

EFFICACY OF BIO-FOLIAR SPRAY ON GROWTH AND BIOCHEMICAL PARAMETERS OF DIFFERENT MULBERRY VARIETIES

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ABSTRACT

The study was undertaken to improve the growth and biochemical contents in an instant available form through foliar combination of spirulina, soybean and vermiwash. 15, 30 and 45 $\mu\text{g mL}^{-1}$ concentrations of bio-foliar were sprayed on five different mulberry varieties namely S-1, S-146, S-1635, BR-2 and AR-14 to know their performance on growth parameters and biochemical constituents. Results of the investigation revealed that, among all experimental concentrations mulberry varieties and treatments, BR-2 and S-1635 varieties were performed well in respect of growth parameters and biochemical constituents. Highest leaf area (234.07 cm^2) and total leaf yield per hectare (51.48 tons) was noticed in BR-2 mulberry variety at 45 $\mu\text{g mL}^{-1}$ followed by S-1635 variety. Further highest protein (0.555 mg g^{-1}), carbohydrate (0.668 mg g^{-1}), carotenoids (0.550 mg g^{-1}), total chlorophyll (2.04 mg g^{-1}) and crude protein content (26.67%) were recorded at 45 $\mu\text{g mL}^{-1}$ concentration by BR-2 variety followed with S-1635 variety as compare to control. The outcome of research implies that, among five mulberry varieties and concentrations, BR-2 and S-1635 varieties with 45 $\mu\text{g mL}^{-1}$ concentration performed well and successfully augments the productivity and biochemical constituents of different mulberry varieties without any side effect.

Keywords: Bio-Foliar, Mulberry Varieties, Biochemicals and Productivity

1. INTRODUCTION

Mulberry leaf forms the basic food material for silkworm, *Bombyx mori* L. and nutrition is the most important growth regulating factor in silkworm, *Bombyx mori* L. Synthetic chemicals, fertilizers, pesticides, herbicides, growth promoters and other inputs though enhance productivity of mulberry but adversely affect the eco-system and increase prices of agricultural inputs (Patil *et al.*, 2006). Under such circumstances the leaf quality may be improved by foliar application of soluble nutrients to mulberry leaves instantly (Shankar *et al.*, 1999). Mulberry (*Morus* spp.) is a deep rooted high biomass producing foliage crop, responds well to foliar nutrition. Foliar application in right time increase level

of application in specific nutrients to the leaf during growth and development (Narahari *et al.*, 1997). Therefore, it is necessary that the nutrients applied to the mulberry through foliar nutrition are made easily available even at later stages of leaf development and it should be cost effective. To mitigate these difficulties proper mode of application of nutrients needs to be developed particularly by natural based formulations which are required in small quantities. Therefore, completely natural based formulation was made in the form bio-foliar and applied for the growth and productivity of mulberry. Bio-foliar is dominating over all kind of foliar sprays which were used for mulberry production due to its natural based product formulation. It is very cost effective, easily can be applied to mulberry

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without harming mulberry ecosystem. Interesting point that for the first time all biological based organisms were used in the current study for making bio-foliar spray namely spirulina, soybean and Vermiwash. The present investigation was carried out to know the efficacy of eco-friendly bio-foliar spray on growth parameters and biochemical constituents of different mulberry varieties.

2. MATERIALS AND METHODS

2.1. Procurement and Analysis of Samples

Spirulina, vermiwash and soybeans were procured from University of Allahabad, Allahabad, Extension Centre of CIMAP, Lucknow and Beej Bhandar Kendra, Seed Market, Lucknow respectively.

Spirulina and soybean seeds were separately air-dried in the laboratory and ground to powder using an electric mixer grinder. About 450 g of the powdered material of each sample was macerated with 2800 mL of ethanol (90%) for 72 h. The extract was filtered and dried by evaporation in a water bath at 40°C to yield 16.94 g of the crude extract, which was used for the preparation of bio-foliar. Vermiwash was prepared as per the procedure described by Karuna *et al.* (1999). The collected vermiwash was centrifuged at 1000 rpm and preserved into sterilized cane for further process. 15, 30 and 45 micrograms per milliliter ($\mu\text{g mL}^{-1}$) concentrations of bio-foliar were prepared with spirulina: Soybean: Vermiwash (3:2:1, w/v) and sprayed on five different mulberry varieties viz; S-1, S-146, S-1635, BR-2 and AR-14 to know their performance on growth and yield. Experiments were conducted three times with four replications in each treatment including control. Moisture percentage and moisture retaining capacity was estimated as defined by Dandin and Jolly (1986). Chlorophyll was estimated by the methodology of Hiscox and Israelstam (1979). Protein content in different mulberry varieties were estimated by method of Lowry *et al.* (1951) while carbohydrate content was estimated by Dubois *et al.* (1956) method.

2.2. Statistical Analysis

Data collected from all experiments with four replicates were subjected to statistical analysis. Two way Analysis of Variance (ANOVA) was carried out to find out the significant differences between the varieties and concentrations. Multiple Comparison of means were made depending on F-ratio and the Critical Difference (CD) values based on student t criteria at 5 and 1% level of significance utilizing the standard methods (Johnson and Wichern, 2007). Pearson's Correlation Coefficients were also computed using the standard method in Statistics.

Computations were made using statistical package under M.S. Excel work sheet.

3. RESULTS

It was found that there were significant changes in the growth, yield and biochemical levels of mulberry due to bio-foliar application of different concentrations on mulberry varieties.

3.1. Effect of Bio-Foliar Spray on Growth of Mulberry Varieties

Results of the present investigations indicated that the different varieties of mulberry did not show any significant effect on shoot length, number of shoot per plant, number of leaves per plant, internodal distance and leaf area after spraying bio-foliar in 10 and 20 days interval. However, the trend improved significantly with same mulberry varieties when treated with 45 $\mu\text{g mL}^{-1}$ concentration of bio-foliar spray after 30 and 40 days.

Among mulberry varieties and treatments, S-1 variety was recorded longest shoot length (103.13cm) and number of leaves per plant (399.00) with 45 $\mu\text{g mL}^{-1}$ as compared to other concentrations. All the treated mulberry varieties have significantly (df:4&12, p:9.46) higher shoot length and number of leaves per plant compared over control.

Number of shoots per plant have increased with S-146 variety (30.50) at 45 $\mu\text{g mL}^{-1}$ of bio-foliar, whereas BR-2 variety showed the largest leaf area (234.07 cm^2) and minimum inter nodal distance (5.74 cm) at 45 $\mu\text{g mL}^{-1}$ when compared to other concentrations and control (Table 1a).

3.2. Effect of Bio-Foliar Spray on Yield of Mulberry Varieties

Observations revealed that highest fresh weight of leaves (475.86 g), leaf yield per plant (2.15 kg) and total leaf yield per hectare (51.48 tons) was noticed in BR-2 mulberry variety at 45 $\mu\text{g mL}^{-1}$ followed by S-1635 variety as compared to 15 and 30 $\mu\text{g mL}^{-1}$ and control. Therefore, the current study clearly revealed that growth and yield of different mulberry varieties were significantly (df:4&12, p: 7.15) enhanced by 45 $\mu\text{g mL}^{-1}$ concentration of bio-foliar formulation (Table 1b).

3.3. Effect of Bio-Foliar Spray on Protein and Carbohydrate Content of Mulberry Varieties

Significantly (df:4&12,p:1.63) increased protein content was noted in 45 $\mu\text{g mL}^{-1}$ concentration of bio-foliar spray in AR-14 (0.523 mg g^{-1}) and BR-2 (0.555 mg g^{-1}) varieties as compared to the rest of the concentrations.

Table 1a. Effect of bio-foliar spray on growth and yield of different mulberry varieties

Mulberry variety	Shoot length (cm)					No. of shoots/plant					No. of leaves/plant				
	15 µg mL ⁻¹	30 µg mL ⁻¹	45 µg mL ⁻¹	Control mL ⁻¹	Mean mL ⁻¹	15 µg mL ⁻¹	30 µg mL ⁻¹	45 µg mL ⁻¹	Control mL ⁻¹	Mean mL ⁻¹	15 µg mL ⁻¹	30 µg mL ⁻¹	45 µg mL ⁻¹	Control mL ⁻¹	Mean mL ⁻¹
S-1	92.200	96.75	103.130	89.31	95.350	17.75	22.50	24.250	18.50	20.750	340.000	341.25	399.000	330.50	352.690
S-146	61.6600	64.90	72.850	58.83	64.560	24.00	25.50	30.500	22.00	25.500	260.750	270.50	287.000	252.75	267.750
S-1635	81.9400	85.28	100.650	81.17	87.260	21.00	23.00	25.500	20.00	22.380	289.250	303.25	325.250	285.50	300.810
AR-14	77.500	80.14	82.510	76.61	79.190	18.00	18.75	20.000	16.00	18.190	216.000	218.25	224.250	209.50	217.000
BR-2	62.400	65.08	74.280	62.09	65.960	21.25	24.00	27.500	20.25	23.250	200.500	213.75	237.750	188.50	210.130
CD @5%	0.998		0.893		1.997	0.717		0.642		1.435	4.091		3.659		8.181
CD @1%	1.328		1.188		2.656	0.954		0.853		1.908	5.44		4.866		10.881

Table 1b. Effect of bio-foliar spray on growth and yield of different mulberry varieties

Mulberry variety	Internodal distance (cm)					Wt. of 100 fresh mulberry leaves (g)					Leaf yield/hectare/year (tons)				
	15 µg mL ⁻¹	30 µg mL ⁻¹	45 µg mL ⁻¹	Control mL ⁻¹	Mean mL ⁻¹	15 µg mL ⁻¹	30 µg mL ⁻¹	45 µg mL ⁻¹	Control mL ⁻¹	Mean mL ⁻¹	15 µg mL ⁻¹	30 µg mL ⁻¹	45 µg mL ⁻¹	Control mL ⁻¹	Mean mL ⁻¹
S-1	5.620	5.85	5.990	5.11	5.640	172.500	196.31	200.780	168.07	184.420	28.220	33.13	35.880	27.15	31.090
S-146	5.280	5.86	6.170	5.22	5.630	235.040	247.86	262.020	231.06	243.990	32.290	34.98	38.390	30.91	34.140
S-1635	6.730	6.89	7.400	6.48	6.870	377.670	387.31	403.240	375.14	385.840	40.120	42.81	46.160	38.34	41.860
AR-14	6.510	7.31	7.830	6.26	6.980	261.100	273.45	299.660	256.28	272.620	29.180	30.02	31.930	28.34	29.870
BR-2	5.760	5.72	5.740	5.74	5.740	445.600	462.87	475.870	423.25	451.900	38.990	41.92	51.480	38.99	42.840
CD @5%	0.075		0.067		0.151	1.567		1.402		3.134	0.526		0.470		1.052
CD @1%	0.100		0.090		0.200	2.084		1.864		4.168	0.700		0.626		1.399

Table 2a. Effect of bio-foliar spray on biochemicals of different mulberry varieties

Mulberry variety	Protein content (mg/g)					Carbohydrate content (mg/g)					Carotenoids content (mg/g)				
	15 µg mL ⁻¹	30 µg mL ⁻¹	45 µg mL ⁻¹	Control mL ⁻¹	Mean mL ⁻¹	15 µg mL ⁻¹	30 µg mL ⁻¹	45 µg mL ⁻¹	Control mL ⁻¹	Mean mL ⁻¹	15 µg mL ⁻¹	30 µg mL ⁻¹	45 µg mL ⁻¹	Control mL ⁻¹	Mean mL ⁻¹
S-1	0.41	0.448	0.488	0.405	0.438	0.521	0.553	0.562	0.508	0.536	0.379	0.394	0.431	0.367	0.393
S-146	0.484	0.498	0.509	0.476	0.492	0.395	0.419	0.433	0.384	0.408	0.510	0.510	0.514	0.503	0.509
S-1635	0.461	0.524	0.534	0.457	0.494	0.559	0.560	0.669	0.547	0.584	0.533	0.541	0.535	0.490	0.525
AR-14	0.513	0.514	0.523	0.513	0.516	0.495	0.515	0.532	0.471	0.503	0.485	0.500	0.511	0.435	0.483
BR-2	0.434	0.548	0.555	0.432	0.492	0.516	0.525	0.668	0.514	0.556	0.523	0.542	0.550	0.527	0.535
CD @5%	0.001		0.001		0.002	0.004		0.003		0.007	0.005		0.005		0.011
CD @1%	0.001		0.001		0.003	0.005		0.004		0.010	0.007		0.006		0.014

Table 2b. Effect of bio-foliar spray on biochemicals of different mulberry varieties

Mulberry variety	Chlorophyll 'a' (mg/g)					Chlorophyll 'b' (mg/g)					Total chlorophyll (mg/g)				
	15 µg mL ⁻¹	30 µg mL ⁻¹	45 µg mL ⁻¹	Control mL ⁻¹	Mean mL ⁻¹	15 µg mL ⁻¹	30 µg mL ⁻¹	45 µg mL ⁻¹	Control mL ⁻¹	Mean mL ⁻¹	15 µg mL ⁻¹	30 µg mL ⁻¹	45 µg mL ⁻¹	Control mL ⁻¹	Mean mL ⁻¹
S-1	0.644	0.683	0.683	0.706	0.679	0.980	0.97	1.040	0.87	0.96	1.628	1.657	1.723	1.582	1.640
S-146	0.674	0.679	0.696	0.704	0.688	1.250	1.24	1.260	0.97	1.18	1.928	1.920	1.963	1.676	1.870
S-1635	0.675	0.691	0.682	0.698	0.686	1.230	1.26	1.240	1.17	1.23	1.910	1.952	1.929	1.876	1.910
AR-14	0.834	0.831	0.852	0.840	0.839	0.970	1.09	0.990	0.92	0.99	1.811	1.923	1.851	1.764	1.830
BR-2	0.680	0.688	0.681	0.677	0.681	1.330	1.34	1.360	1.32	1.34	2.016	2.028	2.046	2.002	2.020
CD @5%	0.004		0.003		0.007	0.013		0.012		0.026	0.012		0.010		0.023
CD @1%	0.005		0.004		0.009	0.017		0.016		0.035	0.015		0.014		0.031

Table 2c. Effect of bio-foliar spray on biochemicals of different mulberry varieties

Mulberry variety	Moisture content (%)					MRC (%)					Crude protein (%)				
	15 µg mL ⁻¹	30 µg mL ⁻¹	45 µg mL ⁻¹	Control mL ⁻¹	Mean mL ⁻¹	15 µg mL ⁻¹	30 µg mL ⁻¹	45 µg mL ⁻¹	Control mL ⁻¹	Mean mL ⁻¹	15 µg mL ⁻¹	30 µg mL ⁻¹	45 µg mL ⁻¹	Control mL ⁻¹	Mean mL ⁻¹
S-1	73.11	74.66	74.400	73.36	73.88	54.07	59.15	59.82	54.16	56.80	24.18	26.35	26.81	22.45	24.950
S-146	73.05	73.77	75.660	71.38	73.46	52.35	54.51	57.81	51.67	54.08	21.85	23.51	24.78	21.59	22.930
S-1635	71.18	72.79	74.810	72.01	72.70	51.29	54.45	55.78	51.72	53.31	24.31	25.64	25.98	22.66	24.600
AR-14	77.14	78.20	80.690	75.90	77.98	51.86	54.39	57.95	52.60	54.20	22.18	23.23	24.21	22.01	22.910
BR-2	74.79	77.98	77.870	73.37	76.00	61.25	64.89	64.94	60.05	62.78	23.75	26.10	26.67	22.50	24.750
CD @5%	0.934		0.835		-	0.88		0.787		-	0.176		0.157		0.351
CD @1%	1.242		1.110		-	1.17		1.047		-	0.234		0.209		0.467

The control of all varieties showed least protein content compared to treated varieties. Similar increased carbohydrate content (0.669 and 0.668 mg g^{-1}) was observed in S-1635 and BR-2 varieties respectively with $45 \mu\text{g mL}^{-1}$ of bio-foliar application. Crude protein content was also shown to be the highest in BR-2 variety at $45 \mu\text{g mL}^{-1}$ concentration as compared to control. Control of all mulberry varieties here again showed least moisture content, moisture retention and crude protein as well (**Table 2a**).

3.4. Alteration of Carotenoid, Total Chlorophyll, Chlorophyll a and Chlorophyll b Content in Mulberry Varieties

Bio-foliar exerted significant (df:4&12, p: 4.75) influence on carotenoids contents. The increased carotenoids content (0.550 mg g^{-1}) was observed in BR-2 variety at $45 \mu\text{g mL}^{-1}$ followed by S-1635 variety, while control of all varieties showed least content of carotenoids.

The highest chlorophyll 'a' (0.852 mg g^{-1}), chlorophyll 'b' (1.365 mg g^{-1}) and total chlorophyll (2.046 mg g^{-1}) was recorded for AR-14 and BR-2 varieties respectively at $45 \mu\text{g mL}^{-1}$ concentration when compared to other concentrations and control. Chlorophyll was found highly significant (df: 4&12, p: 1.11) with bio-foliar application on different mulberry varieties at $45 \mu\text{g mL}^{-1}$ (**Table 2b**).

3.5. Effect of Bio-Foliar Spray on Moisture Retention in Mulberry Varieties

Results indicated that leaf moisture did not show significant (df:4&12, p:6.37) interaction between varieties and various concentrations of bio-foliar. Highest moisture content (80.69%) was observed in AR-14 variety at $45 \mu\text{g mL}^{-1}$ concentration. Nevertheless, maximum moisture retention capacity (64.94%) was noticed with BR-2 variety at $45 \mu\text{g mL}^{-1}$ concentration (**Table 2c**).

4. DISCUSSION

With regard to effect of bio-foliar application, among all mulberry varieties and treatments, S-1 variety has recorded longest shoot length of 103.13 (cm) with $45 \mu\text{g mL}^{-1}$ and it is found to be beneficial over other treatments. The results are in accordance with those of Pramod *et al.* (1999), who opined that higher concentration of foliar application of salicylic acid induced maximum shoot length in soybean. Internodal distance of a variety is one of the parameters which contribute to the total leaf produced by each plant.

Yokoyama (1975) reported that mulberry leaf yield depends on the number and length of the shoots, internodal distance and number and weight of leaves per plant. Fotadar *et al.* (1989) reported that inter nodal distance is an important genotypic attribute which influences the total foliage produced by a plant. Lesser inter nodal distance more will be the number of the inter nodes per plant and in turn more number of leaves per plant. The results are in accordance with those of Moose and Sisco (1994), who have amply demonstrated the most pronounced physiological effect of gibberellins in stem elongation particularly at the elongation of internodes. In the present study, highest leaf area was recorded for BR-2 and S-1635 under bio-foliar treatment at $45 \mu\text{g mL}^{-1}$, which is due to increased of photosynthesis leading to enhanced leaf area and as well better utilization of stored carbohydrates. The results are in accordance with those of Watson (1952) who viewed that different levels of salicylic acid perform better with respect to number of leaves and lamina, which might be due to increasing of photosynthesis leading to enhanced leaf area index. Leaf weight is considered as a more precise index for yield assessment, as leaf to cocoon ratio is calculated by weight (Dandin and Kumar, 1986). Hamada (1956) reported that the yield of mulberry leaves depends upon the length of the shoot and leaf weight. Maximum leaf weight was recorded in BR-2 variety at $45 \mu\text{g mL}^{-1}$ concentration which is due to increased of photosynthesis leading to enhanced leaf area as well as better utilization of stored carbohydrates.

Highest leaf yield was recorded at $45 \mu\text{g mL}^{-1}$ of bio-foliar spray. Bio-foliar induced promontory effect due presence of growth inducing substances, essential amino acids, vitamins and plant nutrients. The results are in accordance with Singhvi *et al.* (2001) who have reported that 15.17% leaf yield was increased by the foliar application of salicylic acid in mulberry.

The improvement in the protein content is directly reflected on the leaf yield. Protein is the main constituent of the mulberry leaf, which contributes for the synthesis of silk (Kawase and Michihiro, 1976) and this improvement indicates the effect of foliar spray. These results are in conformity with Loknath and Shivashankar (1986). Thus, from the study it is concluded that among the four spray formulations including control, $45 \mu\text{g mL}^{-1}$ of bio-foliar spray recorded significant improvement in leaf yield and quality of mulberry. As the concentration of bio-foliar spray was enhanced, leaf yield of different mulberry varieties was also increased due to absorption of higher quantity plant nutrients, antioxidants, vitamins and amino acids because they major constituent of bio-foliar spray which is required for higher growth and productivity of mulberry.

Application of bio-foliar on different mulberry varieties brought a significant increase in the chlorophyll content of leaves over control. Among all mulberry varieties and bio-foliar treatments, BR-2 variety has shown highly significant values ($p > 0.01$), highest total chlorophyll was recorded at $45 \mu\text{g mL}^{-1}$ concentration followed by 30, 15 $\mu\text{g mL}^{-1}$ and control. This is due to an increase in the Fe and Mg content of mulberry leaves. The increase in chlorophyll content with bio-foliar spray confirmed the reports of Singhvi *et al.* (2001) in mulberry and Gunes *et al.* (2007) for maize. Further, Baky *et al.* (2014) showed higher increment of total chlorophyll, chlorophyll a and b and antioxidant low-molecular compounds i.e., glutathione and carotenoids contents by foliar application of green algae and blue green algae (*Spirulina*).

Bio-foliar spray contributed well for leaf moisture when treated with $45 \mu\text{g mL}^{-1}$ concentration. The higher moisture content in the leaves of AR-14 mulberry variety was noticed due to the additional supply of nutrition (bio-foliar) to the leaves and also withstanding the moisture for longer duration. This phenomenon attributed to the fact that bio-foliar application can increase the leaf diffusive resistance and lower transpiration rates. The increased water content in plants treated with foliar spray were also reported for other crops grown under salt stress i.e., barley (El-Tayeb, 2005), tomato (Tari *et al.*, 2002; Szepesi *et al.*, 2005) and cucumber (Yildirim *et al.*, 2008).

Among all mulberry varieties and concentrations, S-1635 variety showed highest carbohydrate content at $45 \mu\text{g mL}^{-1}$ concentration of bio-foliar spray over control. The present observations are in close conformity with findings of Gowda *et al.* (2000) who reported soil application of DAP to mulberry, with foliar application of seriboost to mulberry which increased the total carbohydrates.

Improvement in crude protein was mainly attributed to the foliar spray where nutrients were rapidly absorbed by foliage and evidently the plant metabolism or assimilation have been activated thus contributing for healthy green foliage resulting in synthesis of organic contents.

The results obtained from the proposed study clearly showed that the varieties namely BR-2 and S-1635 emerged as top priority varieties by enhancing biochemical constituents and productivity.

5. CONCLUSION

It is clearly concluded that bio-foliar application of $45 \mu\text{g mL}^{-1}$ concentration on BR-2 and S-1635 mulberry varieties was an effective treatment for growth and productivity of mulberry and it is also able to enhance

biochemical constituents of different mulberry varieties without causing any substantial loss to ecosystem.

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