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Effects of Herbal Essences and Nano-technology on Enzyme Activity of Cut Rose Flowers Cultivar 'Black Majic'

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ABSTRACT: Herbal essences extracted from medicinal plants as well as the materials produced in nanotechnology such as nanosilver and nanocopper having minimum environmental danger. Therefore, an experiment was conducted in order to assess the effects of silver nanoparticles, nanocopper, eucalyptus and thyme essence along with sucrose on catalase and Super oxide dismutase of rose cut flowers. In this experiment, interaction effects of nanosilver (2, 4 and 6 ppm), eucalyptus (25, 50 and 100 ppm), nanocopper (2, 4 and 6 ppm) and thyme (50, 100 and 150 ppm) on variation of these enzymes were evaluated. For this reason, factorial experiment based on completely randomized design with three replications was used. Distilled water and sucrose 3% were used as control. Flowers were excised from maternal stock in bud stage and then transported to laboratory of Islamic Azad University of Khorasgan Branch. Additional leaf and blades were removed from lower part of stem. All cut branches were equaled to 40 cm in long. Analysis of variance showed significant difference among treatments, times and interaction effects between them for catalase and superoxide dismutase activity. The highest catalase activity was observed in the treatments; NS 4E100, NS 6E1, NS 6E2, NS 6E3. For superoxide dismutase the highest activity was revealed in the NS 4E100, NS 6E1, NS 6E2, NS 6E treatments. In conclusion, higher activity of catalase and superoxide dismutase enzymes caused vase life increasing through microbial infection reduction and xylem clogging. Increasingly, the highest concentrations of nanosilver, essences as well as first and second levels of nanocopper produced maximum activity of these enzymes. Furthermore, significant correlation was observed between vase life and activity of these enzymes so measurement of such enzymes are one of the best procedure to determine higher vase life in rose cut flowers.
Key words: Rose (Rosa hybrida L.), enzyme zctivity, catalase, superoxide dismutase, herbal essences, nano-technology.

Introduction

Rose (Rosa hybrida L.) is one of the most beautiful, absorbent and important cut flowers worldwide. The materials produced in nanotechnology science such as nanosilver and nanocopper having minimum environmental danger so nanosilver also is used in food science. The most important factor in increasing postharvest life of cut flowers are environmental conditions and chemical treatments. Among of chemical treatments are germicidals, anti-ethylen componds such as 1-MCP_(1-Methyl cyclopropon), AVG-Amino ethoxy Vinyl glycine, AOA- Amino xyacetic acid and organic acids such as citric acid, tartaric acid have been reported. One of the most important ingredients in a flower preservative solution is sucrose. It can provide the carbohydrates and reduced ethylen production. These solutions increase the vase life, flower size and keep the color of leaves and petals. They have an effect on the quality of the flowers (Raffi ei al, 2006).

Stem end blockage is regarded as a major cause of imbalance between water uptake and water loss from cut flowers (Van Doorn, 1997). The development of such symptoms is considered to be caused by vascular occlusion, which inhibits water supply to the flowers (Van Doorn, 1997). The development of occlusions is thought to be caused by various factors such as bacteria, air emboli and physiological responses of stems to cutting. In many cut flowers, suppression of microbial growth in the vase solution results in delayed wilting (Loubaud and Van Doorn, 2004). Many agents have been used in cut flower vase solutions to extend vase life by improving water uptake. These include silver nitrate (AgNo3), aluminium sulphate (Ichimura and Shimizu-Yumoto, 2007), 8-hydroxyquinoline sulphate in cut rose (Ichimura et al, 1999) and silver thiosulphate (STS) in cut sweet pea. The sugar content is another factor controlling vase life, because the carbon supply is limited in cut flowers (Da-Silva, 2003). Usually, sugars such as sucrose was added to vase water to extend the vase life of cut roses that followed by bacteria growth.

The present study was conducted to evaluate the effect of different concentration of herbal essences extracted from eucalyptus and thymus as well as nano silver and nano copper on catalase and superoxide dismutase and its effect on vase life of cut Rose flower cultivar Black Majic.

Materials And Methods

Plant material of cut rose flower (Rosa hybrida), 'Black Majic' were obtained from a greenhouse in Islamic Azad University, Isfahan, Iran. They were immediately transferred to the postharvest laboratory of horticulture department at Islamic Azad university of Khorasgan (Isfahan), Iran. Buckets containing the flower stems were covered with a plastic film shroud to minimize moisture loss during transportation. The experiments were carried out the same day. The flower stems were re-cut under deionized water to a uniform length of 40 cm. Recutting was to ensure no air blockage of the stem end.

The main block was 3 % sucrose solution. The treatments were combinations of different concentration of Nano copper and Nano Silver (2, 4 and 6 ppm) with eucalyptus essence (25, 50 and 100 ppm) and thymus (50, 100 and 150 ppm) along with distilled water as well as distilled water + sucrose as controls totally twenty treatments. Sucrose was applied in all the treatments except distilled water alone. Cut flowers pulse in these compounds for 2 hours. Then they placed in blocks. The second block was pure distilled water. The measured traits were catalse enzyme, superoxide dismutase enzyme and vase life.

Full factorial experiment with three replications in the layout completely randomized design was used as experimental design. Comparison between the means was achieved by using the Duncan's Multiple Range Test at the 0.05 significance level. Statistical analysis of the data was done by $SAS_{9.2}$ software while the graphs was drawn by Excel2007.

Table 1. Mean comparison for catalase enzyme activity in different days after harvesting rose cut flowers

Treatments	CAT0	CAT3	CAT6
C	3.4067^{a}	2.5967^{g}	1.8000^{f}
Cs	3.4067^{a}	2.6667^{g}	1.8300^{f}
NS2E25	3.4067^{a}	2.9500^{fg}	2.1033^{f}
NS2E50	3.4067^{a}	4.2200^{def}	3.1733^{ef}
NS2E100	3.4067^{a}	4.2500^{def}	3.1667^{ef}
NS 4E25	3.4067^{a}	5.4033^{cd}	4.3467^{b-e}
NS 4E50	3.4067^{a}	5.3833^{cd}	4.3867^{b-e}
NS 4E100	3.4067^{a}	6.4867^{abc}	5.3900^{abc}
NS 6E1	3.4067^{a}	7.2100^{ab}	5.5267^{ab}
NS 6E2	3.4067^{a}	6.4967^{abc}	5.4067^{abc}
NS 6E3	3.4067^{a}	7.2233^{ab}	5.5067^{ab}
Cu2T1	3.4067^{a}	5.3367^{cd}	4.3500^{b-e}
Cu2T2	3.4067^{a}	3.6900^{efg}	3.1133^{ef}
Cu2T3	3.4067^{a}	4.7833^{de}	3.7367^{de}
Cu4T1	3.4067^{a}	5.7867^{bcd}	5.1133^{e-d}
Cu4T2	3.4067^{a}	4.7967^{de}	3.8033^{cde}
Cu4T3	3.4067^{a}	5.7800^{bcd}	5.1033^{a-d}
Cu6T1	3.4067^{a}	7.4167^{a}	6.3967^{a}
Cu6T2	3.4067^{a}	4.8600^{cde}	3.9033^{b-e}
Cu6T3	3.4067^{a}	5.7633^{bcd}	5.1367^{a-d}
In each column, means with the same letter(s) haven't significant difference.			

Results And Discussion

Significant differences was shown by analysis of variance among the treatments for all the measured traits. The highest catalase activity was observed in the treatments; NS 4E100, NS 6E1, NS 6E2, NS 6E3 (Table 1), while for superoxide dismutase the highest activity was revealed in the NS 4E100, NS 6E1, NS 6E2, NS 6E treatments (Table 2).

All together, higher activity of catalase and superoxide dismutase enzymes caused vase life increasing through microbial infection reduction and xylem clogging. Increasingly, the highest concentrations of nanosilver, essences as well as first and second levels of nanocopper produced maximum activity of these enzymes. Over more, significant correlation was observed between vase life and activity of these enzymes so measurement of such enzymes are one of the best procedure to determine higher vase life in rose cut flowers. Variation of the catalase activity over the times (0, 3 and 6 days) revealed the significant effect of treatments, times and its interaction on catalase activity (Figure 1).

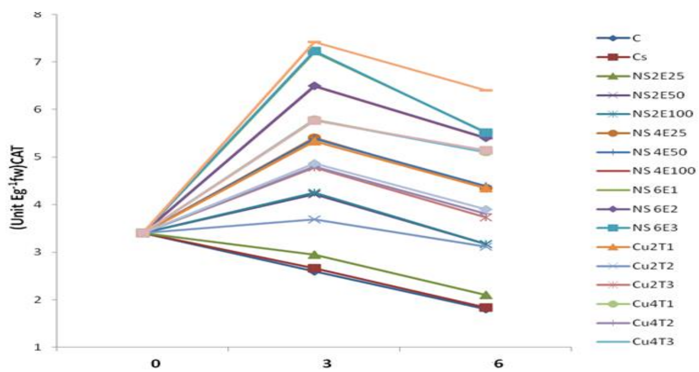

Figure 1. Variation of catalase enzyme over the different times for all the treatments

Variation of the superoxide dismutase enzyme activity for all the times (0, 3 and 6 days) was assessed.

The results dictated on the significant effect of treatments, times and its interaction on superoxide dismutase activity (Figure 2). The treatments NS6E1, NS6E2, NS6E3, Cu2T2, Cu2T3, Cu4T1, Cu4T2 and Cu4T3 showed the highest vase life. Significant differences were observed among these treatments with others. Similar results have been reported by Nair et al (2003) and Song et al (2006).

Table 2. Mean comparison for superoxide dismutase enzyme activity in different days after harvesting rose cut flowers

Treatments	SOD0	SOD3	SOD6
C	5.1067^{a}	3.3500^{e}	2.3533^{de}
Cs	5.1067^{a}	3.5200^{e}	2.3967^{de}
NS2E25	5.1067^{a}	3.5433^{e}	2.2067^{e}
NS2E50	5.1067^{a}	3.5300^{e}	4.0467^{c}
NS2E100	5.1067^{a}	3.5400^{e}	4.0600^{c}
NS 4E25	5.1067^{a}	4.5533^{cd}	5.0433^{ab}
NS 4E50	5.1067^{a}	4.5367^{cd}	4.0900^{c}
NS 4E100	5.1067^{a}	5.5700^{b}	5.6267^{a}
NS 6E1	5.1067^{a}	6.6333^{a}	5.0133^{ab}
NS 6E2	5.1067^{a}	5.5467^{b}	5.5633^{a}
NS 6E3	5.1067^{a}	6.5533^{a}	5.0233^{ab}
Cu2T1	5.1067^{a}	4.5267^{cd}	5.0433^{ab}
Cu2T2	5.1067^{a}	4.2233^{de}	3.0567^{d}
Cu2T3	5.1067^{a}	4.2000^{de}	3.0767^{d}
Cu4T1	5.1067^{a}	5.2800^{bc}	4.4800^{bc}
Cu4T2	5.1067^{a}	4.2300^{de}	4.0300^{c}
Cu4T3	5.1067^{a}	6.0767^{ab}	4.4267^{bc}
Cu6T1	5.1067^{a}	6.8000^{a}	5.4967^{a}
Cu6T2	5.1067^{a}	5.3000^{bc}	4.0333^{c}
Cu6T3	5.1067^{a}	6.0800^{ab}	4.4633^{bc}
In each column, means with the same letter(s) haven’t significant difference.			

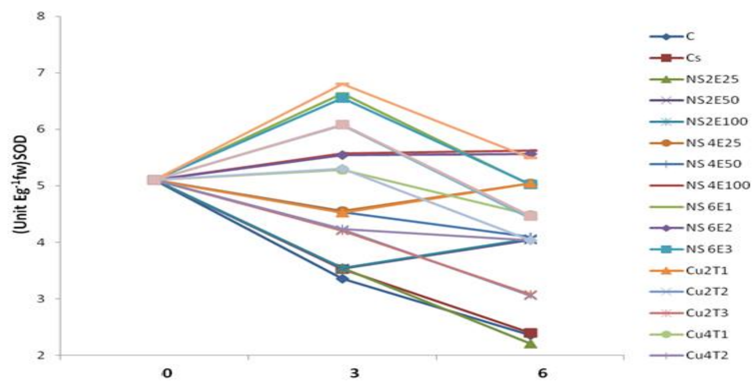

Figure 2. Variation of superoxide dismutase enzyme over the different times for all the treatments

Meman et al (2006) and Kim et al (2005) reported that flowers pulsed with 10 p.p.m. Nano Copper along with 10 p.p.m. Nano Silver had maximum catalase and superoxide dismutase enzyme activity on the seventeenth day. Similar results was reported in Bleeksma and Van Doorn (2003) and Baiges et al (2001) study. They believed that the mechanisms of copper toxicity towards microorganisms is disrupt the integrity of the plasma memberan and DNA. It may be preservative solution microorganisms destroyed by Nano Copper and Nano Silver in our study. Thus vessels remained open and cut flowers were able to absorb more water.

Stem occlusion reduced the water uptake and increased the loss of turgidity (Meman et al, 2006). Liu et al (2009) reported that 20 mg L-1 SNP for 24 hours increased vase life of cut gerbera. Bacterial plugging of the xylem is an alternative cause of early and rapid cut flower senescence (Solgi et al, 2009). The presence of Ag+ ions had a profound effect on the water relations of cut rose flowers. Furthermore, SNP have antibacterial effects (Sondi et al, 2004) and can extend vase life. Therefore, SNP may have positively influence on water uptake because of antibacterial effects of Ag+ ions in SNP may affect regulation of water channel activity via inhibition of sulfhydryl-containing proteins (Niemietz and Tyerman, 2002) and improve solution uptake.

In conclusion, application of the highest concentrations of nanosilver, first and second levels of nanocopper effects lead to enhance vase life in rose cut flowers through microbial infection decline and xylem clogging because of higher activity of catalase and superoxide dismutase enzymes. Over more, significant correlation of vase life with catalase and superoxide dismutase enzyme activity was observed. Therefore, these characters should be determined as criteria to enhance vase life of rose cut flowers cultivar "Black Majic".

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