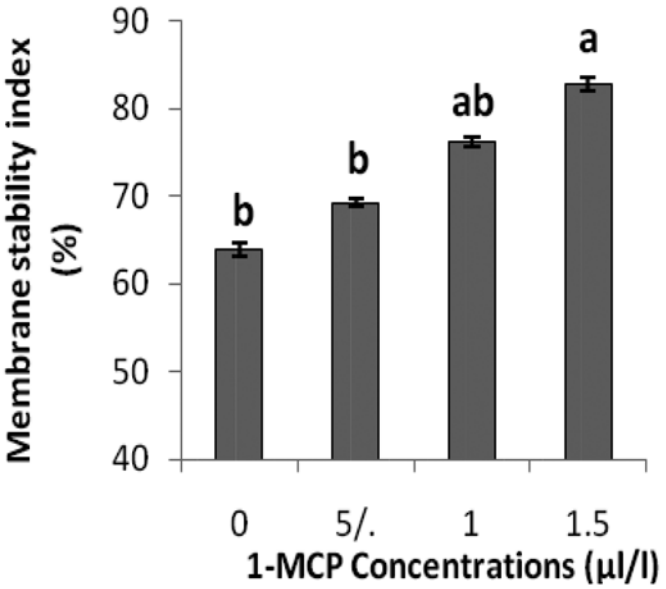

Fig. 2 - Effect of 1-MCP on petal membrane stability of carnation cv. Fortune. Data are means with standard errors (n=3).

most important factor of leaf chlorosis in ornamental plants. In accordance with the present results, 1-MCP treatment at all concentrations decreased ethylene biosynthesis which was followed by reduction of chlorophyll destruction compared to control plants (Asil *et al*., 2013) (Fig. 3). According to Serek *et al*. (1998) 1-MCP inhibited leaf chlorosis in chrysanthemum and geranium cv. Isable (Serek *et al*., 1998). In this case, the effect of 1-MCP was attributed to binding ethylene receptors. A recent study showed that 1- MCP can keep carbon assimilation in a condition of good efficiency and prevent tricarboxylic acid cycle. Under these conditions, by provoking the biosynthesis of gibberellins, the postharvest senescence process is retarded which appeared as preserving the postharvest quality (Wang *et al*., 2014).

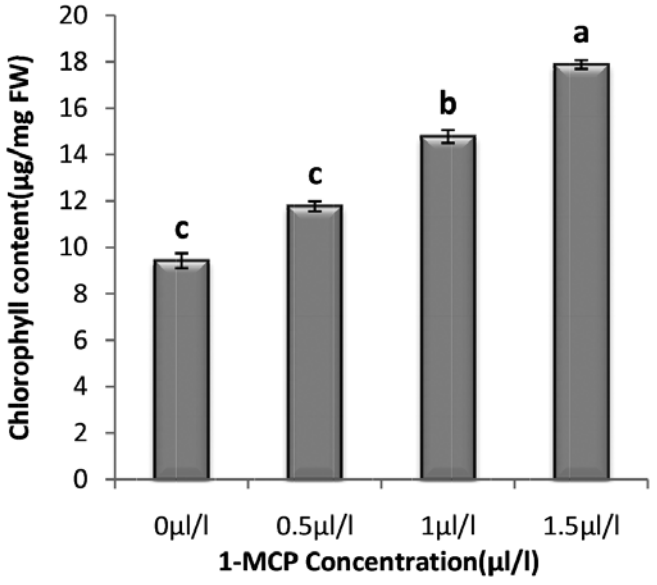

Fig. 3 - Effect of 1-MCP on petal chlorophyll content of carnation cv. Fortune. Data are means with standard errors (n=3).

Anthocyanin content

Our results showed that the highest anthocyanin content on the seventh day after treatment was related to 1.5 µl/l 1-MCP with significant (P<0.01) differences with the other treatments (Fig. 4). Positive effects of 1-MCP on inhibition of external ethylene action, delaying senescence and accordingly protecting suitable cell pH were the factors of anthocyanin photosynthesis pigments. Chutichudet *et al*. (2010) found that Black tulip cut flowers treated with 300 ppb 1-MCP for 8 h had the highest anthocyanin content till the twelfth day of vase life (Chutichudet *et al*., 2010). Usually, postharvest destruction of anthocyanin pigments is as a result of bracteoles' membrane function destruction (Jiang and Chen 1995; Jiang *et al*., 2004). The stability of anthocyanin may be due to the role of 1-MCP, which can decrease membrane destruction of fresh crops (Hershkovitz *et al*., 2005). Vacuole pH is enhanced during senescence and anthocyanin gets free of color before destruction (Zhang *et al*., 2001). Furthermore, anthocyanin destruction occurs as a result of polyphenol oxidase activity (Francis, 1989). The preventing from anthocyanins accumulation in tissues could be ascribed to ethylene action, based on research on *Arabidopsis thaliana* showed negative self-regulation in relationship of ethylene, carbohydrates and anthocyanins. Stress induced by ethylene resulted in reduced absorption of carbohydrates and consequently decreased accumulation of pigments (Das *et al*., 2011).

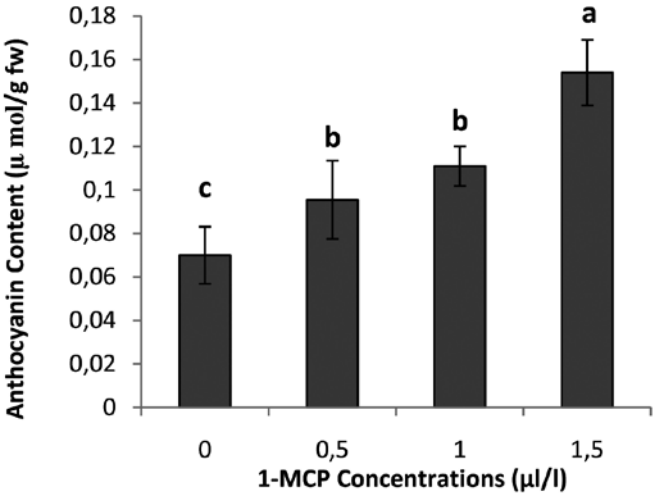

Fig. 4 - Effect of 1-MCP on petal anthocyanin content of carnation cv. Fortune. Data are means with standard errors (n=3).

Antioxidant enzymes

The highest activity of catalase was found in flowers treated with 1 µl/l 1-MCP, however it was not significantly (P<0.01) higher than 1.5 µl/l but was significantly (P< 0.01) higher than control and 0.5 µl/l 1-MCP treatments (Fig. 5). The highest activity of peroxidase was detected in 1 µl/l 1-MCP with significant (P<0.01) differences toward the other treatments (Fig. 6). 1-MCP protected antioxidant enzyme activity by inhibiting ethylene action and biosynthe-

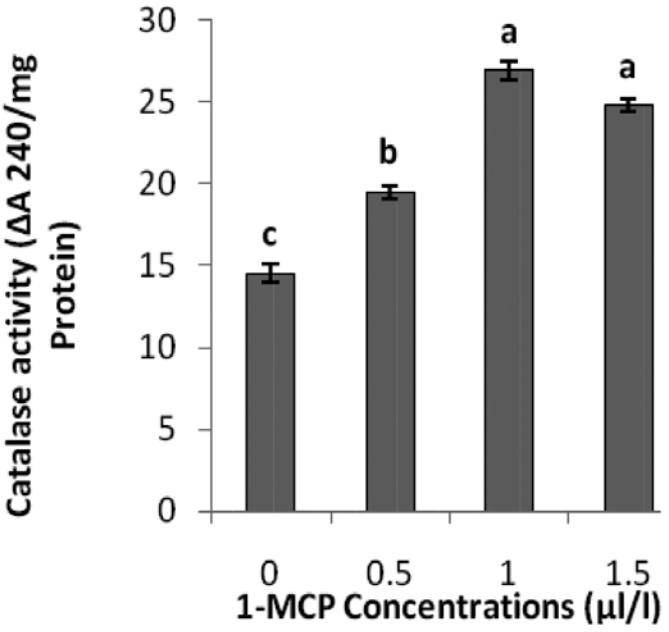

Fig. 5 - Effect of 1-MCP on petal catalase enzyme activity of carnation cv. Fortune.

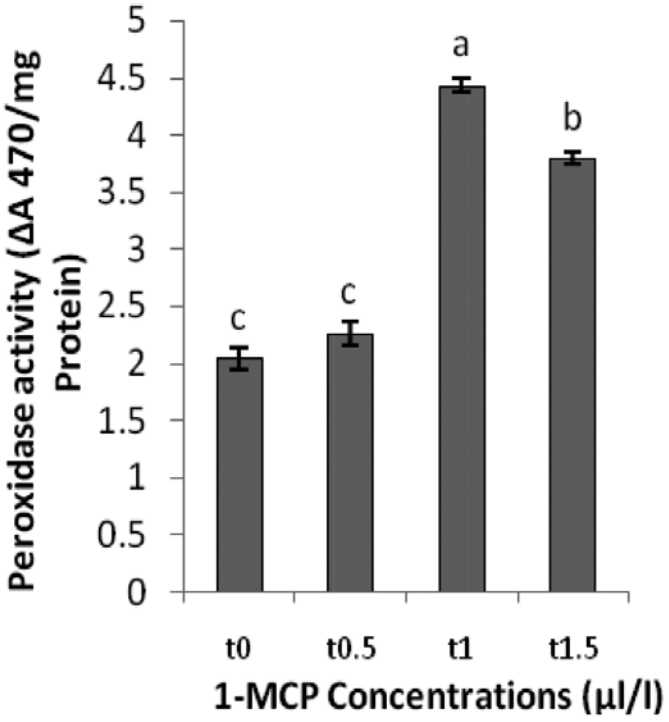

Fig. 6 - Effect of 1-MCP on petal peroxidase enzyme of carnation cv. Fortune.

sis, and subsequently decreased respiration rate. The petal senescence process causes metabolic and physiological changes, which result in the petal death. Senescence begins with the expression of a set of genes related to senescence and it emerges at metabolic level as oxidative processes and often the catabolic processes in senescence increase are irreversible (Buchanan-Wollaston, 1997). In microsomal membranes of carnation, many superoxides are generated during senescence (Mayak *et al*., 1983). Here also, increasing free radicals from ethylene stress in plants untreated with (0 µl/1) 1-MCP damaged to immunity system of antioxidant and reduction of immunity enzymes activity. When treated with different concentrations of 1-MCP, there was more antioxidant enzyme activity because of decreasing effects of external ethylene. Peroxidase has different biological functions, such as detoxification of hydrogen peroxide, lignin biosynthesis, hormonal signaling and response to stress (Gao *et al*., 2010). Catalase is considered an important biological factor and its major function is in the process of superoxide metabolism by playing role in releasing oxygen and hydrogen peroxide free radicals and preventing creation of hydroxyl radicals (Spanou *et al*., 2012). Superoxide dismutase like Cu-Zn superoxide dismutase, Mn superoxide dismutase and outside cell superoxide dismutase play a critical role in inhibition of superoxide (Miao and St Clair, 2009). In fact, peroxidase, catalase and superoxide dismutase play roles in protecting the metabolism balance of oxygen in plant tissues (Xie *et al*., 2003). Superoxide causes lipid peroxidation, cell membrane damage and finally senescence; 1-MCP can affect enzyme activities, which remove superoxide (Li *et al*., 2007). In accordance with our obtained results, increasing antioxidant enzyme (catalase, superoxide dismutase and peroxidase) activity of gladiola florets treated with 1-MCP has been reported (Fig. 7). It seems that this treatment decreases oxidative stresses in cut-flowers (Hassan and Ali, 2014). In other words, activity of these enzymes is a factor for the protection of cells against oxidative stresses (Zhou *et al*., 2014). It should be mentioned that even if ethylene decreases in response to 1-MCP, the activity of antioxidant enzymes will increase. In addition, a considerable increase in peroxidase, catalase and superoxide dismutase activity was observed in petals of carnation flower cv. Lilacon purple treated with 0.5 µl/l 1-MCP. The 1-MCP treatment

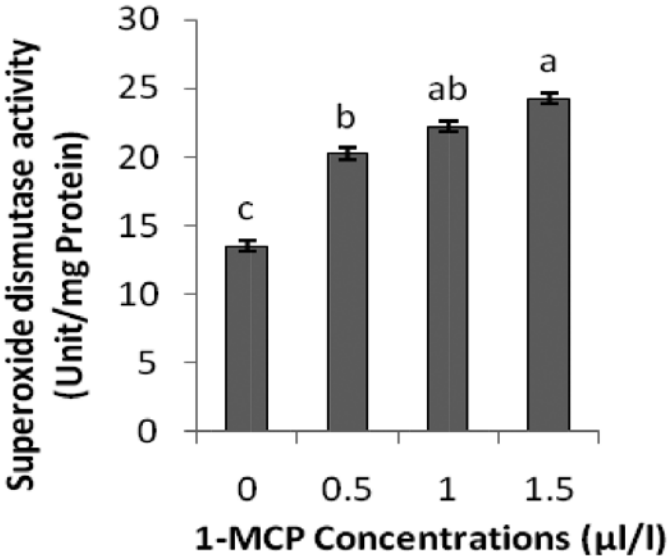

Fig. 7 - Effect of 1-MCP on petal superoxide dismutase enzyme of carnation cv. Fortune.

decreased hydrogen peroxide and superoxide (peroxide anion) compared to control plants (Karimi, 2014). This decreasing may be a result of low ethylene biosynthesis and inhibition of hydrogen peroxide and peroxide anion by peroxide and superoxide dismutase enzymes (Larrigaudiere *et al*., 2004). In asparagus, 1-MCP hindered the ethylene signal transduction and resulted in a delay by affecting ethylene biosynthesis, and enhancing superoxide dismutase activity (Zhang *et al*., 2012). In brief, our study has shown that 1-MCP treatment can delay senescence and increase flower vase life by protecting photosynthesis pigments and increasing antioxidant enzyme activity.

In conclusion, 1-MC prevented the effects of exogenous ethylene and alleviated the stress conditions induced by ethylene in cut carnation flowers. It seems that 1-MC treatment increased the resistance capacity of tissue/organs by boosting the activity of antioxidant enzymes resulting in favorable physiological and biochemical organ activities.

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