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Full Length Research Paper

Regulation of ethylene biosynthesis by nitric oxide and thidiazuron during postharvest of rose

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Nitric oxide (NO) and thidiazuron (TDZ) have been shown to extend the postharvest life of a range of flowers possibly by downregulating ethylene production. In this study, we have evaluated the effect of sodium nitroprusside (SNP), a nitric oxide donor and thidiazuron on postharvest senescence of cut *Rosa* flower. Therefore, we examined the effects of SNP and TDZ on its ethylene production and shelf life. Flowers were treated for 24 h with 0, 20, 40 µmol.L^{-1} TDZ and 0, 20, 40, 60 µmol L^{-1} SNP and then held in the solution, 8-HQS at 300 ppm combination with 1% sucrose. Treatment with NO and TDZ delayed the ethylene production and prolonged shelf life. It was observed that, treating with NO and TDZ at a 40 µMol.L^{-1}concentration and 40 µM.L^{-1} TDZ with 40 µM.L^{-1} SNP decreased ethylene production and senescence of floweres. SNP at 60 µMol.L^{-1} harmed the flowers. It was suggested that NO and TDZ could decrease ethylene output, by inhibiting ACC synthase activity and reducing ACC content.

Key words: Rose, nitric oxide, thidiazuron, ethylene biosynthesis, postharvest.

INTRODUCTION

Rose (*Rosa hybirida*), of the family of Rosaceae, is recognized for its high economic value, and is used in agro-based industry especially in cosmetics and perfumes. Additionally, Rose plays a vital role in the manufacturing of various products of medicinal and nutritional importance. However, the main idea of Rose plant cultivation is to get the cut flowers, which greatly deals with the floricultural business (Butt, 2003). Vase life of cut rose flowers is usually short. Cut flowers wilt and floral axis becomes bent (bent-neck) just below the flower head (Elgimabi and Ahmed, 2009). The development of such symptoms is considered to be caused by vascular occlusion, which inhibits water supply to the flowers (Loub and Van Doorn, 2004). Several methods to increase the vase life of cut flowers and keep their freshness for longer periods have been reported.Thidiazuron (TDZ; N-phenyl-N-l, 2, 3-thiadiazol-5-Urea) is a non-metabolizable phenyl-urea compound with strong cytokinin-like activity (Macnish et al., 2010; Zhua, 2006; Capelle, 1983). Jiang et al. (2009) reported that application of low concentrations of 2 to 10 µM TDZ has been shown to be a very effective means of delaying leaf yellowing in cut flowers such as alstroemeria, stock, lilies and tulips and potted plants, including geranium, freesia, Ornithogalum, and Euphorbia fulgens. Treatment with TDZ has also been reported to prevent leaf senescence in a range of cut flower species including alstroemeria, chrysanthemum, lupin, phlox and tulip (Ferrante et al., 2002, 2003; Sankhla et al., 2005). The mode by which TDZ treatment extends flower longevity has not been determined, although it may act by regulating cytokinin and/or auxin activity (Mok et al., 2000). TDZ, albeit at relatively lower concentrations, can inhibit leaf yellowing and delay the onset of flower senescence in other ornamental plant species (Ferrante et al., 2002, 2003; Sankhla et al., 2005; Macnish et al., 2010). Sankhla et al. (2003, 2005) showed that TDZ treatment could also delay ethylene-mediated floral organ abscission and senescence in phlox and lupin. Mutui et al. (2007) reported that 20 µM TDZ increased ethylene production in pelargonium cutting and can probably

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induce an unknown ACC synthase gene. TDZ is not metabolized by the plants; therefore its activity lasts longer than that of other cytokinins (Genkov and Ivanova, 1995). Moreover, it has been found that TDZ might promote the conversion of cytokinin ribonucleotides to more biologically active Ribonucleosides (Ferrante et al., 2002; Zhua, 2006; Capelle, 1983). Sankhla et al. (2005) suggest that the effect of TDZ has to be studied, considering sensitivity of leaves or flowers. Therefore, the absence of ethylene negative effects may be explained by varying ethylene sensitivity in different species. This hypothesis might be confirmed by considering the relationship between endogenous cytokinins and ethylene sensitivity. Transgenic petunias that over-produce cytokinins showed that the higher level of cytokinins content was correlated with lower flower sensitivity to exogenous ethylene (Chang et al., 2003). Moreover, the climacteric ethylene peak in these plants was delayed and flower life prolonged. Macnish et al. (2010) reportet that pulsing with 0.2 to 1 mM TDZ for 6 to 24 h extended the longevity of iris flowers and leaves and 0.5 mM TDZ stimulated a significant increase in ethylene production by iris flowers during their opening.

Recently, there has been an impressive upsurge in elucidating the physiological and biochemical functions of nitric oxide (NO) in plants (Crawford and Guo, 2005; Del Rio et al., 2004; Lamattina et al., 2003; Neill et al., 2003; Wendehanne et al., 2004).

This enigmatic, but unique diffusible multifunctional plant signal molecule, plays pivotal role in diverse plant processes including hormone modulation, programmed cell death, and wounding and defense responses. Several studies point out that there is a cross talk between NO, ethylene, IAA, abscisic acid, GA, calcium, calmodulin, cGMP and cADPR (Lamattina et al., 2003; Wendehanne et al., 2004). Zhu et al. (2006) reported that in the peaches treated with 5 and 10 µl.L^{-1} NO, 1 aminocyclopropane-1-carboxylic acid (ACC) oxidase activity and ethylene production were reduced. Some previous work has demonstrated that NO could delay ripening and improve the postharvest quality of strawberries (Wills et al., 2000; Zhu and Zhou, 2005), avocados (Leshem and Pinchasov, 2000) and carnations (Bowyer et al., 2003), when applied as short-term fumigation at low concentrations. Although it is suggested that NO might exert a profound influence on fruit by inhibiting ethylene production (Leshem, 2000), the mechanism by which NO affects this process is still not clear. NO has been shown to inhibit ethylene action and synthesis in plants (Leshem and Wills, 1998), (Figure 3) and it has been suggested that NO acts as a natural senescence-delaying plant growth regulator primarily by downregulating ethylene production. NO donors have also been shown to protect a variety of cut flowers from ethylene and dramatically increase the vaselife (Badiyan et al., 2004). The promotion/retardation of flower senescence by NO donors depended on the concentration used and the genotype. Leshem et al. (1998) showed that exogenous NO extends the postharvest life and delays senescence in flowers, fruits, and vegetables.

MATERIALS AND METHODS

Plant material and treatments

Rosa flowers (*Rosa hybrida* cv. 'Sensiro') were picked from shrub growing in commercial greenhouses, Pakdasht, in the fall of 2011 at a bending sepal stage. They were selected for uniformity of size and freedom from defects and mechanical damage. The cut flowers were then transported to the laboratory in Zanjan University and used for experiment. The flower stems were trimmed to 45 cm, and all leaves except for the upper three were removed. Three cut flowers were placed in each of 400 ml beakers, including treatments. The cut flowers were maintained at 19±2°C with natural photoperiods. Flower stems were given pulsing treatment for 24 h with thidiazuron (TDZ) concentrations of 0, 20, 40 µM.L^{-1} and sodium nitroprusside (SNP), a nitric oxide donor with concentrations of 0, 20, 40 and 60 µM.L^{-1}. After pulsing, the assigned samples of flower stems were immediately transferred into the beakers with 300 ppm 8-HQS and 1% socruse. Treatments were arranged in a completely randomized design with 3 replications and analyzed using MSTAT-C. The means were compared by Duncan's multiple range test at $P \leq 0.05$.

Measurement of ethylene production

Ethylene was measured by placing three cut flowers of each treatmant in 18 L airtight chamber at 20°C for 3, 6, 12 and 24 h and then ethylene production level was determined by ICNA56 ethylen biosyntesis bioconfervation.

Measure method of flower longevity

Longevity trait was measured by utilization of submitted method, and by attention to such traits as: flower wilting, flower color change, petals number opening, bending of flower neck and flowers freshness that are due to flowers without senescence and measured on base of percent (Fernando et al., 1999).

RESULTS AND DISCUSSION

TDZ effects on ethylene production and flower longevity

This experiment result shows that ethylene production increased during postharvest for all TDZ-treated flowers and the control (Figure 1). TDZ treatments decreased ethylene production; between 20 and 40 µM.L^{-1} TDZ treatments had significantly effeciency ($P<0.05$). Cut flowers treated with 40 µM.L^{-1} TDZ slowed ethylene production down. Solution whitout TDZ had higher level of ethylene production during postharvest. The vase life of cut rosa flowers was efficiently increased by pulse treatments with 20 µM.L^{-1} TDZ, which inhibited flower senescence during the whole experimental period

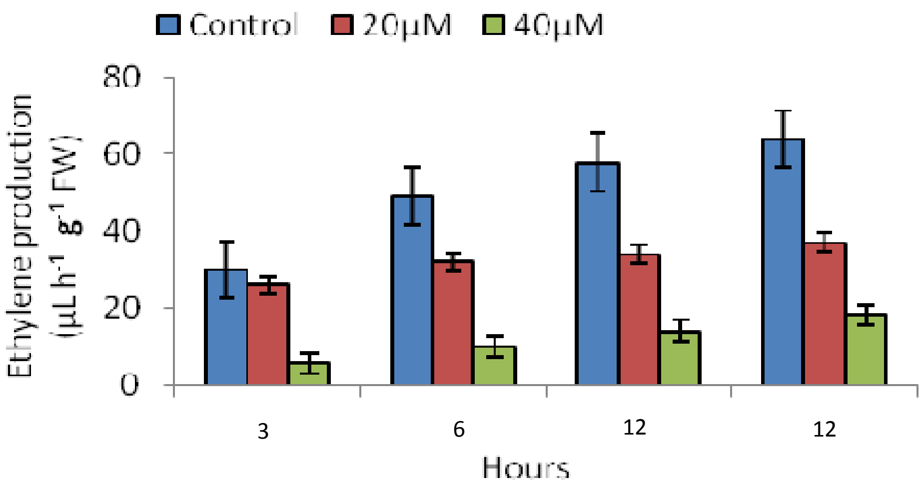

Figure 1. Effect of thidiazuron on ethylene production in rosa flower during postharvest.

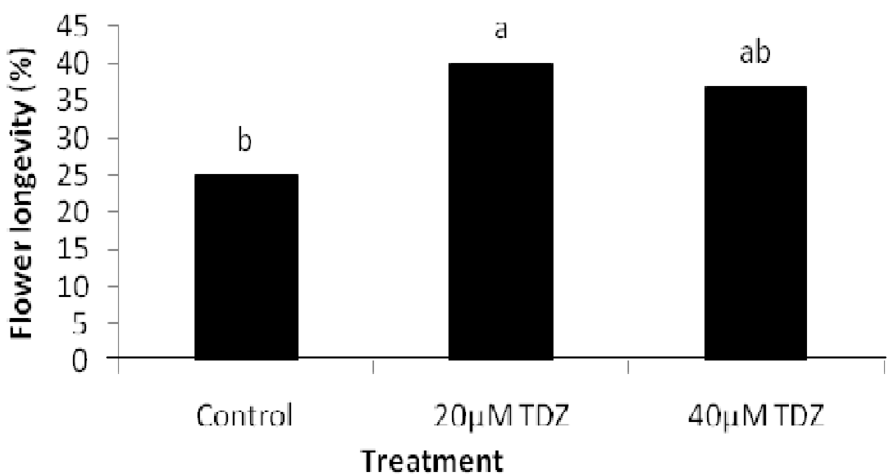

Figure 2. Effect of thidiazuron on flower longevity in rosa flower during postharvest.

(P<0.05), (Figure 4). In fact, treated cut flowers with TDZ increased flower longevity by decreasing ethylene production. Thidiazuron has high cytokinin-like activity.Although the exact mode of TDZ action is not known, evidence suggests that it can regulate endogenous cytokinin biosynthesis and/or metabolism (Mok et al., 2000). It is unclear whether TDZ acts to invoke cytokinin responses by interacting directly with cytokinin receptors in the leaves or indirectly by stimulating conversion of cytokinin nucleotides to their biologically active ribonucleosides or by inducing accumulation of endogenous adenine- based cytokinins which could be due to inhibition of cytokinin oxidase (Ferrante et al., 2002). Thus, TDZ treatment, by reducing ethylene production, increases vaselife of cut flowers. This experiment results are in agreement with Sankhla et al. (2005) and Macnish et al. (2010), but not with Mutui et al. (2007).

SNP effects on ethylene production and flower longevity

The results show that SNP treatments significantly affect (P<0.05) the ethylene production in rosa flowers. Ethylene production rates decreased during the postharvest period in treated flowers and increased untreated flowers with SNP (Figure 2). Ethylene production in 40 and 60 µM.L^{-1} SNP-treated Rose was lower than those of the controls.Pulse treatments with solutions containing SNP slightly delayed the flower senescence of cut rosa flowers compared to controls and

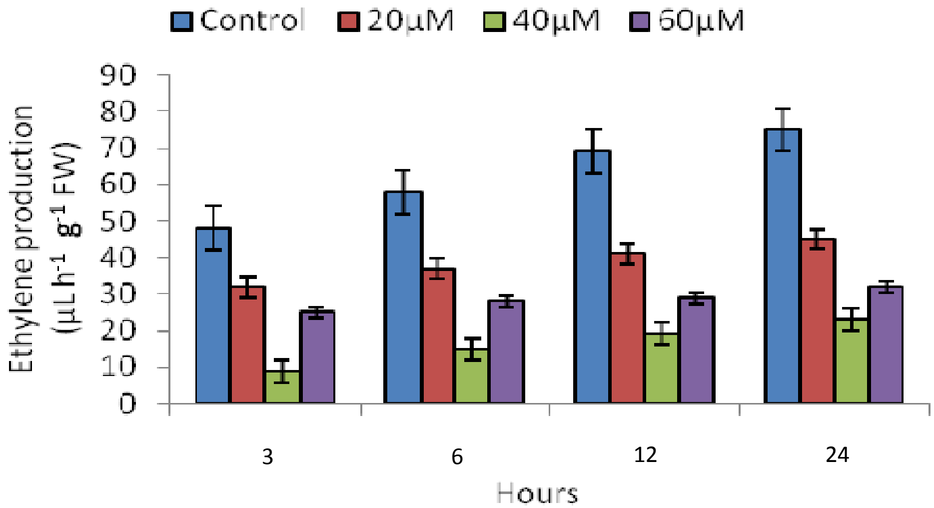

Figure 3. Effect of nitric oxide on ethylene production in rosa flower during postharvest.

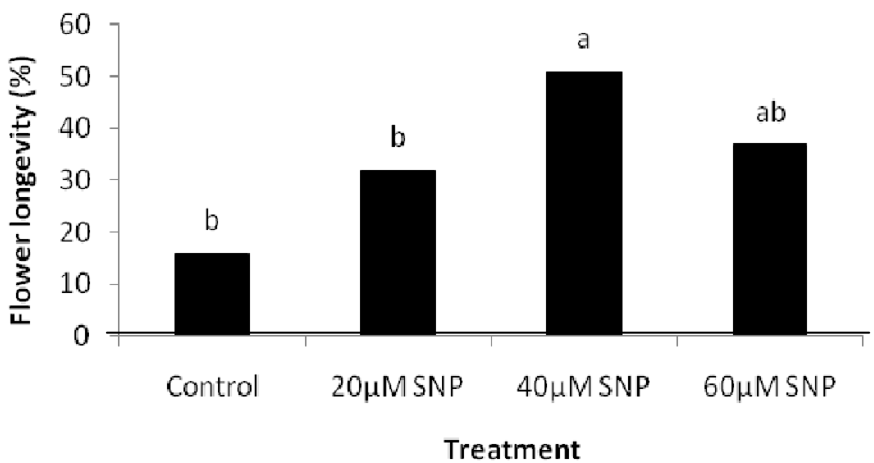

Figure 4. Effect of nitric oxide on flower longevity in rosa flower during postharvest.

on the conversion of ACC to ethylene; it only prevented ACC synthesis through ACS deactivation. Through this slightly increased the vase life ($P<0.05$). The highest vase life was observed at 40 µM.L^{-1} SNP-, while vase life at 20 µM.L^{-1} SNP and control was reduced; but between this two treatments there was no significant difference.Exogenous application of SNP extended the postharvest life of fresh horticultural produced by inhibiting ethylene production (Leshem et al., 1998). Recently, it has been demonstrated that endogenous NO and ethylene production maintained an inverse correlation during the ripening of strawberries and avocados (Leshem and Pinchasov, 2000). NO has been shown to extend the postharvest life of a range of flowers, fruits and vegetables possibly by down-regulating ethylene production (Badiyan at al., 2004; Leshem and Wills, 1998). In phlox, although SNP in the vase solution promoted the abscission of open flowers, the younger buds continued to open even in the presence of high SNP concentrations (Sankhla et al., 2003). In this experiment 40 µM/L^{-1} SNP compared to 60 µM.L^{-1} SNP had low ethylene production. Also results show that higher SNP concentration induced ethylene production. In high SNP concentrations (>50 µM), the leaves became either yellow, or more frequently turned progressively black and senesced (Sankhla et al., 2003). In conclusion, the results presented here demonstrate that NO has the potential to regulate ethylene biosynthesis. Also, physiological changes associated with *Rosa* cut flower senescence could be halted or delayed by inhibiting ethylene production. Ethylene production during senescence is tightly regulated by the two key enzymes, ACS and ACO (Barry et al., 1996, 2000; Rottmann et al., 1991). NO treatment was hypothesized to have no effect

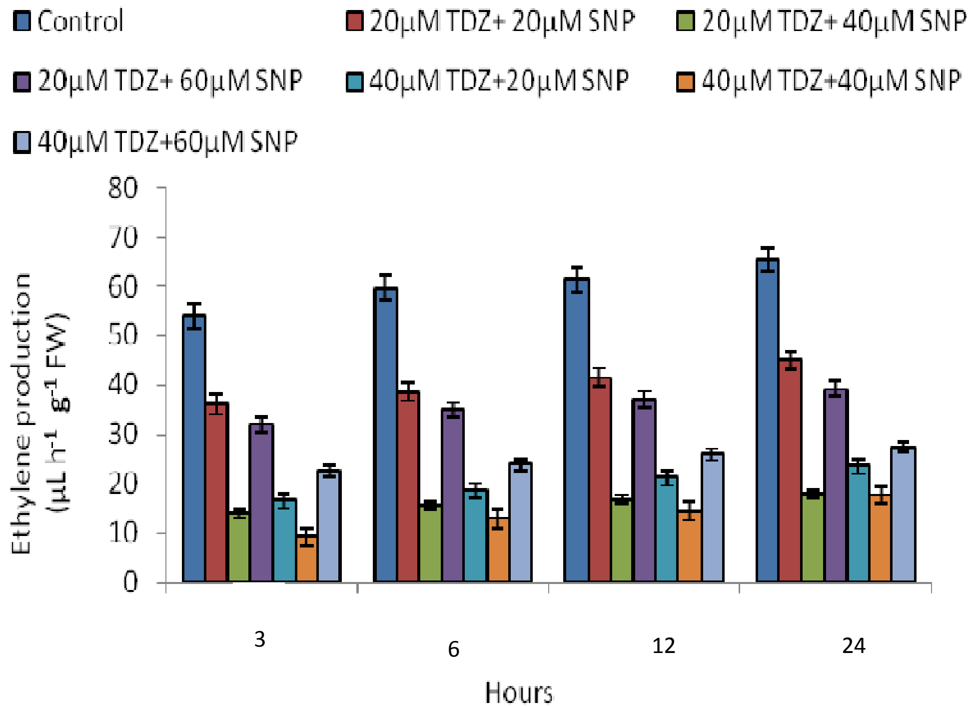

Figure 5. Effect of thidiazuron and nitric oxide on ethylene production in rosa flower during postharvest.

mechanism, NO might inhibit ethylene biosynthesis (Eum et al., 2009). NO may lower ACO activity by inhibition of ACC transport. In the process of ethylene biosynthesis, ACC is proposed to be synthesized in the cytoplasm byACS, and then transported to ACO. ACO is a membrane-bound enzyme and located at the external face of the plasma membrane (Wang et al., 2006; Rombaldi et al., 1994). NO delayed the softening of strawberry throughout the storage period and reduced the rate of ethylene production compared to untreated fruit (Eum and Lee, 2007). This experiment results are similar with that of Eum and Lee (2007); Badiyan at al. (2004) and Leshem and Wills (1998).

TDZ and SNP effects on ethylene production and flower longevity

In the present study, TDZ and SNP concentrations significantly ($P \leq 0.5$) inhibited the ethylene production rate during postharvest (Figure 2). Pulse treatment with 40 µM.L^{-1} TDZ plus 40 µM.L^{-1} SNP reduced ethylene production compared to the other treatments. The vase life of cut Rosa flowers was significantly increased by SNP treatment ($P<0.5$). Exposure of flowers to 40 µM.L^{-1} SNP concentration was more effective than other treatments in increasing the flower longevity during postharvest. Badiyan et al. (2004) show that DETA/NO, the NO donor extended the vase life of cut flower such sa Snapdragon, Delphinium, Chrysanthemum, Tulip, Gerbera, oriental Lily, Rose and Iris.

Leshem et al. (2000) also suggested that NO may inhibit ACO activity by oxidative inactivation of its cofactors, ascorbate and Fe_2. In addition, it was suggested that NO affects ethylene production through direct regulation of ACS or ACO enzymes, and the regulation of ACS and ACO genes (Wills et al., 2000) (Figures 5 and 6).

Conclusion

Finally, it was concluded that treatment with NO and TDZ delayed the ethylene production and prolonged shelf life. It was observed that, treating with NO and TDZ at a 40 µMol.L^{-1} concentration and 40 µM.L^{-1} TDZ with 40 µML^{-1} SNP decreased ethylene production and caused senescence of floweres. SNP at 60 µMol.L^{-1} harmed the flowers. It was suggested that NO and TDZ could decrease ethylene output, by inhibiting ACC synthase activity and reducing ACC content.

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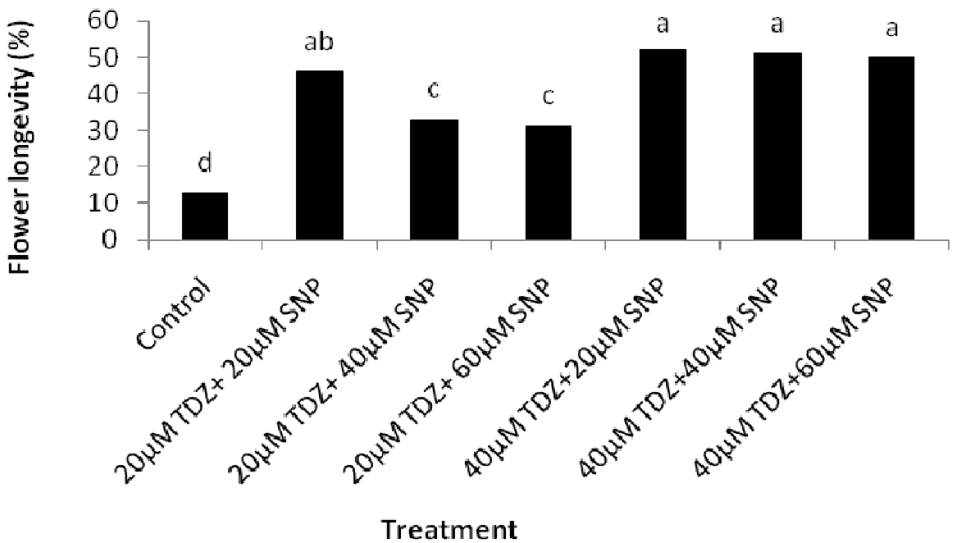

Figure 6. Effect of thidiazuron and nitric oxide on flower longevity in rosa flower during postharvest.

REFERENCES

Badiyan D, Wills RBH, Bowyer MC (2004). Use of a nitric oxide donor compound to extend the vase life of cut flowers. HortSci., 39: 1371-1372.

Barry CS, Llop-Tous MI, Grierson D (2000). The regulation of 1-aminocyclopropane-1-carboxylic acid synthase gene expression during the transition from system-1 to system-2 ethylene synthesis in tomato. Plant Physiol., 123: 979-986

Bowyer MC, Wills RBH, Badiyan D, Ku VVV (2003). Extending the postharvest life of carnations with nitric oxide–comparison of fumigation and in *vivo* delivery. Postharvest Biol.Technol., 30: 281-286.

Butt SJ (2003). A Review on prolonging the vase life of Rose. Pakistan Rose Annual. Published by Pak. Nat. Rose Soc., 49-53.

Capelle SC, Mok DWS, Kirchner SC, Mok MC (1983). Effects of thidiazuron on cytokinin autonomy and the metabolism of N6-(Δ2-isopentenyl)(8-14C) adenosine in callus tissues of Phaseolus lunatus L. Plant Physiol., 73: 796-802.

Chang H, Jones ML, Banowetz GM, Clark DG (2003). Overproduction of cytokinins in petunia flowers transformed with PSAG12-IPT delays corolla senescence and decreases sensitivity to ethylene. Plant Physiol.,132: 2174-2183.

Crawford NM Guo FQ (2005). New insights into nitric oxide metabolism and regulatory functions. Trends Plant Sci.,10: 195-200.

Del-Rio LA, Corpas FJ, Barroso JB (2004). Nitric oxide and nitric oxide synthase activity in plants. Phytochem., 65: 783-792.

Elgimabi MN, Ahmed OK (2009). Effects of Bactericides and Sucrose-Pulsing on Vase Life of Rose Cut Flowers (*Rosa hybirida*). Bot. Res. Int., 2 (3): 164-168,

Eum HL, Kim HB, Choi SB, Lee SK (2009). Regulation of ethylene biosynthesis by nitric oxide in tomato (Solanum lycopersicum L.) fruit harvested at different ripening stages. Europian Food Res. Technol., 228: 331-338.

Fernando IF, Monica MC, Jjose GB, Paulo CRF (1999). Influence of ethephon, silver thiosulfate and sucrose pulsing on bird of – pardise vase lif. Revista brasileria defisiologia vegetal., 11(2): 119-122

Ferrante A, Hunter DA, Wesley PH, Reid M S (2002). Thidiazuron - a potent inhibitor of leaf senescence in Alstroemeria. Postharvest Biol.Technol., 25: 333-338.

Ferrante A, Mensuali-Sodi A, Serra G, Tognoni F (2003). Treatment with thidiazuron for preventing leaf yellowing in cut tulips, and chrysanthemum. Acta Horticultur., 624: 357-363.

Ferrante A, Mensuali-Sodi A, Serra G (2009). Effect of thidiazuron and gibberellic acid on leaf yellowing of cut stock flowers. Central European J. Biol., 4(4): 461-468

Genkov T, Ivanova I (1995). Effect of cytokinin-active phenylurea derivatives on shoot multiplication, perioxidase and superoxide dismutase activities of in vitro cultured carnation, Bulg. J. Plant Physiol., 21: 73-83.

Jiang CZ, Wu L, Macnish AJ, King A, Yi M, Reid MS (2009). Thidiazuron, a Non-Metabolized Cytokinin, Shows Promise in Extending the Life of Potted Plants. Acta. Horticultur., 847: 59-65

Lamattina L, Garcia-Mata C, Graziano M, Pagnussat G (2003). Nitric oxide: the versatility of an extensive signal molecule. Ann. Rev. Plant Biol., 54: 109-136.

Leshem YY Wills RBH (1998). Harnessing senescence delaying gases nitric oxide and nitrous oxide: a novel approach to post harvest control of fresh horticultural produce. Biol. Plant, 41: 1-10.

Leshem YY (2000). Nitric Oxide in Plants: Occurrence, Function and Use. Kluwer Academic, Dordrech, The Netherlands.

Leshem YY, Pinchasov Y (2000). Non-invasive photoacoustic spectroscopic determination of relative endogenous nitric oxide and ethylene content stoichiometry during the ripeneing of strawberries *Fragaria anannasa* (Dutch.) and avocados *Persea americana* (Mill.). J. Exp. Bot., 51: 1471-1473.

Loub M, Doorn GV (2004). Wound- induced and bacteria-induced xylem blockage in Rose, Astible and Viburmum. Horticultral Sci., 32: 281-288.

Macnish AJ, Jiang CZ, Reida MS (2010). Treatment with thidiazuron improves opening and vase life of iris flowers. Postharvest Biol. Technol., 56: 77-84.

Mok MC, Martin RC, Mok DVS (2000). Cytokinins: Biosynthesis, metabolism and perception. *In vitro* Cell. Biol. Plant, 36: 102-107.

Mutui TM, Mibus H, Serek M (2007). Influence of thidiazuron, ethylene, abscisic acid and dark storage on the expression levels of ethylene receptors (ETR) and ACC synthase (ACS) genes in Pelargonium. Plant Growth Regulator, 53: 87-96.

Neill SJ, Desikan R, Hancock JT (2003). Nitric oxide signalling in plants. New Phytol., 159: 11-35.

Rottmann WH, Peter GF, Oeller PW, Keller JA, Shen NF, Nagy BP, Taylor LP, Campbell AD, Theologis A (1991). 1-aminocyclopropane-1-carboxylate synthase in tomato is encoded by a multigene family whose transcription is induced during fruit and floral senescence. J. Mol. Biol., 222: 937-961.

Sankhla N, Mackay WA, Davis TD (2005). Effect of thidiazuron on

senescence of flowers in cut inflorescences of Lupinus densiflorus Benth. Acta Horticultur., 669: 239-243.
Wendehanne D, Durner J, Klessig DF (2004). Nitric oxide: a new player in plant signaling and defense responses. Curr. Opin. Plant Biol., 7: 449-455.
Wills RBH, Ku VV, Leshem YY (2000). Fumigation with nitric oxide to extend the postharvest life of strawberries. Postharvest Biol.Technol.,18: 75-79
Zhu SH, Zhou J (2005). Effect of nitric oxide (NO) on ripening and senescence of strawberry. Sci. Agric. Sin., 38: 1418-1424 (in Chinese).
Zhua S, Liu M, Zhou J (2006). Inhibition by nitric oxide of ethylene biosynthesis and lipoxygenase activity in peach fruit during storage. Postharvest Biol. Technol., 42: 41-48.