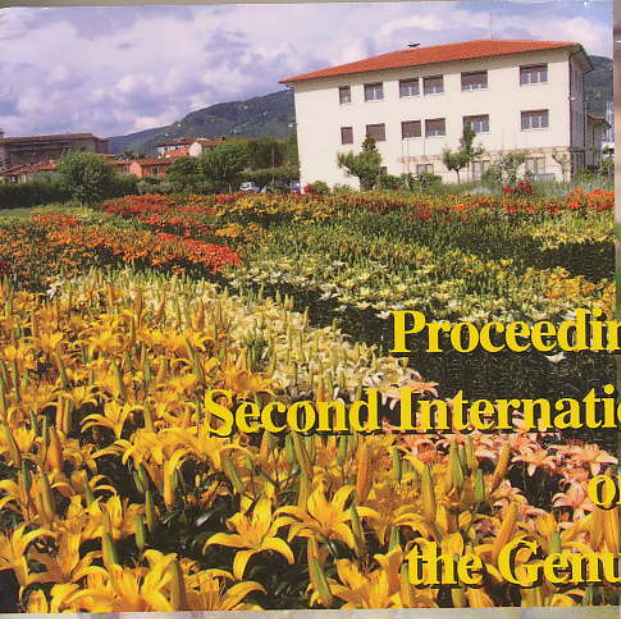

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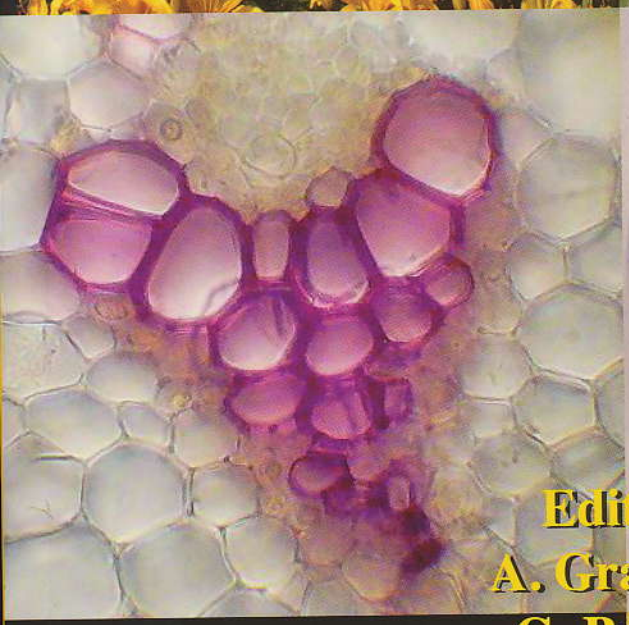

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Effect of $FeSO_4$, Methanol and H_2SO_4 Sprays on Chlorophyll Content of *Lilium* 'Brunello'

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Keywords: cuticle, foliar nutrition, foliar absorption, iron chlorosis

Abstract

In order to investigate the possible effect on iron nutrition status, three levels of $FeSO_4$ (0, 0.5, 1% w/v) and H_2SO_4 (0, 0.05, 0.1% v/v) and two levels of methanol (0, 5% v/v) were investigated in a randomized factorial design with three replications. Methanol was used to test the possible interaction with $FeSO_4$ and H_2SO_4 foliar absorption via assumed effect on cuticle permeability. The measured parameters were height, mean floret size, stem diameter, vase-life, chlorophyll content, mean bulbil formation and mean bulbil weight. The sprays were done two times during the plant growth period. $FeSO_4$ and H_2SO_4 in applied concentrations caused visible injuries as necrotic spots on the plant and caused no positive effect on any of the measured parameters. The result of factorial analysis indicates that H_2SO_4 and methanol significantly decreased and increased the chlorophyll content respectively, while H_2SO_4 had no effect. A significant correlation ($p \leq 0.01$) between methanol and chlorophyll content supports this conclusion. A positive interaction between 0.05% concentration of $FeSO_4$ and 5% methanol on increasing chlorophyll content was observed.

INTRODUCTION

Iron (Fe) deficiency as observed by leaf chlorosis is the major nutritional stress affecting growth and development in various crops in calcareous soils in the Mediterranean area (Abadía et al., 1999). Apoplastic pH is one of the factors controlling Fe acquisition from the leaf apoplast (Mengel, 1994). Acidic solutions have been used to remobilize pre-existing Fe pools. This practice is sometimes, but not always, effective (Abadía et al., 2002). Sulfuric acid was found to be as effective as Fe (III)-EDDHA in correcting chlorosis in garden pea (Sahu et al., 1987). Foliar sprays of aqueous 10-50% methanol are reported to improve growth and development of C_3 photosynthetic pathway crops under arid conditions (Nonomura and Benson, 1992). Methanol increased chlorophyll content, blooming, and fruit number and retarded senescence, especially in tomato plants derived from cuttings. Its whole action was similar to the action of growth retardants. It is known that methanol in small quantities is a natural plant product and source of carbon (C) atoms for plant metabolism (Koukourikou-Petridou and Koukounaras, 2002). Foliar application of 14-21% (v/v) methanol increases sugar yield of sugar beets (Nadali et al., 2010). Methanol alone or in combination with chloroform is used regularly as a cuticle solvent and therefore, this experiment was designed to find any possible effect on penetration of accompanying substances, $FeSO_4$, through the cuticle layer.

MATERIALS AND METHODS

The bulbs of *Lilium* × *elegans* Thunb. 'Brunello' Asiatic hybrid lily were planted in pots filled with a growing medium composed of peat moss, sand and garden soil (2:2:1 by volume) on 5 February 2009 and first time irrigated by 5,000 ppm benomyl plus 2,000 ppm diazinon solution. Plants were irrigated twice a week. To avoid any interference with results, no fertilizer was applied during the experiment period. Shoots emerged on 12 and 13 February 2009, and 15 days later the flower buds were observed.

Plants were sprayed twice, first on 9 April and the second time 8 days later.

The experiment was conducted in an experimental greenhouse of the Agriculture Faculty (Islamic Azad University, Karaj Branch). Three levels of $FeSO_4$ (0, 0.5, and 1% w/v), H_2SO_4 (0, 0.05, and 0.1% v/v), and two levels of methanol (0 and 5% v/v) were applied as foliar sprays in a 3×3×2 randomized factorial design with three replications.

The flower stems were harvested when the buds reached the puffy stage showing color. To evaluate postharvest performance, cut flowers were placed in a vase solution containing 10% sucrose, 150 ppm hydroxyquinoline sulfate and 50 ppm silver nitrate. The chlorophyll content was measured by a portable chlorophyll meter (SPAD-502, Minolta Camera Co. Ltd., Japan) at the time of harvest. The flower's vase life was determined for all 54 cut flowers, as the number of days after harvest until florets' wilting. The other recorded parameters after harvest included shoot length and diameter, flower size, the number and weight of bulblets.

The results were analyzed using SPSS 16.0 Software (IBM Inc.). The factor effect was evaluated with general linear model (GLM) module and the mean separation was done by Duncan Analysis option. The cross correlation analysis was done with the same software.

RESULTS AND DISCUSSION

A week after spraying, burning spots were observed on the plants sprayed by all treatments containing sulfuric acid. While there was no significant difference between 0 and 0.05% levels, the chlorophyll content was reduced significantly in 0.1% concentration ($p \leq 0.05$) levels of sulfuric acid. The plant height was adversely affected by all levels of sulfuric acid ($p \leq 0.05$). Other parameters including floret size, bulbil number and weight and vase life were not affected significantly by different levels of sulfuric acid application. Plants which were sprayed with 1 and 0.05% percent of $FeSO_4$ levels were all adversely affected significantly in terms of plant height and bulbil weight. In higher application level, burning spots on the leaf appeared as well.

Application of 5% methanol increased chlorophyll content significantly ($p \leq 0.05$) (Fig. 1). A significant correlation ($p \leq 0.01$) between methanol and chlorophyll content supports this conclusion (Table 1). The interaction effect between 5% methanol and 0.5% $FeSO_4$ was significant ($p \leq 0.01$) and concluded to be positive on chlorophyll content (Fig. 2).

In this study, the applied levels of $FeSO_4$ and H_2SO_4 were not promising to have any desirable effect (Fig. 3). Methanol effects on growth indices were previously reported by levels of 10-50% by Nonomura and Benson (1992) and increasing of chlorophyll was previously reported by Koukourikou-Petridou and Koukounaras (2002) at 30% application level. In our study, the lower level of 5% showed promising effect on increasing chlorophyll concentration and could be considered a potential agent in management of chlorophyll content at least in combination with $FeSO_4$. Methanol evaporates readily so its absorbance could depend on the climatic conditions, the difference between the better results obtained at controlled environment versus field environment reported by Hernandez et al. (2004) supports this conclusion. We assume that the observed effect could be attributed to a carrier effect of methanol as a non-polar agent which could translocate through the lipophilic cuticle more easily and which could have affected on penetration of $FeSO_4$.

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Tables

Table 1. Pearson cross-correlations among various treatments and parameters.

	$FeSO_4$ (%)	H_2SO_4 (%)	Methanol (%)	Stem length	Mean floret size	Mean chlorophyll content	Vase life
$FeSO_4$ (%)	-						
H_2SO_4 (%)	.206	-					
Methanol (%)	.171	.086	-				
Stem length	-.313	-.402*	-.261	-			
Flower size	-.052	-.239	-.311	.569**	-		
Chlorophyll content	.063	-.326*	.424**	.293	.066	-	
Vase life	.099	-.179	.000	.207	.135	.478**	-

*, **: Correlation is significant at the 0.05% and 0.01% level, respectively (2-tailed).

Figures

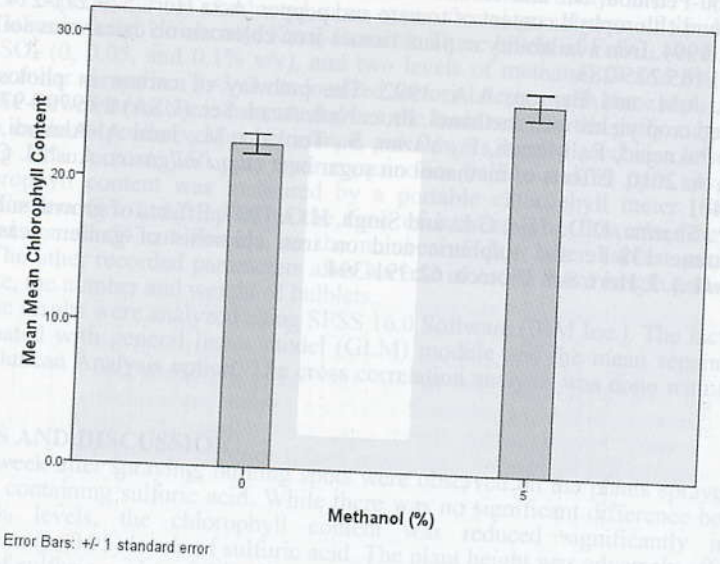

Fig. 1. Effect of methanol on chlorophyll content. Mean of all treatments (factor-levels) without methanol is compared with all treatments containing methanol.

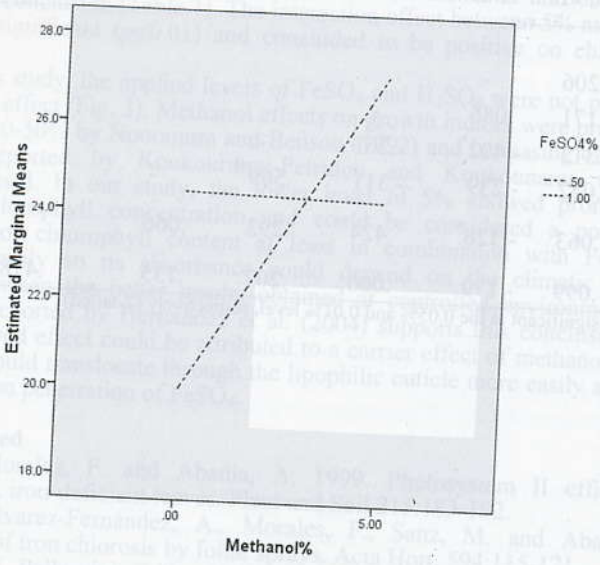

Fig. 2. Estimated marginal means for chlorophyll content (SPAD reading) showing the positive interaction between methanol and $FeSO_4$.

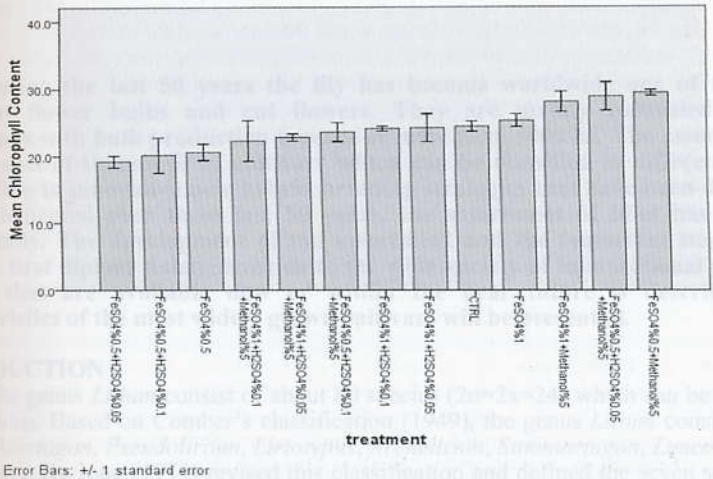

Fig. 3. Effect of different combinations of $FeSO_4$, methanol and H_2SO_4 on chlorophyll content of cut flowers.

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