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EFFICIENCY OF ESSENTIAL OILS AND NANO-MALATE IN REDUCTION OF ETHYLENE PRODUCTION AND EXTENSION OF VASE LIFE OF CUT *EUSTOMA GRANDIFLORUM* MARIACHII. CV. BLUE FLOWERS

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Abstract

The effect of essential oils and nano-malate in extending the vase-life of *Eustoma grandiflorum* Mariachii. cv. Blue flower was investigated. The treatment with 3 mM nano-malate increased flower longevity as compared to control. Nano-malate treatment increased chlorophyll, proline and carbohydrate content and membrane stability, while descreasing ACO (ACC-oxidase activity) and MDA (malondialdehyde) content and delay of senescence and peroxidation of lipids. Thyme oil was slightly effective significantly. The application of nano-malate as preservative solutions for *E. grandiflorum* flowers maintained the vase life of flowers for a longer period.

Vase life of cut flower is most attractive and economic components of cut flower (Chakrabarty *et al.* 2009). The main problems of ornamental perishables usually are flowers or leaf senescence (Kazemi and Shokri, 2011). During senescence marked changes occur in the biochemical and biophysical properties of the cell membranes. Ethylene plays a central role in the senescence of many cut flowers (Zagory and Reid 1989). The post harvest quality of many flowers is reduced by ethylene. Ethylene causes premature wilting, color fading, abscission of flower petals and leaf yellowing (Celikel *et al.* 2002). Essential oils are also used as flavoring agents in food industry. Numerous studies have reported the antimicrobial activity and chemical composition of essential oils (Tepe *et al.* 2004, Kodali *et al.* 2005).

Nano-malate is a well-known organic acid that can reduce the number of bacteria in the solution and decrease ACC-oxidase activity which causes delay in the onset of hydrolysis of structural cell components (Kazemi *et al.* 2010). In this study, effects of thyme oil and nano-malate on the vase life of cut *Eustoma grandiflorum* Mariachii. cv. blue flowers is reported.

Eustoma grandiflorum Mariachii. cv. Blue were harvested in open stage in the morning by a grower in Tehran, Iran (2013-2014) and chlorophyll content, membrane stability, malondialdehyde content and ACC-oxidase (ACO) activity were measured. Treatments were of thyme oil (25, 50 and 75 mg/l) and nano-malate (0.5, 1.5 and 3 mM), in a factorial test with complete randomized design with six replications. Distilled water was used for the controls and placed in chambers at 19°C. The relative humidity was about 70% while 14 hrs photoperiod was maintained using fluorescent lamps with a light intensity of 15 µmol m^{-2} s^{-1} at the top of the corolla. The vase life of the inflorescence was considered terminated when 50% of the open flowers had wilted. Total chlorophyll (a + b) content was measured by chlorophyll meter (SPAD-502, Minolta Co. Japan) which is presented by SPAD value. Average of 3 measurements from different spots of a single leaf was considered. Anthocyanin leakage was measured based on the method of Poovaiah (1979). ACO was measured based on the method of Moya-Leon and Herrera

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Table 1. Mean comparisons of chlorophyll content, vase life, MDA, SOD activity, membrane stability and ACC oxidase activity in thyme oil and nano-malate treatments.

Treatment		Vase life (day)	Chlorophyll total (a+b) content (spad reading)	ACC oxidase activity (nmol/gFW/h)	Membrane stability (Antocyanin leakage OD 525)	MDA (µmol/mg protein)	SOD (U/g protein)	Proline (µmol/g FW)
Control	0	5d	1.22c	70.45a	178.96a	134.76a	65.45c	45.66d
Nano-malate (mM)	0.5	6c	1.8b	64.78b	104.45b	107.56b	65.67c	60.56c
	1.5	10b	2b	35.54c	87.56c	96.56c	89.12b	65.12b
	3	18a	5.42a	21.11d	64.34d	66.8d	100a	80.78a
Thyme oil (mg/l)	25	7c	2.03b	35.45c	87.9c	96c	95.12ab	64.98b
	50	10b	2.1b	35.32c	87.56c	95.89c	96ab	65b
	75	9bc	2.23b	35c	87.11c	98.8c	101.45a	65.01b
F-test probabilities	Nano-malate	0.001	0.002	0.001	0.001	0.001	0.03	0.003
	Thyme oil	0.05	0.061	0.049	0.05	0.05	0.001	0.05

Means in each column followed by similar letters are not significantly different at 5% level

Table 2. Effect of nano-malate and thyme oil on carbohydrate content (per mg dry weight) for petals of cut flowers.

Treatment	1st day			5 st day			18 st day		
	Fructose	Glucose	Sucrose	Fructose	Glucose	Sucrose	Fructose	Glucose	Sucrose
Control (0)	0.43	1.43	1.84	0.33	0.54	0.64	-	-	-
Nano-malate 0.5 mM	0.45	1.56	1.89	0.21	0.76	0.67	-	-	-
Nano-malate 1.5 mM	0.41	1.59	1.85	0.3	1	1.01	-	-	-
Nano-malate (3 mM)	0.42	1.5	1.87	3.98	5.87	4.16	4.76	6.11	5
Thyme oil 25 (mg/l)	0.46	1.49	1.67	1.31	1.76	1.98	-	-	-
Thyme oil 50 (mg/l)	0.43	1.45	1.69	1	1.65	1.78	-	-	-
Thyme oil 75 (mg/l)	0.43	1.4	1.71	1.08	1.6	1.8	-	-	-

(2004). Oxidative damage to lipids was measured based on the method of Heath and Packer (1968). The activity of superoxide dismutase (SOD) was measured based on the method of Beauchamp and Fridovich (1971). Carbohydrates were measured based on the method of Hassan (2005). Proline were measured based on the method of Bates *et al*. (1973). Analysis of variance was performed on the data collected using the general linear model (GLM) procedure of the SPSS software (Version 16, IBM Inc.). The mean separation was conducted by Tukey analysis in the same software ($p = 0.05$).

The nano-malate at concentration 3 mM prolonged the vase life of cut *E. grandiflorum* flowers, while thyme oil was slightly effective significantly ($p < 0.05$) as compared to that control (Table 1). The results indicate that 3 mM nano-malate treatment caused a significant increase in chlorophyll content (1.22 to 5.42), membrane stability (178.96 to 64.34), SOD (65.45 to 100), and proline (45.66 to 80.78), while, reduced ACO (70.45 to 21.11) and MDA (134.76 to 66.8). On the other hands, thyme oil has slightly effective significantly ($p < 0.05$) (Table1). Carbohydrate contents in petals decreased rapidly in present cut flowers in solutions containing control while flowers in the solutions containing 3 mM nano-malate showed the minimum decrease in carbohydrate contents at the end of day 18 ($p \leq 0.05$) (Table 2). In agreement with our result, Kazemi *et al*. (2010) found that application of MA on cut flower increased vase life and enzyme antioxidant activity. According to the texts, ethylene, reduced vase-life of cut flowers. Ethanol increased vase-life by inhibiting ethylene synthesis and sensitivity to ethylene action. It also inhibited conversion of ACC into ethylene (Wu *et al.* 1992). Kazemi *et al.* (2011) reported that treatment with malic acid and salicylic acid significantly extends the vase life with reduced the anthocyanin leakage and ACO activity. Antibacterial agents will keep the water free from bacteria and other microorganisms (Van Doorn *et al*. 1994). Essential oils have strong antimicrobial properties against some pathogens and bacteria because of high levels of phenolic compounds such as carvacrol, thymol and eugenol. The improved vase life by using Essential oils treated preservative solutions might be due to their role in inhibiting the microbial growth and preventing bacterial plugging. These results are in agreement with those of Saini *et al*. (1994) and Kazemi *et al.* (2010) who showed that the vase life of tuberose and carnation cut flowers increased when placed in solutions with different concentrations of essential oils. Reduction in membrane integrity, destruction of enzymatic systems involved in energy production and cellular structure components are the main mechanisms of these compounds in mitigating microbial infection (Sikkema *et al.* 1995). The application of nano-malate as preservative in solutions *E. grandiflorum* flowers maintained the vase life of flowers for a longer period.

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