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Evaluation of Synthetic Zirconyl Complex as Potential Floral Preservative on Postharvest Quality of Cut Roses *Rosa hybrida* L. Cv. Grand prix

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ABSTRACT

Floral preservative biocides in most cases consider toxic at the affective concentration to both plant and microbial proliferation. Therefore, in order to find nontoxic biocide, this experiment was conducted to evaluate a recent Synthetized Zirconyl Complex as a potential floral preservative biocide for cut roses (*Rosa hybrida* L. cv. Grand Prix). To assess the effect of Zirconyl biocide as potential preservative, cut roses was treated with 0, 10, 20 and 50 ppm plus 20% sucrose as pulse solution for 24 hours before transferred into holding solution 10 times dilution from the pulsing solution. The flower quality such as the vase life, flowers relative fresh weight and maximum flower diameter were observed. Also, flower water relation parameters were determined, besides evaluating the biochemical responses such as chlorophyll degradation and florescence, total soluble sugars, free amino acids, DPPH free radical scavenging activity, total phenolic compounds and total flavonoids were determined. Results indicated the possibility to use Zirconyl complex biocide as floral preservative at 20-50 ppm concentration to extend the cut rose flowers vase life of from 8.88 to 13 day through improving flower water relation parameters and reducing oxidative damage.

Keywords: Rose, Zirconyl, Vase life, water relation, chlorophyll, fluorescence, DPPH.

INTRODUCTION

Roses (*Rosa hybrida* L.), the most important ornamental species of *Rosaceae* family, are recognized for their high economic value. Vase life of rose cut flowers is usually short Shaman, (2012). Floral preservative solution consider is a preferred environment for microbial proliferation as it holds needed resources of water and sugars essential for reproduction. The microbial proliferation surrounding flowers stems rabidly causes vascular occlusion which reduce and/or prevent water uptake Jowkar *et al*., (2012) which consider the major cause of quality deterioration and vase life reduction.

Therefore, the target of cut flower market is to control and minimize microbial proliferation to extend quality and longevity of cut flowers, mainly roses. Conversely functional biocides are expected to affect flowers physiological properties particularly their photosynthetic system function by their toxic compounds during aging and senescence Jowkar *et al*., (2012). None of the biocides had a consistent and high anti-bacterial effect at concentrations that are not toxic to flowers. Knee, (2000).

Some of these biocides such as silver nitrate and silver thiosulphate are considered hazard chemical and risk to environment and health Damunupola and Joyce, (2006). A recent Zirconyl Complex based on azo dye ligands have been Synthetized on the Department of Chemistry, Faculty of Science, Tanta University. The Synthetic Zirconyl complex were tested *in-vitro* for their antimicrobial activity against different types of bacteria and fungi. In most cases, the ligands and complexes were active against both tested bacteria and fungi. The importance of the hetyerocyclic azodyes stems from their biological activities such as inhibition of DNA, RNA, protein synthesis, carcinogenesis and biological activity Khedr *et al*., (2014).

The biocidal role of the complex had been investigated, but how plant tissues respond to it and if it toxic to plants or not wasn't investigated. To be used as floral preservative biocide, plant physiological responses such as chlorophyll degradation, chlorophyll fluorescence and plant antioxidant mechanisms still need more evaluation. Therefore in order to found an easy to use, nontoxic and inexpensive compound for large scale application; we have focused on the mentioned physiological properties beside flower quality assessment to evaluate biocidal efficacy of Zirconyl Complex as floral preservative biocide for cut rose flowers (Rosa hybrida L. cv. Grand Prix)

MATERIALS AND METHODS

The experiment toke place at the Botany Laboratory, Faculty of Science Tanta University at January 2014 to evaluate the recent synthetic Zirconyl Complex as a potential preservative biocide in the cut flower market of *Rosa hybrida* L. cv. Grand Prix.

Plant material: Cut rose (Rosa hybrida L. cv. Grand Prix), flowers were purchased from a flora mix Egypt commercial cut flower growers in Giza, Egypt. The cut flowers were harvested at normal harvest maturity when sepals starting to reflex Shaman, (2012). They were immediately stood upright into buckets partially filled with tap water and transported within 3 hours to the botany laboratory at faculty of science Tanta University. To minimize moisture loss, the cut flowering stems were enclosed with plastic film during transport.

In the laboratory, uniform flowers free of visual defects were used in the experiments. Prior to treatments, the stems were re-cut under distilled water to 35 cm length to eliminate air blockage in the stem. Lower leaves were removed from each stem to insure that leaves out of the vase are used to hold the flowers. Rose flower stems were held individually in 200 ml glass vases each containing 150 ml of vase solution. The vase opening was covered with a low density polyethylene film sheet to minimize contamination and prevent evaporation.

Preservative solution preparation: Solutions were freshly prepared at the beginning of experiments.by dissolving the Zirconyl complex in 0.2% DMSO before reaching the final volume using distilled water. The Complex Molecular formula and analysis are presented in Table (1) quoted after Khedr *et al*., (2014), the complex structure is presented in Fig (1).

Table 1. Complex formula and characteristics:

Complex Molecular formula	Mol. Wt. (Cal. M. Wt.)	Color (Δ_m)	Microanalysis Found % (Calcd.)				
			%C	%H	%N	%Cl	%M
$[ZrO(L^4)_2]$	641.76	Brown	29.65	1.99	11.33	6.02	14.62
$(C_{16}H_{10}BrN_{10}O_3Zr)$	(641.35)	(2.18)	(29.96)	(1.57)	(10.92)	(5.53)	(14.22)

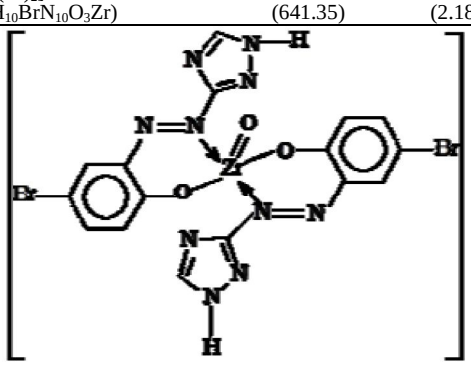

Fig. 1.Structure representation of Zirconyl (II) complexes.

Experimental design and treatments:

Flowers were arranged in a completely randomized design of 4 treatments. The treatment were replicated three times and each replicate consisting of nine flowers. Treatments applied as vase solutions were 0, 10, 20 and 50 ppm plus 20% sucrose as pulse solution for 24 hours, then flowers were transferred into holding solution 10 times dilution from the pulsing solution plus 2% sucrose. During the experiment, the flowers were held in controlled growth cabinet at 20 ± 2°C, relative humidity of 60 ± 10%, and light from cool white florescent tubes with 12 h of light.

Flower quality assessment:

Vase life was evaluated by checking the flowers appearance and condition, termination of vase life was recorded when wilting of the outer 5 petals occurred or bent neck was observed.

Maximum flower head diameter (cm) were daily measured to evaluate the bud size difference between treatments.

Assessment of water relation of cut roses: The fresh weight of cut flowers and the amount of water uptake were measured daily. The weight of vases without and with the flower was separately recorded in order to calculate:-

Relative fresh weight (RFW**)** of stems was calculated as % of initial fresh weight using He *et al*., (2006) equation RFW = (FWt/FWt-0) ×100, where, FWt is the fresh weight of stem (g) at t = day 2, 3, 4,…10, and FWt-0 is the fresh weight of the same stem (g) at t = day 0

Daily water uptake (µL day^{-1} g^{-1} fresh weight) was calculated as water uptake (µL day^{-1} g^{-1} fresh weight) = (St-1)-St, where, St is weight of vase solution (g) at t = day 2, 3, 4… 10, and St-1 is weight of vase solution (g) on the previous day. Ahmad *et al*., (2011).

Daily water loss was calculated as water loss (±µL day^{-1} g^{-1} fresh weight) = (Ct-1) - Ct, where, Ct is the combined weights of the cut stem and vase (g) at t = day 2, 3, 4, …,10, and Ct-1 is the combined weight of the stem and vase (g) on the previous day.

The difference between water uptake and water loss was expressed as the water balance

Flower Physiological responses:

Chlorophyll Degradation: Total chlorophyll were determined in detached leaf samples on day 2, 4, 6, 8, and 10 using 85% cold acetone for extraction and their color intensities were measured by using a spectrophotometer (SPECTRONIC 20 D) as mg/g fresh weight following Sadasivam and Manickam, (1992) method.

Chlorophyll Fluorescence: The quantum efficiency of open photosystem II centers (Fv/Fm), was measured according to method described by Jowkar *et al*., (2012) on all the stems leaves on day 2, 4, 6, 8, and 10 with Opti-Sciences OS-5P pulse amplitude fluorimeter (Opti-Sciences INC., Hudson, NH, USA).

The total soluble sugars concentrations mg/g D.W. were determined in detached leaf on day 8 when the vase life of control flowers was terminated according to Herbert *et al*. (1971).

DPPH Radical Scavenging Assay: The DPPH assay was used to determine the radical scavenging activity of the seed methanolic extracts according to the method reported by Brand-Williams *et al*., (1995). The percentage of decolourisation was obtained spectrophotometrically at 517 nm using JENWAY 6315 UV/Visible Spectrophotometer (Japan). DPPH radical scavenging activity (%) was calculated as [(Abs Control-Abs Sample)/Abs Control] ×100

Analysis of total phenolic compounds content mg/g D.W. was quantitativeley estimated by the method described by Jindal and Singh, (1975) using a standard curve prepared by using different concentrations of gallic acid

Estimation of total flavonoids content was performed following the Aluminium chloride colorimetric technique Chang *et al*., (2002) as mg/g D.W.

Determination of free amino acids content mg/g D.W was analyzed by Ninhydrin assays using glycine as a standard according to Lee and Takahashi, (1966).

Statistical analysis of results: Four treatments of three replicates each, were arranged in a randomized complete (RCD) design. The experiment was performed twice and the results were combined for two experiments (*n=6*) and analysis of variance (ANOVA) was performed using MSTAT-C program, USA Bricker, (1991). Means were compared by the LSD test at P = 0.05 according to Sendecor and Cochran, (1982).

RESULTS

Effect of potential preservative Zirconyl Complex on Vase life and flower quality.

Results indicated that Zirconyl complex was successful to prolong the vase life of rose cut flowers (Fig. 2). All treatment significantly recorded longer vase life compared to untreated flowers. The maximum vas life 13 days resulted from 20 ppm treatment compared to 8.88 days for the control treatment.

In the same time, Zirconyl treatment enhanced the quality of rose cut flowers by maximizing the flower diameter. All treatments increased flower diameter compared to control. The maximum flower diameter was reached by the 8th day. The maximum flower diameter was recorded for 20 or 50 ppm treatment without significant different. The maximum value in this concern was recorded for 20 ppm treatment as recorded 9.68 cm diameter compared to 8.53 cm for control treatments.

Treatments of Zirconyl complex treatment not only enhanced the vase life and maximum flower diameter, but also maintained higher relative fresh weight. The highest relative fresh weight was reached at the 6_{th} day then declined again till the end of vase life. Treatment of 20 ppm followed by 50 ppm maintained the highest relative fresh weight (131.25 and 128.77 % of initial fresh weight, respectively).

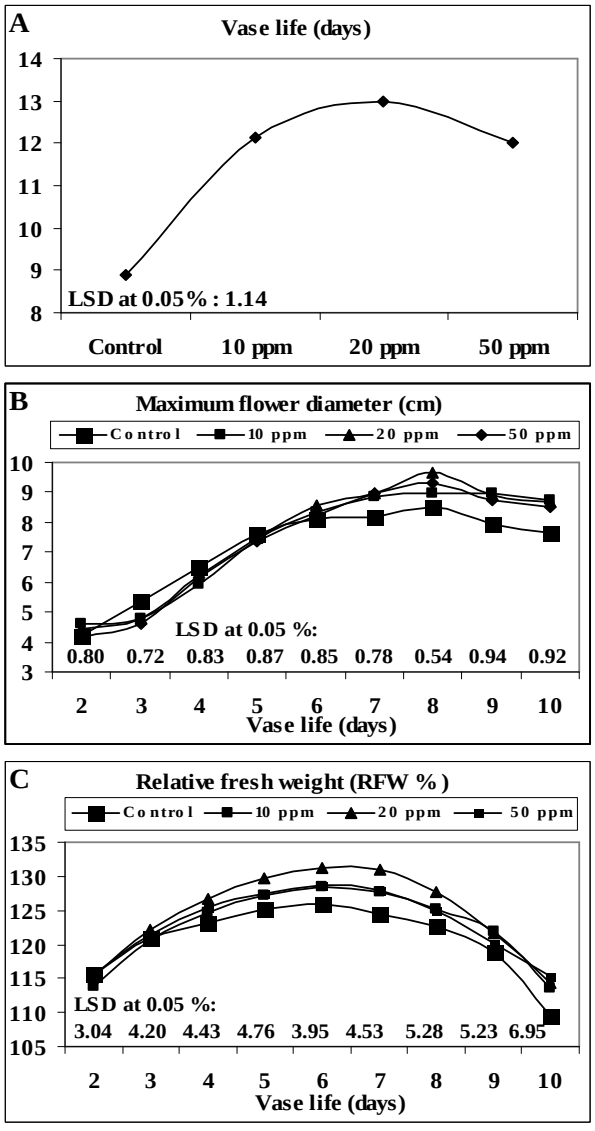

Fig. 2. Effect of Zirconyl complex treatment on vase life (A) maximum flower diameter (B) and relative fresh weight (C) of *Rosa hybrida* L. cv. Grand Prix cut roses

Effect of potential biocide Zirconyl Complex on rose cut flower water relations.

Using Zirconyl complex as postharvest biocides significantly enhanced water uptake and loss by *Rosa hybrida* L. cv. Grand Prix cut roses (Fig. 3). All levels of Zirconyl complex minimized water uptake and loss by flowers compared to control. Both water uptake as well as loss was decreased within the flower vase life to reach its minimum at the end of the experiment. Begging from the fifth day of flower vase life the lower values for water uptake and water loss (220 and 209 µL/day/g F.W.) was recorded for 50 ppm treatment followed by 20 ppm treatment in the second place for the rest of the flower vase life whereas all treatment recorded the same significant values compared to control treatment.

The balance between water uptake and water loss reveal the status of internal tissues activities of flower. As long as the water balance is positive, means that the flower is not suffering from water losing from tissues. All treatments recorded positive water balance till the sixth day before the water loss become greater than water uptake. By the end of vase life only 20 and 50 ppm treatments significantly minimized water loss values compared to other treatments.

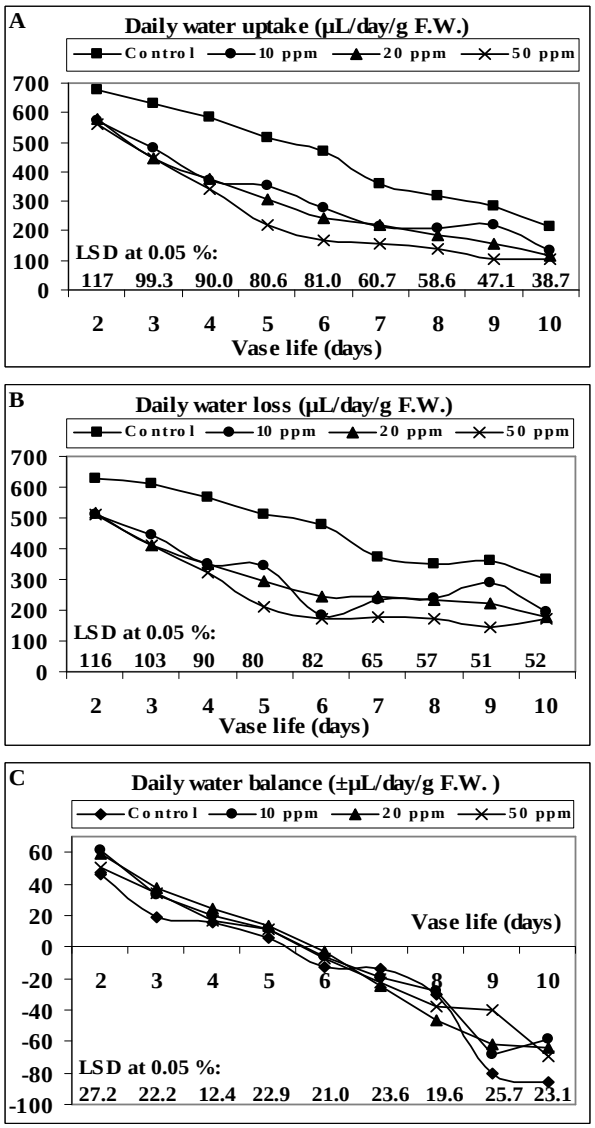

Fig. 3. Effect of Zirconyl complex treatment on daily water uptake (A), daily water loss (B) and daily water balance (C) of *Rosa hybrida* L. cv. Grand Prix cut roses.

Effect of potential biocide Zirconyl Complex on rose cut flower physiological parameters

Using Zirconyl Complex as a preservative component affected chlorophyll degradation during rose vase life (Fig.4). Till the 6th day all plants treated with Zirconyl Complex maintained higher chlorophyll content compared to control without significant differences between treatments. By the end of vase life only higher concentration was potent in this respect.

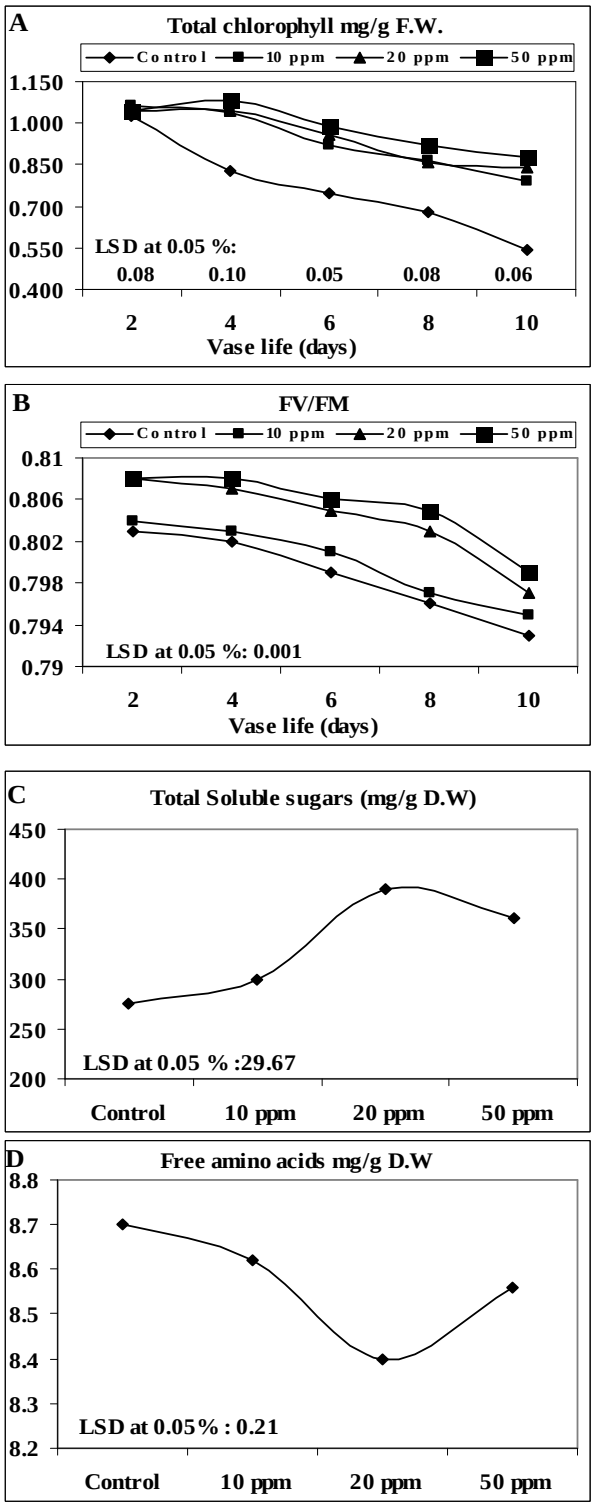

Fig. 4. Effect of Zirconyl complex treatment on total chlorophyll degradation (A), FV/FM (B), total soluble sugars (C) and free amino acids content (D) of *Rosa hybrida* L. cv. Grand Prix cut roses.

Zirconyl preservative not only retarded chlorophyll degradation but also enhancement of PSII photochemical efficiency measured by Fv/Fm. Rose flowers treated with 50 and 20 ppm respectively kept higher Fv/Fm values till the end of the experiment. As indicated in Fig. (4) Leaves of flowers treated with higher concentration of Zirconyl preservative recorded higher concentration of total soluble sugars, treatment of 20 ppm recorded the highest values of T.S.S. Meanwhile the same treatment recorded the lowest concentration of free amino acids 8.4 mg/g D.W. while other treatment had no significant difference compared to untreated plant.

Effect of potential biocide Zirconyl Complex on rose cut flower antioxidant responses

Treatment of Zirconyl not only affected water relation of Grand Prix cut roses but also recorded significant differences for all plant physiological responses (Fig. 5).

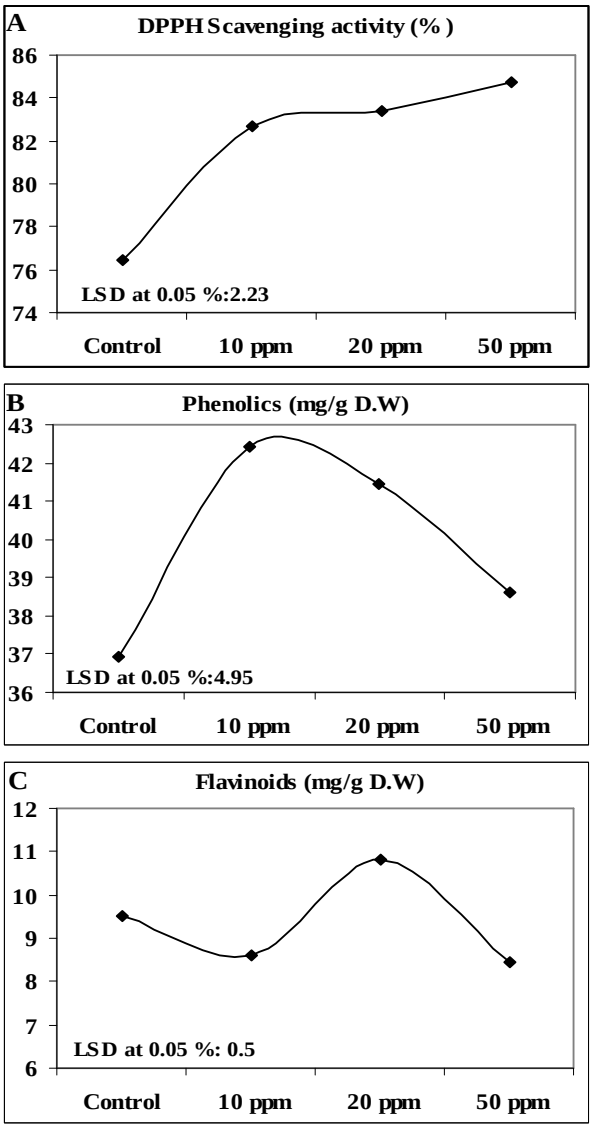

Fig.5. Effect of Zirconyl complex treatment on DPPH Scavenging activity (A), phenolic contents (B), and flavonoid content (C) of *Rosa hybrida* L. cv. Grand Prix cut roses.

Free radical scavenging activity (DPPH activity) shows a gradual increase within increasing Zirconyl

complex concentration. However the uppermost DPPH activity 84.73 % was recorded for 50 ppm treatment without significant differences between 10 and 20 ppm.

Meanwhile Phenolic compounds as a potent antioxidant compounds showed hewed a non-significant variations between all of Zirconyl complex concentrations. Only the treatment of 10 ppm recoded significant increase compared to control.

Phytochemical flavonoids, the low molecular weight polyphenolic secondary metabolic compounds seems to follow almost similar pattern. Flower treated with 20 ppm recoded the highest flavonoids content 10.81 mg/g D.W. followed by 9.5 mg/g D.W. for 50 ppm treatment.

DISCUSSION

Generally, Zirconyl complex proved to be effective floral preservative biocide without obvious toxicity symptoms. In most cases, effective biocides can be toxic to flower Jowkar *et al*., (2012). In our experiment, by the end of flower life a visual growth of lateral auxiliary buds were seen elongated in the flower stems and leaves were turgid and fresh. As an active biocide tested against both bacteria and fungi Khedr *et al*., (2014**)**, Zirconyl complex succeeded to prolong Grand Prix Cut rose's vase life. All treatment increased vase life by 46.4 to 35.13 % compared to control treatment.

In the same time the flower quality was maximized, the flower diameter was increasing for all treatment including control till the sixth day as control flowers shows no further increase after that. Meanwhile other treatment continued increasing flower diameter to reach its maximum at day 8. Zirconyl complex treatments not only delayed flower opening but also increase the flower diameter that the flower can grow to reach. In this experiment the flower lasted for about 4 days before it begins to welt down to declare ending vase life. The previous observation can be referred to the higher relative fresh weight of the flower stems during the vase life. Decline of relative fresh weight can causes some physiological disorders such as life reduction, lack of flower opening Bleeksma and van Doorn, (2003) and wilting of the leaves accompanied by improper opening and wilting of flowers Torre and Fjeld., (2001).

Relatively higher fresh weight is mostly due to inhibition of microorganism proliferation in vase solution and thus preventing stem basal end occlusion by microbes Van Doorn, (1996). Therefore, controlling or reducing microbial proliferation using Zirconyl complex extended the vase life of roses via maintaining higher water relation and balance. The data indicated that rose stems kept in Zirconyl floral preservative exhibited high water balance while minimized the water loss by the flower stem. As seen in (Fig. 3) a gradual decrease in water uptake and loss was observed till the sixth day with a positive water balance for all treatment followed by a negative balance after that with the minimum negative water balance in favor of Zirconyl complex treatments. Increasing water loss is the main cause of flower quality degradation, water loss is mostly due to stem blockage by the bacteria Liu *et al*. (2009) or by malfunction of membrane permeability caused by toxic biocide compounds during postharvest development and aging Jowkar *et al***.,** (2012).

On the other hand, applied biocides could also severally affect other physiological aspects of cut flowers i.e. photosynthetic function and by their toxic compounds during postharvest development and aging Jowkar *et al*. (2012). Flower stems under Zirconyl complex floral preservative stunted chlorophyll degradation and displayed higher content PSII photochemical efficiency (Fv/Fm). Delaying chlorophyll content degradation in cut flowers may be due to water relation improvement as indicated in (Fig. 3). Chlorophyll degradation is associated with of PSII photosynthetic efficiency DeEll *et al*., (1999), the Fv/Fm is normally in the range of 0.75 to 0.85 for non-stressed plants Bolhar-Nordenkampf *et al*., (1989). This is confirm our assumption that Zirconyl complex preservative created non stress environment for the flower stems. Chlorophyll fluorescence indicated the quantum yield of PSII during vase life, this fact and our results explains the higher levels of total soluble sugars in rose stem leaves. Another evidence explains that Zirconyl exhibit minimum toxicity to flower tissue is minimum, is free amino acids contents as plant treated with Zirconyl share the same values compared to control without significant differences. This means that, Zirconyl cause no further stress on plant tissues as plant under different stress condition tend to increase in the level of free amino acids Solanki and Sarangi, (2014).

The results in (Fig. 5) also indicates that the DPPH free radical Scavenging activity, of cut rose flower preserved with Zirconyl complex was initially low at the control treatment and increased gradually with increasing Zirconyl concentration. This effect was suggested by Schmitzer *et al*., (2010). Results also support the opinion of Lama *et al*., (2013) that, at the final stages of flower vase life, the low phenol content makes the flower more susceptible to oxidative stress. Plant treated with Zirconyl complex recorded lower phenolic contents except of 10 ppm treatment. Meanwhile, total flavonoids content was enhanced by 20 ppm treatment for efficiently mitigate oxidative stress management.

CONCLUSION

Form physiological point of view supported with the previous results, we could confirm that beside vase life improvement, using of Zirconyl complex as a floral preservative at low concentration 20-50 ppm improving flower water relation parameters as well as physiological and biochemical responses. As for our knowledge, this the first time to evaluate Zirconyl complex as a floral preservative. Further study is still needed to study its relation regarding antioxidant enzymes activity and stomata behavior.

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تقييم استخدام مركب الزركونيل التخليقى فى محاليل الحفظ وجودة أزهار الورد بعد الحصاد

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تعتبر معظم المركبات المستخدمة فى محاليل حفظ الزهور فى ظل التركيز الفعال سامة للنبات كما هى سامة للكائنات الممرضة. ولهذا أجريت هذه التجربة لتقييم استخدام أحد مركبات الزركونيل المخلقة حديثا كمركب محتمل لاستخدامه فى صناعة حفظ زهور القطف. لتقييم المركب تم حفظ الازهار فى محاليل تحتوى على المركب بتركيزات صفر، ١٠، ٢٠، ٥٠ جزء فى المليون بالاضافة الى سكر السكروز بمعدل ٢٠ % لمدة ٢٤ ساعة قبل نقلها لمحاليل مخففة بنسبة ١:١٠ من المحاليل الأولية. خلال التجربة تم تقييم عمر الأزهار، الوزن الطازج النسبى و الحد الأقصى لقطر الزهرة. بالاضافة لذلك تم حساب العلاقات المائية للأزهار مع تقييم الإستجابات الكيموحيوية متمثلة فى تحلل الكلوروفيل، الفلورة الضوئية، المحتوى الكلى للسكريات الذائبة، الأحماض الأمينية الحرة، النشاط الكلى لمضادات الاكسدة DPPH، محتوى الفينولات والفلافينات الكلية. أوضحت النتائج إمكانية استخدام مركب الزركونيل التخليقى فى محاليل حفظ ازهار الورد بتركيزات منخفضة حيث ادى استخدام المركب بتركيزات من٢٠ الى ٥٠ جزء فى المليون فى اطالة عمر ازهار الورد لتصل ال ١٣ يوم مقارنة ب ٨.٨ يوم للمعاملة الضابطة من خلال تحسين العلاقات المائية للازهار بالإضافة الى تقليل الضرر التأكسدى. مع عدم ظهور اى اثار واضحة لسمية المركب على الأزهار حيث لوحظ فى نهاية التجربة نمو فى البراعم الجانبية مع إحتفاظ أوراق الساق الزهرية بنضارتها.

كلمات البحث: الورد، الزركونيل، عمر الازهار، العلاقات المائية، الكلوروفيل، الفلورة الضوئية، DPPH.