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Effects of Chemicals on Vase Life of Cut Carnation (*Dianthus caryophyllus* L. 'Delphi') and Microorganisms Population in Solution

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Abstract

The vase life of cut flowers and foliage is often shortened by vascular occlusions that constrict vase solution supply. Reduction in stem conductivity is typically caused by blockage of cut stem ends and xylem conduits by microbes, physiological plugging, and disruption of water columns in xylem vessels by cavitations and air emboli. Cut flower and foliage longevity can be greatly affected by the chemical composition of the vase solution. A broad range of biocides has been suggested to prevent the proliferation of microorganisms in vase solutions; however, their assumed antimicrobial action may be confounded by their other physicochemical effects. the effect of some chemicals on postharvest longevity and microorganisms in solution of cut carnation 'Delphi' evaluated in a randomized complete block design with three replications. Flowers harvested in paint brush stage and recutted to 60 cm stem length. Vase life evaluated in 20±2 °C temperature, relative humidity 60% and 1800 lux light intensity. The results showed that flowers longevity has significant different ($P \leq 0.01$) and copper sulfate and Halamid® (Sodium N-Chloro-para-Toluenesulfonamide) were the best treatments. Population ($P \leq 0.001$) and relative water content ($P \leq 0.05$) were significantly affected by treatments and Halamid® was the best treatment to microorganisms control and water content. Highly significant negative correlation of relative water content and the bacterial population in solution indicate that the main effect of bacteria in reducing the water uptake.

Keywords: Carnation (*Dianthus caryophyllus* L. 'Delphi'), Copper sulfate, Halamid, HQC, Microorganism, Postharvest, STS.

INTRODUCTION

Carnation is one of the most important cut flower nowadays as well. Therefore it is important to ensure the longest vase life of the flowers. Various factors influence the postharvest performance and the vase life of cut flowers (Ichimura *et al*., 2002; Mayak *et al*., 1974). It is influenced by genetics, the growing conditions, flower handling (Pizano, 2009), carbohydrate content, blockage of xylem vessels, ethylene, the composition of the atmosphere, and the chemical solutions of the preservatives.

The principle antimicrobial compounds that have been used to extend the vase life of cut flowers are: (i) chlorine and bromine compounds, such as sodium hypochlorite (NaOCl) (van Doorn *et al*., 1990); (ii) hydroxyquinoline (HQ) compounds, such as 8-hydroxyquinoline citrate (HQC) (Knee, 2000; Marousky, 1969; van Doorn *et al*., 1990) and 8-hydroxyquinoline sulphate (HQS) (Hussein, 1994); (iii) quaternary ammonium compounds, such as benzalkonium chloride (n-alkyl dimethylbenzyl ammonium chloride) (iv) silver compounds, such as silver nitrate ($AgNO_3$) (Fujino *et al*., 1983) and, (v) a range of iscellaneous compounds including aluminium sulphate ($Al_2(SO4)_3$) (Put *et al*., 1992; Ruting, 1991); sodium benzoate (Knee, 2000); bromopropanediol (Knee, 2000); and, thiabendazole or 2-(4-thiazolyl)-benzimidazole (TBZ) (Apelbaum and Katchansky, 1977; Halevy *et al*., 1978). Each of these potential biocides has advantages and disadvantages (Faragher *et al*., 2002), and many of them have other functions added to antimicrobial.

The microorganisms on stems of cut flowers, foliage and in vase solutions are typically composed of yeasts, filamentous fungi, and bacteria (van Doorn, 1997). These microorganisms vary in their response to biocidal agents. For example, mycobacteria are relatively resistant to biocides, and then Gram-negative and Gram-positive bacteria being most sensitive (Maillard, 2002). Moreover, the developmental stage of a microorganism may result in a differential response to a biocidal agent. For instance, fully mature spores of *Bacillus subtilis* are much less susceptible to biocides than non-sporulating bacteria or vegetative cells (Turner *et al*., 2000). The differential response of microorganisms to biocides may be ascribed to variations in morphological structure (e.g. vegetative cell versus mature spore) and chemical composition (e.g. different types of peptidoglycans in bacterial spores) of the individual microorganism (Maillard, 2002; Turner *et al*., 2000). To be effective, an antimicrobial treatment must function in all conditions, including across varying vase solution composition (Knee, 2000), and against the prevalent microorganism, such as a specific bacterial species (Turner *et al*., 2000). In some studies were indicated that among 25 microorganisms, which are found in *Dianthus caryophyllus* L. 'Improved White Sim', three of them reduced vase life of them. Some of these microorganisms reduced vase life of cut rose "Cara Mia", *chrysanthemum* × *morifloium* Ramat. 'May Shoesmith' and other carnation cultivars such as 'Improved Red Sim' and 'Improved Pink Sim'. They also reported that it is possible that genetic structure of microorganisms that cause to reduce vase life of cut flowers, allow us to introduce a stimuli which cause to begin reduce vase life process. Understanding of starting stage of this process can delay senescence and increase vase life of cut flowers (Zagory and Reid, 1986; Ansari *et al*., 2011).

Both DICA and household bleach or sodium hypochlorite (NaOCl) are widely used in experimental flower handling and vase solutions (Faragher *et al*., 2002; He *et al*., 2006; Knee, 2000; van Doorn *et al*., 1989, 1990). Chlorine action involves the oxidation of cellular components in microorganisms, including essential enzymes in cell membranes and protoplasm (Bloomfield and Arthur, 1989, Dychdala, 1983). Five to 10 mg L^{-1} free available chlorine (FAC) helps control bacteria in preservative solutions (Xie *et al*., 2007). When chlorine compounds are added to preservative solutions, a portion of the chlorine is consumed by water impurities (chlorine demand), which include inorganic reducing agents, like Fe^{2+}, Mn^{2+}, NO^{2-}, and H_2S, as well as organic compounds, like amino acids. The chlorine atom ceases to maintain oxidising capacity upon reduction to chloride.

Silver is typically applied as the nitrate salt (Ketsa *et al*., 1995; van Doorn *et al*., 1991a); however, Ag+ can act as an antimicrobial agent (van Doorn *et al*., 1990), as an inhibitor of aquaporins in plants (Niemietz and Tyerman, 2002), and/or as an ethylene-binding inhibitor during ethylene synthesis and action (Beyer, 1976; Serek *et al*., 2006; Veen, 1979, 1983). It was not clear why the effectiveness of $AgNO_3$ as a biocidal agent was highly variable. van Doorn *et al*. (1990) noted that $AgNO_3$ cannot be used in water containing chlorine due to immediate precipitation of AgCl. Moreover, even in DI and distilled water, $AgNO_3$ will slowly undergo photochemical oxidation leading to a black Ag_2O deposit. $AgNO_3$ should be present in the vase solution in order to prolong vase life. Study on the mechanism of inhibitory action of Ag+ on microorganisms revealed that the expression of cellular proteins and enzymes that is necessary for ATP production, was inactivated with Ag+ (Yamanaka *et al*., 2005); also, DNA loses its replication ability. In contrast, HQS probably acts principally by its chelating ability with metal ions, and thereby disruption of bacterial cell enzyme function (Weinberg, 1957).

The ability of STS to inhibiting the ethylene action is utilized to prolong the vase life of cut carnations and other floricultural products (Cameron and Reid, 1983; Joyce, 1992; Mor *et al*., 1984; Premawardena *et al*., 2000; Yapa *et al*., 2000). Application of Ag+ as STS, substantially reduced binding activity by substitution for Cu^{2+} (Beyer, 1976). Cu^{2+} is involved in enzymatic reactions related to biosynthesis and action of ethylene (Himelblau and Amasino, 2000). The using of STS on cut flowers is of concern with regard to the disposal of waste silver solutions (Macnish *et al*., 2004). van Doorn *et al*., (1991b) found that STS (656, 1312, and 2624 mg L^{-1} for 4 h) did not reduce the number of bacteria in petioles of *Adiantum raddianum* fronds. In contrast, $AgNO_3$ (12.5 and 25.0 mg L^{-1}) reduced the number of bacteria in the petiole to zero. Biocides and other poisonous substances, including heavy metal compounds (e.g. Ag+), should not be disposed into the environment. Such chemicals need to be managed by an accredited/licensed waste contractor or through a chemical industry disposal program (Damunupola and Joyce, 2008).

Copper ions have been used in flower vase solutions as a biocide (Halevy and Mayak, 1981; van Doorn, 1997) and a wound reaction enzyme inhibitor (Vaslier and van Doorn, 2003). Van Meeteren *et al*., (1999) suggested that an artificial tap water solution containing low concentrations of $CuSO_4$ (50 μM), $CaCl_2$ (0.7 mM), and $NaHCO_3$ (1.5 mM) is appropriate as a standardised vase solution. CuSO4 had a pronounced positive effect on the time to wilting in dry-stored *Bouvardia* 'Van Zijverden' (Vaslier and van Doorn, 2003). While a non-specific inhibitor of peroxidase, Cu^{2+} also inhibits other enzymes, such as phenylalanine ammonium lyase (PAL) (Kim *et al*., 1996). PAL is also involved in cut stem wound reactions.

This paper examines Halamid® as a new biocide which is used in cut flower and foliage postharvest handling. Halamid® is a disinfectant based on a chemical substance known as Sodium N-Chloro-para-Toluenesulfonamide, ($C_7H_7ClNNaO_2S$, $3H_2O$) or "Chloramine T". Halamid® ionizes when dissolved in water. Because Halamid® attacks microbes through a process of oxidation, they cannot build up a resistance to it. In addition the chloramine T is highly stable and remains active over an extended period of time.

MATERIALS AND METHODS

Cut carnations 'Delphi' were harvested from a commercial greenhouse in Mahallat. The experiment was carried out in the laboratory of National Research Center of Ornamental Plants during April 2010. The flowering stems were trimmed to a uniform length of 60 cm and putted in 0.5 lit. glass jars (disinfected with sodium hypochlorite 10%) contain 330 ml solution. Treatments were 10 solutions include:

Water (Control)
Ethanol 8%

Silver thiosulfate (STS) 300 ppm
$CuSO_4$ 300 ppm
$CuNO_3$ 300 ppm
8-hydroxyquinoline citrate (HQC) 200 ppm
$CaNO_3$ 300 ppm
Citric acid 300 ppm
Sodium hypochlorite (NaOCl) 300 ppm
Sodium N-Chloro-para-Toluenesulfonamide (25% active chlorine) (Halamid) 400 ppm

3% sucrose was added to all treatments. Halamid® manufactured by Axcentive company and other chemicals by Merck company. Each treatment had 3 replications of 5 flowers in a glass jar. Vase life evaluated in 20±2 °C temperature, relative humidity 60% and 1800 lux fluorescent light intensity. During the experiment, traits including, vase life, quality, relative water content and the number of microorganisms in the solution were recorded.

Vase Life

In this experiment, the lifetime days from harvest to aging was measured. Vase life of cut carnations determined by observing senescence symptoms, i.e., in-rolling of petals or wilting of one third of petals in each flower.

Quality

The quality of cut flowers evaluated and scored by important parameters including all attributes associated with market-friendly such as stem stability and color changes in leaves, sepals and petals. Score was 10 for the highest quality and no signs of aging at the beginning of the experiment. During the lifetime, symptoms of low quality appeared and the assigned number was decreased. Scores for each glass jar summed at the end of vase life.

Relative Water Content (RWC)

At sixth day of experiment, one gram petal were isolated from four flowers in each jar (FWT). Then petals placed 24 hours between two layers of completely wet filter paper inside petri dish in 20 °C dark place. After that moistured petals weighed (SWT). Petal back into petri dishes, dried in 80 °C oven and weighed again after 24 hours (DWT). Relative water content was determined using the following equation:

$RWC\% = (FWT-DWT) / (SWT-DWT) \times 100$

Microorganisms Population

Microorganisms in solution incubated on general bacterial medium (NA), and were evaluated by repeated dilution method to 1:10000 (serial dilution). Number of colonies per petri dish was counted accurately.

Statistical Analysis

The experiment was laid out in randomized complete block design(RCBD) with three replications. A two-way analysis of variances (ANOVA) was done by using statistical method (MSTAT-C). The difference was quantified by Duncan's multiple range test (DMRT) ($P \leq 0.05$).

RESULTS AND DISCUSSION

The results showed that flower longevity have significant different ($P \leq 0.01$)and copper sulfate and Halamid® (Sodium N-Chloro-para-Toluenesulfonamide) were the best treatments. Correlation between flower longevity and other traits were significant and reasonable. Microorganisms

that grow in a solution are containing bacteria, yeasts and fungi. The activity of microorganisms in water can cause (1) vascular occlusion in cut stems (2) release of toxic metabolites (3) ethylene production and (4) reactions leading to the induction of PCD[1] (Edrisi, 2009). Marousky (1969) called physiological responses to unknown factors as vascular occlusion.

The Chloramine T ion in halamid, reacts with organic material like proteins or enzyms and destroys cell material or disrupts essential cell processes, quickly. Halamid® can not change pH preservative solution. Edrisi (2009) found that effects of Halamid® on longevity of gerbera was about 20% higher than sodium hypochlorite and 200% higher than control (tap water) . halamid 400, 600 ppm also increased about 110% vase life and quality compared to control in carnation. In rose 'Moroussia', Halamid® had toxic effect on leaves and its use is not recommended. Effects of Halamid® on flower longevity appears similar to the HQC and in some cases may be better because its cost is lower, easy to use and lack of odor and color of the solution in comparison with the HQC.

8- HQS promotes stomatal closure in addition to having biocidal activity (Burge *et al*., 1996). Stoddard and Miller (1962) demonstrated that 8-HQS closes stomata and thereby reduces water loss. HQS and HQC may also promote flower longevity by acidifying the vase solution (Halevy and Mayak, 1981). Acidic quinoline esters in solution (Weinberg, 1957) and 8-HQC may inhibit stem plugging by reducing solution pH. Since physiological plugging is mediated enzymatically (van Doorn, 1997), the presence of 8-HQC may influence the activity of enzymes by altering pH (Marousky, 1969, 1971). Marousky (1972) considered that while 8-HQ compounds could help prevent microbial occlusion, their ability to reduce vascular blockage can be due to their ability to inactivate enzyme systems through pH adjustment.

Another mode of action of quinoline esters in inhibiting vascular blockage in rose stems may be their chelating properties (Weinberg,1957). Of seven isomeric mono-HQs, only 8-HQ can chelate metallic ions and is thus the only HQ which is antibacterial. Zentmyer (1943) suggested that with increasing of H+ concentration, chelating and biocidal activity are increased. Interestingly, a low concentration of Fe2+, Cu2+, or Cd2+ is required for toxicity against Gram-positive bacteria species such as *Micrococcus pyogenes*, for example, a 2 : 1 ratio of HQ-Fe^{2+} is more toxic than HQ-Fe^{2+} ratios < 2 : 1.

The results showed that the relative water content of the treatments have significant difference ($P \leq 0.05$) and Halamid® was the best treatment for water content. Highly significant negative correlation of relative water content and the bacterial population in solution indicates that the main effect of bacteria is reducing of water uptake. The highest and the lowest bacterial population observed in control and Halamid® treatment respectively. Since Halamid® attacks microbes through a process of oxidation, they cannot build up a resistance to it. In addition, the Chloramine T ion is highly stable and remains active over an extended period of time. The disinfectant property of Cl− reduced (Dychdala, 1983). Consequently, effects of chlorine in postharvest vase solutions may decrease rapidly. High initial concentrations can be used to meet chlorine demand, but may be phytotoxic (van Doorn *et al*., 1990). Joyce and Beal (1999) suggested that if symptoms of phytotoxicity was appeared, addition of 0.5–1.0% (w/v) sucrose plus 25 mg l^{-1} Cl may be appropriate to reduce phytotoxic damage as well as to extend vase life.

Based on the hypothesis that CuSO4 can inhibits enzymatic activities related to physiological stem occlusion and also inhibits bacterial growth, Loubaud and van Doorn (2004) tested its effect on rose 'Red One', *Astilbe* × *arendsii* 'Glut' and 'Erica', and *Viburnum opulus* 'Roseum' as pulse (2 and 10 mM) and continuous (0.25, 0.50, and 1.0mM). As noted by the authors, 2mM $CuSO_4$ pulse treatment delayed the time to wilting in all plants. In contrast, 10 mM pulse treatment had small positive effect. $CuSO_4$ continuous treatments of 0.25 and 0.50 mM for flowers that had not been stored dry also delayed wilting. At 1mM, $CuSO_4$ in the vase solution was toxic to flowers

1- Programmed Cell Death

in all treatments. The bacterial count after 3 days of vase life in vase water containing rose 'Red One' was lower with 0.25 mM $CuSO_4$ as compared with the water control (2.7×10^5 and 1.8×10^8 cfu/L, respectively).

Therefore, Cu^{2+} at 0.25 mM delayed wilting and inhibited the growth of bacteria in the vase solution in addition to any effects on physiological stem plugging. It was observed that Cu2+ inhibited enzymes involved in plant induced occlusion in *chrysanthemum* (van Doorn and Vaslier, 2002) and *Bouvardia* (Vaslier and van Doorn, 2003).

Edrisi and Kalaei (2004) found that copper compounds specially copper nitrate have most and aluminum sulfate least effect on the cut carnation longevity and quality. Economical comparison by benefit–cost ratio method determined that $CuNO_3$ 500 mg l^{-1} was the most profitable treatment. Van Meeteren *et al*., (1999) observed that tap water may vary in mineral composition and contain Cu^{2+}. In all experiments, Cu^{2+} (> 0.30 mg l^{-1}) reduced bacterial growth in cut open vessels, thereby leading to an increased relative fresh weight of *chrysanthemum* cut flowers.

The effect of treatment on flowers opening and its correlation with other traits were not significant . But some flowers such as rose, carnation, *chrysanthemum* and gladiolus need a solution containing sugar (sucrose) for the opening (Edrisi, 2009).

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Tables

Table 1. Correlations of traits.

	Vase life (days)	Quality	Microorganism (colony.cm^{-3})	RWC%
Vase life (days)	1	0.835***	-0.722***	0.472**
Quality		1	-0.541**	ns 0.328
Microorganism(colony.cm^{-3})			1	-0.585***
RWC%				1

** , *** Significance at 1% , 0.1% respectively and ns means no significant.

Figures

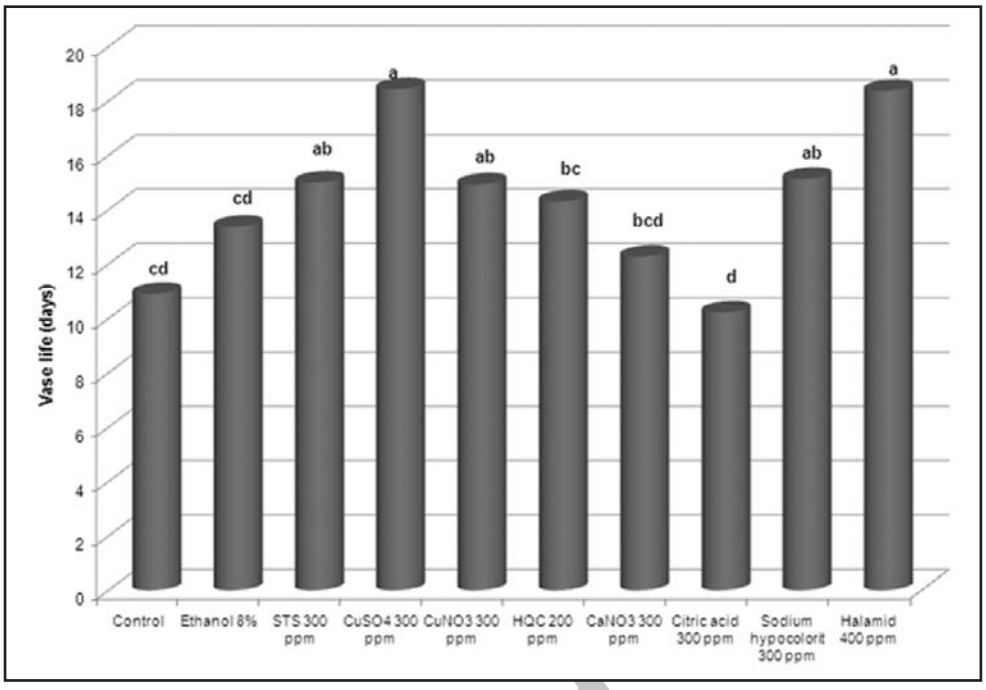

Fig.1. Vase life in treatments. Different letters indicate significant differences (P ≤ 0.05)

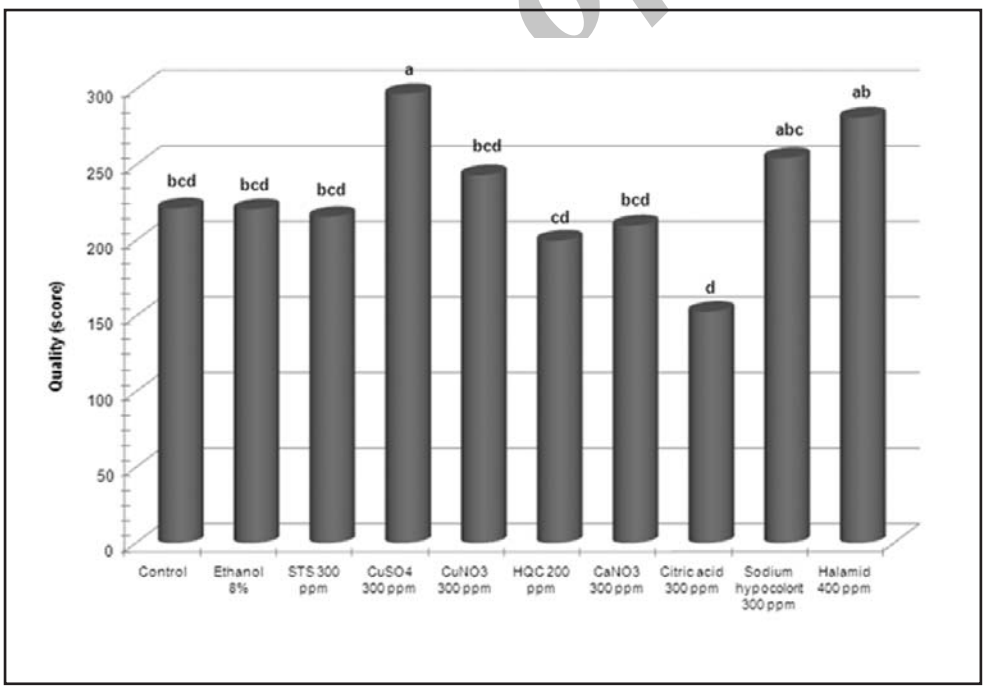

Fig. 2. Quality of flowers during the life time. Different letters indicate significant differences (P ≤ 0.05).

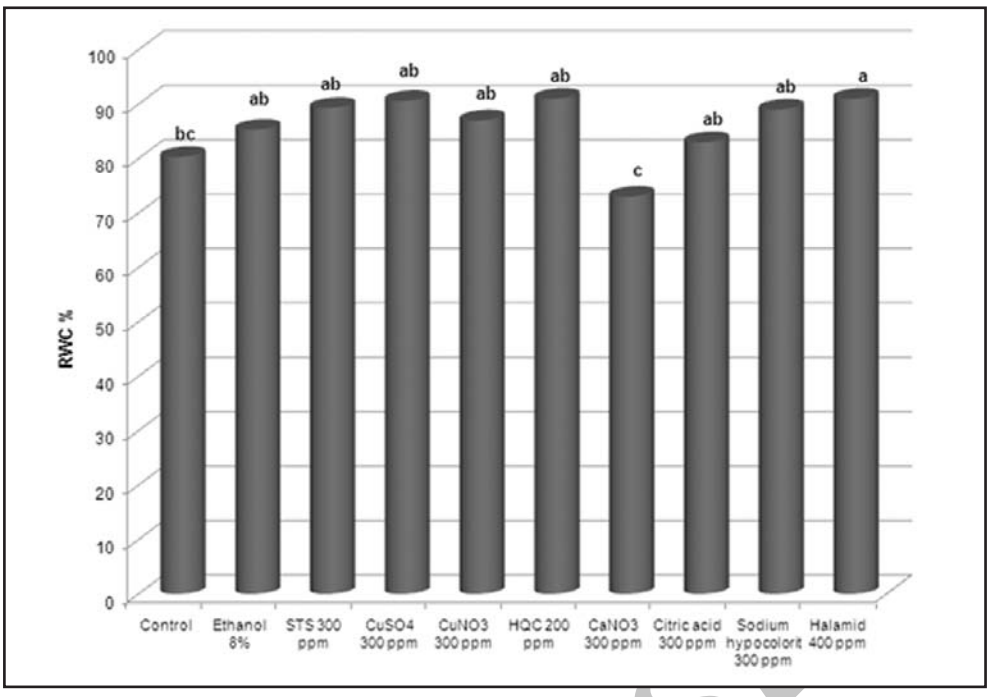

Fig. 3. Relative water content (RWC) in sixth day after harvest. Different letters indicate significant differences (P ≤ 0.05).

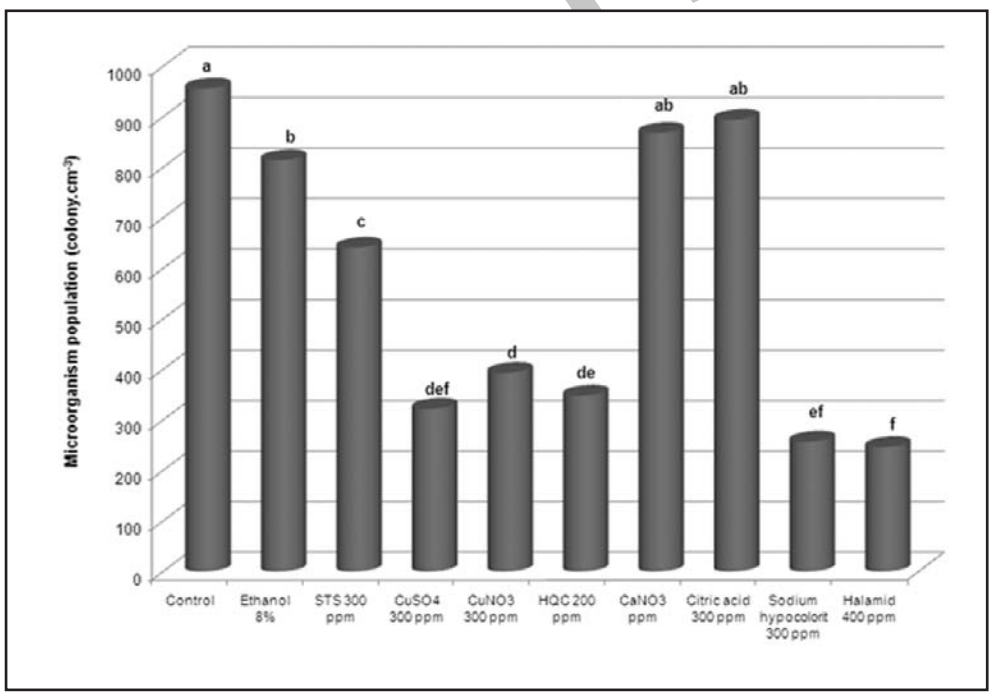

Fig. 4. Microorganisms population in solution. Different letters indicate significant differences (P ≤ 0.05).