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RESEARCH ARTICLE

Physiological and Biochemical Changes in Mulberry (*Morus alba* L.) as Influenced by Nutrients

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ABSTRACT

Physiological and biochemical changes in mulberry due to the combined application of biofertilizers and micronutrients were studied in a field experiment. In this study, biofertilizers were given as soil application and micronutrients were applied either by soil or foliar spray depending upon the treatments. Among the treatments, 100 % RDF + Soil application of $ZnSO_4$, $FeSO_4$, $MnSO_4$ and $MgSO_4$ @ 25 kg ha^{-1} each + Recommended dose of Biofertilizers (Azos: *Azospirillum lipoferum*, Phospho: *Bacillus megaterium* var. *Phosphaticum* and Potash mobilizer: *Frateuria aurantia* @1500 ml/ha each) has significantly influenced the physiological and biochemical parameters of mulberry as compared to other combinations of nutrients and application methods. This treatment recorded the highest values for physiological parameters *viz.,* chlorophyll content, photosynthetic rate and the lowest in transpiration rate. Similarly, soluble protein, carbohydrates, total phenolics and nitrate reductase (NR) activity were found to be higher in this treatment compared to other treatments.

Keywords: *Mulberry, Micronutrients, Biofertilizers, Physiological and biochemical characters.*

INTRODUCTION

Sustainability and any improvement in sericulture as a venture require optimal use, management and preservation or reconstruction of soil fertility and physical properties both of which rely heavily on soil biological processes and maintenance of biodiversity. Mulberry plant can be cultivated for several years due to its perennial characteristics and leaves are to be harvested five times in a year. Due to periodical pruning, this crop removes the soil nutrient reserves and need proper nutrient management for a successful crop.

Several researchers have demonstrated the beneficial effect of combined use of chemical and organic fertilizers to mitigate the deficiency of many secondary and micronutrients in mulberry. The potential of Plant Growth Promoting Rhizobacteria (PGPR) reduces dependence on high levels of fertilizer inputs (Lucy *et al.,* 2004). The PGPR plays a very important role in yield improvement by synthesizing different phytohormones including auxins, cytokinins and gibberellins which can positively influence plant growth by enhancing physiological and biochemical parameters of plant and that can modulate plant growth and development (Glick, 2012). Plant lifecycle depends on the supply of essential elements at the optimum quantity and at an appropriate stage of plant growth. All essential elements havea specific impact on physiological and biochemical parameters of plant growth. Hence present investigation was undertaken to find out the influence of the application of micronutrients along with biofertilizers on mulberry.

MATERIAL AND METHODS

A field experiment was conducted at D. Perumapalayam village of Salem district, Tamilnadu in an established mulberry garden with variety (V1) Victory 1 planted at a spacing of (5+3) x 2 feet in paired row system. The mulberry plants pruned for 5 times in a year with a time interval of 70 days. The following treatments were imposed after each pruning. The experiment was laid out in a randomized block design and replicated thrice with 12 treatments.

T_1 -Control (100% RDF)

T_2 -T_1+ Soil application of $ZnSO_4$, $FeSO_4$, $MnSO_4$ and $MgSO_4$ @ 25 kg ha^{-1} each.

T_3 - T_2+ Recommended dose of Biofertilizers (RDBF) (Azos, Phospho and Potash mobilizer)

T_4 - 75 % RDF + Soil application of $ZnSO_4$, Fe

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SO_4, $MnSO_4$ and $MgSO_4$ @ 25 kg ha^{-1}+ RDBF (Azos, Phospho and Potash mobilizer)

T_5 - T_1+ Soil application of $ZnSO_4$, $FeSO_4$, $MnSO_4$ & $MgSO_4$@ 15 kg ha^{-1}

T_6 - T_5+ RDBF (Azos, Phospho and Potash mobilizer)

T_7 - 75 % RDF + Soil application of $ZnSO_4$, $FeSO_4$, $MnSO_4$ and $MgSO_4$ @ 15 kg ha^{-1}+ RDBF (Azos, Phospho and Potash mobilizer)

T_8 - T_1+ 0.5 % Foliar spray of $ZnSO_4$, $FeSO_4$, $MnSO_4$ and $MgSO_4$

T_9 -T_1+ 0.2 % Foliar spray of $ZnSO_4$, $FeSO_4$, $MnSO_4$ and $MgSO_4$

T_{10} -T_8+ RDBF (Azos, Phospho and Potash mobilizer)

T_{11} -T_9+ RDBF(Azos, Phospho and Potash mobilizer)

T_{12} -75 % RDF + 0.2 % Foliar spray of $ZnSO_4$, $FeSO_4$, $MnSO_4$ and $MgSO_4$ + RDBF (Azos, Phospho and Potash mobilizer)

Young leaves were selected at random from each treatment on 65 DAPR (Days after pruning) and analyzed for physiological and biochemical parameters.

Biofertilizers

The bio-fertilizer inoculants Azos: *Azospirillum lipoferum*, Phospho: *Bacillus megaterium* var. *Phosphaticum* and Potash mobilizer: *Frateuria aurantia* used in this study were mass multiplied on the respective medium and the bacterial inoculants cells were separated and concentrated by tangential flow filtration system (PALL Life Sciences Inc.) and formulated in a liquid-based cell encapsulation medium with declared cell count of 1 x 10^8 CFU ml^{-1} and given as soil application at recommended dosage of 1500 ml ha^{-1} in Bio-fertilizer Production Unit, Department of Agriculture, Salem .

Mulberry leaves were analyzed for different physiological and biochemical parameters at 65 DAPR of each crop following the prescribed standard procedures. The chlorophyll content of mulberry leaf was estimated by adopting the procedure outlined by Yoshida *et al.* (1971), photosynthetic rate, stomatal conductance and transpiration rate were recorded by using LI-6400-XT portable photosynthesis system. Total carbohydrate content (Yemm and Willis, 1954), soluble protein content (Lowry *et al*., 1951), total phenol content (Mallick and Singh, 1980) and NR activity (Nicholas *et al*., 1976) of mulberry leaves were also estimated. Statistical scrutiny of the experimental data was done by the method of analysis of variance as suggested by Gomez and Gomez (1992).

RESULTS AND DISCUSSION

Physiological Parameters

Chlorophyll content

The fractions of chlorophyll a and chlorophyll b, as well as total chlorophyll content, were estimated in leaf samples collected from various treatments and presented in Table 1. Among the treatments, the highest total chlorophyll content was registered with

Table 1. Influence of micronutrients and biofertilizers on physiological parameters of mulberry

Treatments	Chl a (mg g^{-1})	Chl b (mg g^{-1})	Total Chl (mg g^{-1})	Transpiration rate (mmol H_2O $m^{-2}s^{-1}$)
T_1	1.68	0.69	2.37	6.80
T_2	1.98	1.11	3.09	6.60
T_3	3.04	1.38	4.42	6.09
T_4	2.38	1.67	4.05	6.17
T_5	1.79	0.88	2.67	6.78
T_6	2.25	1.26	3.51	6.42
T_7	2.41	1.15	3.56	6.27
T_8	1.87	.84	2.71	6.65
T_9	1.83	.82	2.65	6.75
T_{10}	2.18	1.29	3.47	6.46
T_{11}	1.94	1.16	3.10	6.51
T_{12}	2.05	1.03	3.08	6.54
Mean	2.12	1.10	3.22	6.99
SEd	0.07	0.04	0.13	0.21
CD (0.05%)	0.14	0.08	0.25	0.42

the treatment T_3 (3.35 mg g^{-1}) followed by T_4 (3.38 mg g^{-1}) and T_7 (2.88 mg g^{-1}). Increase in chlorophyll content at 25, 40 and 60 DAPR of mulberry due to the combined application of *Azospirillum*, *Rhizobium* and effective microorganism (EM) in soil was reported by Vinoj (2008) and as foliar spray of EM alone in mulberry (Gnanaselvi, 2007) which might be due to synergistic interaction of biofertilizers and EM. The findings are also in tune with the report of Singh *et al*. (1991), Das *et al*. (1994) and Ramarethinam *et al*. (2005). Similar results obtained due to the application of biofertilizer and nutrients with increased intercellular CO_2 concentration in mulberry (Pannerselvam *et al*., 1997) and increased photosynthesis leading to better utilization of stored carbohydrates (Ahmed *et al*., 2017). The increased amount of chlorophyll content in leaves indicated the photosynthetic efficiency and it can be used as one of the criteria for quantifying photosynthetic rate in mulberry (Sujathamma and Dandin, 2000). Higher levels of chlorophyll content are indicative of higher photosynthetic efficiency of plants (Patil *et al*., 1998; Watson, 1952; Raj and Tripathy, 1999).

Table 2. Influence of micronutrients and biofertilizers on biochemical parameters of mulberry

Treatments	Soluble protein (mg g^{-1})	Total Phenolics (mg g^{-1})	Nitrate reductase (NO_2 g^{-1} hr^{-1})
T_1	14.01	0.79	73.62
T_2	18.81	1.30	89.15
T_3	22.19	1.95	124.90
T_4	21.78	1.90	120.59
T_5	15.22	0.79	80.84
T_6	20.65	1.82	103.91
T_7	20.75	1.86	110.86
T_8	17.18	1.13	84.24
T_9	16.35	0.98	81.36
T_{10}	20.14	1.73	99.51
T_{11}	18.50	1.65	94.07
T_{12}	18.39	1.46	92.88
Mean	18.71	1.45	96.33
SEd	0.60	0.05	3.15
CD (0.05%)	1.22	0.10	6.40

Photosynthetic rate

With respect to photosynthetic rate, the treatment T_3 exhibited higher efficiency to record the highest value of *Pn* (28.62 µmol CO_2 m^{-2} s^{-1}) followed by T_4 (27.73 µmol CO_2 m^{-2} s^{-1}) which was on par with T_3. Significant differences were found among other treatments also (Fig.1). However, the lowest photosynthetic rate of 17.06 µmol CO_2 m^{-2} s^{-1} was recorded in treatment T_1. In the present study, application of biofertilizer leads to an increase in mobilization of NPK and all micronutrients. High nitrogen content in the leaf tissue allows the plant to have more chlorophyll, RuBisCo and triggering a higher rate of photosynthesis (Osaki *et al*., 1995). Phosphorus is an element that directly affects the process of photosynthesis (Warren, 2011) and phosphorus has been reported to affect the dark reactions of photosynthesis, the apparent quantum efficiency, and starch accumulation, but the rate of electron transport and stomatal conductance is not affected (Brahim *et al*., 1996). Potassium is an element that directly involved in translocation of photosynthate from source to sink. Photosynthesis is an important component of the plant's capacity in the utilization of atmospheric CO_2 and correlated with nutrients (Ma *et al*., 1995).

Transpiration rate

The transpiration rate of mulberry (Table 1) was significantly influenced by the application of micronutrients and biofertilizers and the lowest transpiration rate of 6.09 mmol H_2O m^{-2} s^{-1} was recorded in treatment T_3 followed by T_4 and T_7 with 6.17 and 6.27 mmol H_2O m^{-2} s^{-1} respectively and the highest transpiration rate was observed in T_1 followed by T_5 with 6.80 and 6.78 mmol H_2O m^{-2} s^{-1} respectively. When photosynthesis was high, transpiration was low especially in the micronutrient and biofertilizer due to much of H_2O used for photosynthesis before the water vapor was released in the transpiration process. When water absorbed by the plant roots from the soil is not entirely used to produce dry matter because most of the total water absorbed by the roots (90%) is lost *via* transpiration (Sterling, 2004). A similar finding was also made by Ahmed *et al*. (2017) in the leaves of AR-14 mulberry variety attributing to the fact that foliar application of nutrients can increase the leaf diffusive resistance and lower transpiration rates. It is therefore apparent from various studies and also from the present study that application of micronutrient and biofertilizer leads to the reduction in transpiration rate which might enhance the relative water content and in turn higher leaf yield.

Stomatal conductance

Stomatal conductance is the measure of the rate of passage of carbon dioxide (CO_2) entering or water vapor exiting through the stomata of a leaf. The higher stomatal conductance was registered in treatment T_3 imposed plants which recorded the gaseous exchange of 0.902 mmol H_2O m^{-2} s^{-1}

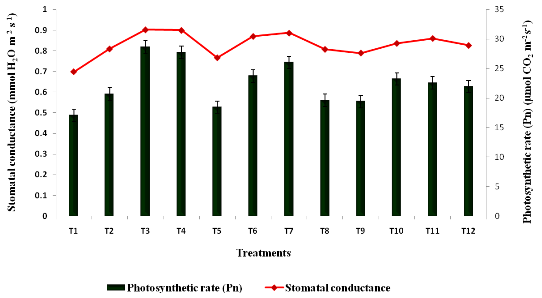

Figure 1. Influence of micronutrients and biofertilizers on physiological parameters of mulberry

followed by T_4 with 0.899 mmol H_2O $m^{-2}s^{-1}$ (Fig. 1). The treatment T_3 recorded 30.91 per cent increase in stomatal conductance over control which was in corroboration with the findings of Warren (2011) who observed significantly increased stomatal conductance in mulberry plants due to the application of *Azotobacter chroococcum* + *Azospirillum brasilense* + *Bacillus megaterium*.

Biochemical parameters

Soluble protein

A positive and significant effect of micronutrients and biofertilizers on mulberry was witnessed insoluble protein also (Table 2). Among the treatment, T_3 accumulated the highest soluble protein (22.19 mg g^{-1}) followed by T_4 with 21.78 mg g^{-1} and these two treatments were significantly different from other treatments. The lowest soluble protein content of 14.01 mg g^{-1} was observed in treatment T_1. The N_2 fixing property of *Azospirillum* increased the N availability which in turn increased protein content. The increased soluble protein content due to micronutrient and biofertilizer application was strongly supported by Wu and Tiedemann (2001) and Sori *et al*. (2008). Also, Chakraborty *et al.* (2008) observed the same results with the combined application of poultry manure with biofertilizers. Hence, a combined application of nutrient and bio fertilizers are not only improving higher RuBP case level but also effective in increasing in sugars and soluble protein in mulberry leaves are very much needed for the growth of young and late age silkworm. Significant increase in total soluble protein content in leaves denotes an increase in the nutritional status of mulberry leaves in terms of biochemical contents through the application of microorganisms (Madhubabu *et al*., 1992). Further, the nitrogen-fixing activity of microorganisms increased the nitrogen availability which in turn might have increased the protein content in the leaves.

Total carbohydrates

Data pertaining to total carbohydrate content of mulberry under different micronutrient and biofertilizer treatments revealed a significant variation compared to control and among the treatments, maximum total carbohydrate content was recorded in T_3 (21.61 mg g^{-1}) followed by T_4 with 21.27 mg g^{-1} and the lowest carbohydrate content was observed in T_1 (Fig. 2). Increase in the nutritional status of the mulberry leaves in terms of biochemical contents through the application of fertilizers and biofertilizers have also been recorded by earlier workers (Sengupta *et al.,* 1972; Loganath and Sivashankar, 1986; Subburethinam and Sulochanachetty, 1991; Madhubabu *et al.,* 1992). The synergistic action of introduced organisms increased the vigor and vitality of plant which resulted in enhanced metabolic activities of the plant.

Total phenolics

The total phenolics content (Table 2) of mulberry in treatment T_3 was the highest(1.95 mg g^{-1}) followed by T_4 and T_7 with 1.90 mg $g^{-1}$and 1.86 mg g^{-1}, respectively which were on par with each other. The lowest total phenolics content was recorded in T_1 (0.79 mg g^{-1}) received only the recommended dose of fertilizers. Phenolics are physiologically active secondary compounds produced by higher plants and are involved in the modulation of cell wall plasticity (Wallace and Fry, 1994). In the present study, higher phenolics content observed in T_3 might be due to decreased polyphenol oxidase activity in the treated plants which have resulted in high phenol content and this is in accordance with the findings of Jaleel *et al*. (2009) and Lakshmanan *et al*. (2007) in turmeric. Cheynier *et al*. (2013) also reported that the application of nutrient and biofertilizers increased the total phenolics in mulberry. Phenolic constituents of plants have anti-oxidant activity and offer protection against oxidative damage (Evans *et al.,* 1997).

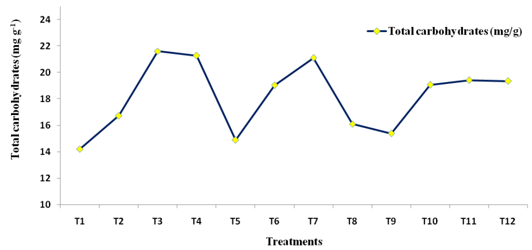

Figure 2. Influence of micronutrients and biofertilizers on total carbohydrates (mg g^{-1}) of mulberry

Nitrate reductase (NR) activity

Elevated NR activity was found in the treatment T_3 (124.90 µg NO_2 g^{-1} h^{-1}) followed by T_4 (120.59 µg NO_2 g^{-1} h^{-1}) (Table 2). The importance of minerals like Fe, Mg, Zn and Mn for enzyme activation is well recognized and reported earlier (Broyer and Stout, 1959; Ghosh and Srivastava, 1993). They also reported that this enzyme is found to be stimulated in rice plants by monovalent cations such as Na^+, K^+ and in *Vigna mungo* by a divalent cation such as Ca^{++}. Increase in NR activity by magnesium, zinc and molybdate also observed by Johnson *et al*., (2005) and reported that, NR activity is to considered as a predictive index of crop yield through proteins of foliage.

CONCLUSION

From the experiment, it is concluded that the treatment consisted of 100 % RDF + Soil application of $ZnSO_4$, $FeSO_4$, $MnSO_4$ and $MgSO_4$ @ 25 kg ha^{-1} each + Recommended dose of Biofertilizers (Azos: *Azospirillum lipoferum*, Phospho: *Bacillus megaterium* var. *Phosphaticum* and Potash mobilizer: *Frateuria aurantia*) (T_3) has showed significant influence on physiological (chlorophyll content, photosynthetic rate, transpiration rate, stomatal conductance) and biochemical parameters (soluble protein, total carbohydrate, total phenolics, NR activity) which in turn positively correlated with the quantity and quality of mulberry leaves for better growth and development of silkworm.

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