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IMPACT OF FOLIAR APPLICATION OF MICRONUTRIENTS ON SOME BIOCHEMICAL PARAMETERS OF MULBERRY (*Morus alba* L.)

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ABSTRACT

The present study was aimed to assess the impact of foliar application of various micronutrients *viz.*, Fe, Zn, Mn, B and Mo, sprayed individually and in combination, on the qualitative characteristics of mulberry leaves. The experiment was conducted in 2011-2012 and quality parameters assessed 45 days after pruning. The results revealed that all the individual applications of above micronutrients significantly improved moisture, moisture retention capacity, chlorophyll 'a' and 'b' and total chlorophyll, carotenoids, nitrogen, phosphorus, potassium, zinc, iron, boron, manganese, crop growth rate and net assimilation rate over control. The combined foliar application of 0.5% $ZnSO_4$ + (1.0% $FeSO_4$ + 0.1% citric acid) + 0.2% boric acid+ 0.5% $MnSO_4$ + 0.01% Na_2MoO_4 was most effective in improving mulberry leaf quality.

Key words: Chlorophyll, foliar spray, leaf quality micronutrients, mulberry

INTRODUCTION

Mulberry (*Morus alba* L.) is a perennial, deep rooted, fast growing and high biomass producing foliage plant. Mulberry leaf is a sole source of food for silkworm so the success of sericulture industry depends mainly on the quality and quantity of leaves produced and fed to silkworms. It is well-established fact that, in sericulture, more than 60% of the total cost of cocoon production goes towards mulberry production alone (Yogananda Murthy *et al.*, 2013). The quality of mulberry leaves alone contributes about 38.2% towards quality cocoon production (Sabhat *et al*., 2011). About 92.2% of the silk produced worldwide is procured from mulberry silkworm *Bombyx mori* L., reared solely on mulberry leaves (Central Silk Board, 2014). With the increasing demands for silk, the stress is laid on improving the leaf productivity both in terms of quality and quantity.

Among the various factors influencing the vertical development of mulberry production, the contribution by micronutrients is quite remarkable. Micronutrients play a major role in several metabolic activities responsible for protein, sugar and enzyme synthesis leading to better quality mulberry leaf production. In case of multiple deficiency of micronutrients, the yield may be reduced even upto 50% (Manimala, 1995). Foliar application of micronutrients reportedly improves the yield, quality and micronutrient status of mulberry leaf (Dhiraj and Kumar, 2011) as well as the quantitative traits of silkworm (Bose and Majumder, 1996).

General improvement in the quality of mulberry leaf with foliar application of micronutrients and enhanced plant uptake of major and minor nutrients has earlier been reported by Lokanath and Shivashankar (1986). Foliar application of urea and micronutrients improved the yield and quality of mulberry leaf thereby showed good response to foliar spray (Yeasmin *et al.*,1995) and foliar fertilizers are known to immediately deliver nutrients to the tissues and organs of the crop (Baloch *et al.*, 2008). Venkatesh Kumar *et al.* (2012) reported foliar application to be 8 to 10 times more effective than soil application with 90% foliar nutrients present in small root of mulberry within 60 min of application.

Mulberry needs micronutrients like Fe, Zn, Mn, B and Mo in very small quantities for its nutrition in order to meet its optimum growth and development, wherein play an important role in enzymatic reactions. The micronutrients also affect the activities of several groups of micro-organism there by modifying the microbial processes influencing the supply of micronutrients in available form in one way or the other (Shankar, 1997). Micronutrients in general, zinc and iron in particular stimulate the metabolic activity in silkworm leading to improved rearing performance, silk content and higher fecundity rate (Bose *et al*., 1995). An attempt was made to know the effect of foliar application of micronutrients on the biochemical components and photosynthetic pigments of mulberry leaves.

MATERIALS AND METHODS

The present study was conducted in the Experimental fields of Department of Sericulture, TNAU, Coimbatore, Tamilnadu (India) during the years 2011-2012. The soil at the experimental site was sandy clay loam with 27.3% coarse sand, 31.5% fine sand, 8.5% silt and 32.7% clay. The soil had pH 8.1 (1:2.5 soil: water suspension), electrical conductivity 0.39 dSm^{-1}, available nitrogen 259 kg ha^{-1}, available phosphorus 11 kg ha^{-1}, available potassium 439 kg ha^{-1}, zinc 1.25 mg kg^{-1}, iron 6.31 mg kg^{-1}, manganese 2.11 mg kg^{-1} and boron 0.27 mg kg^{-1}. The 15 year old mulberry plants cv. 'Victory 1' were chosen for the study. The experiment comprised of 10 treatments *viz*., i) zinc sulphate [$ZnSO_4$ (0.5%)], ii) ferrous sulphate [$FeSO_4$ (1.0%)] + citric acid (0.1%), iii) boric acid (0.2%), iv) manganese sulpahte [$MnSO_4$ (0.5%)], v) sodium molybdate [Na_2MoO_4 (0.01%)], vi) $ZnSO_4$ (0.5%) + [$FeSO_4$ (1.0%) + citric acid (0.1%)], vii) $ZnSO_4$ (0.5%) + [$FeSO_4$ (1.0%) + citric acid (0.1%)] + boric acid (0.2%), viii) $ZnSO_4$ (0.5%) + [$FeSO_4$ (1.0%) + citric acid (0.1%)] + boric acid (0.2%) + $MnSO_4$ (0.5%), ix) $ZnSO_4$ (0.5%) + [$FeSO_4$ (1.0%) + citric acid (0.1%)] + boric acid (0.2%) + $MnSO_4$ (0.5%) + Na_2MoO_4 (0.01%), and x) control (water spray). The zinc, iron, boron, manganese and molybdenum were sprayed, individually or in combinations, as per the treatment. The plants were pruned on 5th August, 2011 and micronutrients applied as foliar spray on 10th and 20th day after pruning (DAP) of mulberry. The plot size was 10 m^2 with 12 plants in each treatment replication. The experiment was laid out in a randomized block design with three replications. The plant leaves at 45 days after last pruning were taken for various biochemical studies. Four fresh leaves from middle shoot region in each treatment were plucked for the estimation of chlorophyll 'a', 'b' and total contents spectrophotometrically (SL210 UV VIS model, type 232) at 663, 645 and 653 nm, respectively, as per the method of Hiscox and Israelstam (1979). The chlorophyll 'a', 'b' and total and chlorophyll a/b ratio were expressed in mg g^{-1} fresh weight (Arnon, 1949).

$$\text{Chlorophyll 'a'} = (12.7 \times OD_{663}) - (2.69 \times OD_{645}) \quad \frac{V}{W} \times 1000$$

$$\text{Chlorophyll 'b'} = (2.69 \times OD_{645}) - (4.68 \times OD_{663}) \quad \frac{V}{W} \times 1000$$

$$\text{Total chlorophyll} = \frac{OD_{652} \times 1000}{34.5} \quad \frac{V}{W} \times 1000$$

Where, OD = optical density, V = final volume of supernatant liquid, and W = weight of leaf sample

The carotenoid content in mulberry fresh leaves was estimated as per the method of Arnon (1949) using the following formula:

$$\text{Carotenoids} = (7.6 \times OD_{480}) - (1.49 \times OD_{510}) \times W \ \frac{V}{1000}$$

The crop growth rate (CGR) and net assimilation rates (NAR) were estimated as per the methods suggested by Watson (1952) and Radford (1967), respectively.

$$\text{CGR (g m}^{-2}\text{ day}^{-1}) = \frac{W_2 - W_1}{P\,(t_2 - t_1)}$$

$$\text{NAR (mg g}^{-1}\text{ day}^{-1}) = \frac{W_2 - W_1}{t_2 - t_1} \times \frac{\log_e L_2 - \log_e L_1}{L_2 - L_1}$$

Where, W_2 and W_1 are dry weight of plants at time t_2 and t_1, respectively; P = ground area in m^2; t_1 and t_2 are time in days; L_1 and L_2 are leaf area of plant at time t_1 and t_2, respectively.

The available soil nitrogen content was estimated as per alkaline permanganate method (Subbiah and Asija, 1956). The available soil phosphorus was estimated by using 0.5 M sodium bicarbonate as soil extract (Olsen *et al.*, 1954). The available potassium in soil was estimated by using the neutral normal ammonium acetate (pH 7.0) (Stanford and English, 1949). The crude protein content in leaf samples was calculated by multiplying nitrogen content with factor 6.25 (Lowry *et al.*, 1951). The leaf moisture was calculated by subtracting dry weight from fresh leaf weight. For leaf moisture retention capacity, fresh leaf weight, leaf weight 6 hr after shade drying under ambient conditions and leaf weight after micro-oven drying till constant weight were estimated and moisture retention capacity calculated as per the formula given by AOAC (1970). The observations on biochemical parameters, macronutrients (N, P and K) and micronutrients (Zn, Fe, B and Mn) in mulberry leaves were recorded. The oven-dried leaf samples were digested using tri-acid mixture (nitric acid: sulphuric acid: perchloric acid) in 9:2:1 ratio for the analysis of Zn, Fe and Mn. Zn, Fe and Mn contents were estimated by atomic absorption spectrophotometric method (Jackson, 1973). Boron was estimated by wet digestion method as per Banuelos *et al.* (1992). The data was subjected to statistical analysis (Panse and Sukhatme, 1978).

RESULTS AND DISCUSSION

Moisture content and moisture retention capacity in mulberry leaves

The individual and combined foliar spray of micronutrients exerted significant impact on leaf moisture content and moisture retention capacity in mulberry. Maximum moisture content was observed in mulberry leaves sprayed with 0.5% $ZnSO_4$ + (1.0% $FeSO_4$ + 0.1% citric acid) + 0.2% boric acid + 0.5% $MnSO_4$ + 0.01% Na_2MoO_4 (79.02%), followed by 0.01% Na_2MoO_4 (76.01%) and found to be superior to all other treatments (Table 1). The findings are supported by Bose *et al.* (1995) who reported that Mo increased the leaf moisture content.

The foliar spray of 0.5% $ZnSO_4$+ (1.0% $FeSO_4$ + 0.1% citric acid) + 0.2% boric acid + 0.5% $MnSO_4$ + 0.01% Na_2MoO_4 recorded highest moisture retention capacity (84.28%) followed by 0.01% Na_2MoO_4 (80.41%) and were statistically superior over the leaves harvested from mulberry without foliar application (76.37%) [Table 1]. The increased moisture content and moisture retention capacity are due to higher nutrient contents of leaves and keeping quality (Dandin and Kumar, 1989).

Table 1: Biochemical parameters of mulberry leaves on 45 DAP

Treatments	Moisture content (%)	Moisture retention capacity (%)	Chlorophyll (mg g^{-1})			Crude protein (%)	Caroteinoid (mg g^{-1})
			'a'	'b'	Total		
Zinc sulphate (0.5%) [a]	72.68	78.44	1.52	0.31	2.05	18.63	0.36
Ferrous sulphate (1.0%) + citric acid (0.1%) [b]	70.57	77.76	1.56	0.35	2.15	19.09	0.39
Boric acid (0.2%) [c]	72.22	77.43	1.50	0.30	2.07	17.40	0.34
Manganese sulphate (0.5%) [d]	71.38	77.50	1.51	0.29	2.10	17.16	0.32
Sodium molybdate (0.01%) [e]	76.01	80.41	1.54	0.34	2.14	16.91	0.31
a + b	72.65	78.66	1.57	0.37	2.16	21.53	0.41
a + b+ c	73.01	79.40	1.58	0.39	2.18	21.72	0.42
a + b + c +d	74.26	80.39	1.61	0.40	2.19	23.32	0.48
a + b + c + d + e	79.02	84.28	1.85	0.56	2.44	24.21	0.53
Control	69.36	77.33	1.35	0.27	1.89	15.78	0.30
SE	2.01	1.81	0.05	0.02	0.11	0.30	0.02
$CD_{0.05}$	4.21	3.81	0.12	0.05	0.24	0.62	0.04

Photosynthetic pigments in mulberry leaves

The combined foliar application of micronutrients (0.5% $ZnSO_4$ + (1.0% $FeSO_4$ + 0.1% citric acid) + 0.2% boric acid + 0.5% $MnSO_4$ + 0.01% sodium molybdate) had significant influence on leaf chlorophyll and crude protein (Table 1) at all the growth stages of mulberry. This could be due to adequate supply and timely application of micronutrients to mulberry plants. This findings are akin to the report of Richards *et al.(*2001) who reported that micronutrients play key role in many plant processes, including the promotion of growth hormones, starch formation, seed maturation and production, function as metello-enzymes, chlorophyll formation, photosynthesis, protein and carbohydrate metabolism, N metabolism and assimilation and cellular division and development (Richards *et al.*, 2011). Viswanath (1979) noticed increased chlorophyll content in mulberry with foliar spray of zinc sulphate while studying the effect of foliar spray of zinc on flag leaf of rice. However, Lokanath (1981) made observation in micronutrients treated plants and reported that foliar spray of ferrous sulphate showed higher chlorophyll content.

Improvement in crude protein may mainly be attributed to improved uptake of nitrogen and evidently more plant metabolism/assimilation might have been activated so contributing to healthy green foliage production (Singhvi *et al.*, 2000, Vijaya *et al.*, 2009). Vishwanath (1979) and Lokanath (1981) suggested that foliar spray of zinc sulphate increases the moisture content in mulberry leaves. The moisture content determines the nutritive quality of leaves and plays an important role in the production of quality of cocoon (Dandin and Kumar, 1989).

Nutritional status of mulberry leaf

Biochemical analysis of leaf samples revealed positive impact of various treatments on nutritional status of mulberry. Significantly higher N, P and K contents were observed in mulberry leaves treated with 0.5% $ZnSO_4$+ (1.0% $FeSO_4$ + 0.1% citric acid) + 0.2% boric acid + 0.5% $MnSO_4$ + 0.01% Na_2MoO_4 over the mulberry plants grown without foliar micronutrient supply (Table 2). The increase in nitrogen and phosphorus may be due to more moisture absorption coupled with higher Zn uptake which is involved in the biosynthesis of plant hormones and is a component of a variety of enzymes like carbonic anhydrase and protein synthesis and helps in the utilization of nitrogen and phosphorus (Thakkar and Randhava, 1978). These observations are in line with those of Malakouti (2008) who reported that micronutrients like Fe, B, Mn, Cu and Mo have the ability to enhance crop yield (most to least effect). Supply of micronutrients to the mulberry, either through soil application, foliar spray or seed treatment, not only improved leaf yield and quality but also

Table 2: Effect of nutrient sprays on the nutritional status of mulberry leaf

Treatments	N	P_2O_5	K_2O	Zn	Fe	B	Mn
	(kg ha^{-1} $harvest^{-1}$)			(g ha^{-1} $harvest^{-1}$)			
Zinc sulphate (0.5%) [a]	17.52	3.73	7.13	168.0	1415.6	20.0	413.1
Ferrous sulphate (1.0%) + citric acid (0.1%) [b]	14.99	2.58	7.62	100.4	1642.7	19.8	399.4
Boric acid (0.2%) [c]	13.86	2.88	6.42	84.7	1443.3	23.1	396.5
Manganese sulphate (0.5%) [d]	14.89	2.99	6.66	111.7	1421.5	18.0	455.9
Sodium molybdate (0.01%) [e]	13.29	2.74	6.32	75.5	1587.5	19.5	381.5
a + b	18.42	3.07	7.47	180.5	1686.3	26.5	484.7
a + b+ c	18.55	3.86	9.45	197.3	1794.6	30.3	519.3
a + b + c +d	18.68	4.75	9.85	216.3	1873.2	31.1	540.2
a + b + c + d + e	20.32	5.12	10.96	249.3	2020.6	35.8	584.0
Control	10.99	2.65	5.52	83.5	1071.6	16.5	296.3
SE	0.78	0.15	0.48	6.58	56.89	1.54	15.62
$CD_{0.05}$	1.63	0.32	1.01	13.76	118.86	3.23	32.64

macro-nutrient-use-efficiency. Foliar feeding is a relatively new and controversial technique of feeding plants by applying liquid fertilizer directly to their leaves and is known to immediately deliver nutrients to the plant tissues and organs (Ashfaq and Aslam, 2006; Baloch *et al.*, 2008). Viswanath (1979) reported that foliar application of Zn and Fe increased NPK uptake in mulberry.

The mulberry plants sprayed with combined nutrients 0.5% $ZnSO_4$+ (1.0% $FeSO_4$ + 0.1% citric acid) + 0.2% boric acid + 0.5% $MnSO_4$ + 0.01% Na_2MoO_4 had higher micronutrient content than the plants grown without foliar application (Table 2). It appears that foliar fertilization increased nutrient uptake from the soil as it helped the plant to pump more sugars and other exudates from their root rhizosphere (Baloch *et al.*, 2008; Vijaya *et al.*, 2009).

Crop growth rate and net assimilation rate

Foliar application of micronutrients [0.5% $ZnSO_4$ + (1.0% $FeSO_4$ + 0.1% citric acid) + 0.2% boric acid + 0.5% $MnSO_4$ + 0.01% Na_2MoO_4] significantly influenced physiological parameters like crop growth rate and net assimilation rate at all the growth stages of mulberry (Table 3). It seems that increased protoplasmic constituents accelerated the process of cell division and elongation which,in turn, improved the physiological processes. Improved growth and physiological parameters have been observed due to the application of micro- and macro-nutrients in mulberry Lokanath (1981).

Table 3: Effect of foliar spary of micronutrients on physiological parameters of mulberry

Treatments	Crop growth rate (g m^{-2} day^{-1})		Net assimilation rate (mg g^{-1} day^{-1})	
	Stage I	Stage II	Stage I	Stage II
Zinc sulphate (0.5%) [a]	20.8	11.8	0.128	2.090
Ferrous sulphate (1.0%) + citric acid (0.1%) [b]	21.5	12.8	0.129	2.107
Boric acid (0.2%) [c]	21.1	12.1	0.126	1.993
Manganese sulphate (0.5%) [d]	21.0	11.8	0.127	2.003
Sodium molybdate (0.01%) [e]	21.0	11.4	0.125	2.040
a + b	21.5	14.0	0.130	2.133
a + b + c	21.6	14.8	0.131	2.137
a + b + c + d	21.7	16.9	0.133	2.163
a + b + c + d + e	23.1	19.1	0.148	2.373
Control	18.0	10.8	0.103	1.433
SE	0.59	0.93	0.0064	0.096
$CD_{0.05}$	1.23	1.94	0.0134	0.0201

*Stage 1 = 15-30 DAP; stage II = 30-45 DAP

The presence of increased Zn content in leaves not only enhanced nitrogen and phosphorus uptake but also moisture uptake. Zinc spray helped in retaining the leaf freshness for longer periods. The increase in nitrogen and phosphorus by means of more moisture absorption is attributed to the involvement of Zn in biosynthesis of plant hormones and enzymes (Thakkar and Randhawa, 1978). The application of Zn yielded higher amount of N in plants which, however, was at par with other treatment *viz.,* 1.0% $FeSO_4$ + 0.1% citric acid. The increase in yield components is the outcome of high concentration of nutrients in leaf and its partitioning towards favorable biomass accumulation in mulberry. Application of fertilizers without foliar spray of micronutrients did not perform well. Therefore, the supplementation of all micronutrients through foliar spray appears essential to boost biochemical components in plants. It could reasonably be explained that combined foliar spray of micronutrients leads to the higher availability of macronutrients viz., N, P and K as well as specific micronutrients to mulberry. In the present study one more feature noted was that individual foliar application of micronutrients was not sufficient to increase the quality of leaf than compared to combined foliar application.

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