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Responses of fresh leaf yield and quality variables of M_5 mulberry to bio-inoculants, farm yard manure (FYM) and inorganic fertilizers under rain-fed conditions

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An experiment was conducted to know the effect of integrated use of bio-inoculants (*Azotobacter* sp. at 20 kg/ha/year, *Aspergillus awamori* at 25 kg/ha/year and *Trichoderma harzianum* at 20 kg/ha/year), farm yard manure (FYM) and inorganic fertilizers (IF, that is, Nitrogen-phosphorus-potassium (NPK) on fresh leaf yield and quality variables of M_5 mulberry under rain-fed condition during 2006-07. The experiment was laid out using randomized complete block design with eight treatments replicated thrice. The results revealed that combined use of treatment components had a positive effect on fresh leaf yield and quality variables of mulberry on par with the standard check (100:50:50 kg/ha/year NPK and 12 MT/ha/year FYM). Application of the recommended dosage of fertilizers recorded maximum fresh leaf yield per plant (403.60 and 538.13; 718.74 and 867.57 g) at 45 and 60 days after pruning in the first and second crops, respectively. However, it was consistently and statistically on par with T_4 (397.80 and 530.40; 614.07 and 795.20 g). Further, the leaf quality variables namely, moisture percentage (71.89 and 70.90; 71.50 and 68.90 at 45 and 60 days after pruning for crop I and II, respectively), chlorophyll (a) (1.45 mg/g), chlorophyll (b) (0.69 mg/g), total chlorophyll (2.14 mg/g), crude protein (18.21 mg/g), total soluble protein (8.82 mg/g), total soluble sugar (7.95 mg/g) and NPK (2.98, 1.72 and 1.33%, respectively) content of the leaf were maximum in the standard check which was on par with T_4. Thus, N and P inorganic fertilizers can be reduced to the tune of 25% without affecting the fresh leaf yield and quality variables of M_5 mulberry by amending the same through bio-inoculants under rain-fed conditions.

Key words: M_5 mulberry, bio-inoculants, yield and quality variables, rain-fed condition.

INTRODUCTION

Mulberry (*Morus* spp.) is the sole food plant for the silkworm (*Bombyx mori* L.). Besides climate, rearing technique, silkworm race and silkworm eggs, are known to influence successful cocoon production by mulberry silkworm (Singhal et al., 1999). Thus, nutritive value of mulberry leaf plays a predominant role in better crop performance of mulberry silkworm. However, the nutritive value of mulberry leaf is influenced by the soil nutrient status (Bongale, 2006). Frequent use of inorganic fertilizers for a prolonged period results in poor soil health and affects availability of nutrients to be absorbed by plants (Subbaswamy et al., 1994). It was reported that feeding of nutrient deficient mulberry leaves to developing silkworms led to 17.11% cocoon production loss(Singhvi et al., 2007). Use of 20 kg/ha/year of *Azotobacter* was effective in minimizing the application of

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Table 1. Month wise weather data in the study area during the study period.

Months	Total rain fall (mm)	Temperature (°C)		Relative humidity (%)		Wind speed (km/h)
		Maximum	Minimum	Morning	Noon	
Year 2006						
June	112.60	29.20	19.60	90.00	49.00	10.60
July	84.40	28.10	19.30	90.00	52.00	14.10
August	63.20	28.50	19.00	93.00	49.00	11.10
September	61.00	29.20	19.40	91.00	47.00	9.00
October	36.00	28.80	18.70	91.00	48.00	8.00
November	111.80	27.00	17.80	95.00	53.00	7.10
December	1.80	26.40	13.70	93.00	49.00	8.10
Year 2007						
January	0.00	28.10	13.80	90.00	42.00	7.70
February	0.80	29.80	14.80	90.00	40.00	7.90
March	0.00	33.20	18.40	82.00	33.00	7.60
April	109.60	33.80	20.20	83.00	33.00	7.50
May	85.40	33.20	21.20	85.00	38.00	9.10
June	60.30	29.60	19.70	91.00	49.00	11.20
July	149.20	28.40	19.90	90.00	50.00	12.10

inorganic fertilizers 'N', thereby, reducing the cost of mulberry cultivation under irrigated conditions without adverse effect on leaf quality (Das et al., 1994). Therefore, it was deemed necessary to test the influence of bio-inoculants on different functions of mulberry yield and quality variables under rain-fed conditions by complementing it with inorganic fertilizers and farm yard manure (FYM).

MATERIALS AND METHODS

The experiment was superimposed on already established 10 years old M_5 mulberry to determine the influence of bio-inoculants (*Azotobacter* sp. at 20 kg/ha/year, *A. awamori* at 25 kg/ha/year and *T. hazianum* at 20 kg/ha year), FYM at 12 MT/ha/year and inorganic fertilizers (N from urea, P from single super phosphate and K from muriate of potash at 100:50:50 kg/ha/year and reduced dosages of NP plus the bio-inoculants) on fresh leaf yield and quality variables of M_5 mulberry under rain-fed condition at Gandhi Krishi Vignana Kendra. The study was established at Bangalore in the Department of Sericulture premises which is located at a coordinates of 77°35' E longitude and 12°58' N latitude at an altitude of 930 m above mean sea level. The weather data on rainfall, temperature, relative humidity and wind speed that prevailed during the cropping period were compiled and presented in Table 1. The rain fall was maximum in the month of July 2006/2007 (149.20 mm) and there was no rain during January and March 2006/2007. The temperature ranged between 13.70 to 33.80°C; the hottest month being April (33.80°C (Max.)) and 20. 20°C (min.)); however, the coldest month was December (26.40°C (Maximum) and 13.70°C (minimum)). Maximum relative humidity of 95.00% was recorded during November and minimum was during March (82.00%). Maximum wind speed of 14.10 km/hr was recorded in July but minimum of 7.10 km/h was registered in the month of November.

Bio-inoculants were obtained from bio-fertilizers scheme, Department of Agricultural Microbiology, UAS (B), GKVK and were mixed with FYM under wet condition and applied to the root zone of mulberry two weeks after the application of inorganic fertilizers to avoid possible contact. The experiment was laid-out in a randomized complete block design with eight treatments each with three replications. A single plot size of 4 x 4 m (16 m^2) was used for each replication. A spacing of 60 x 60 cm between plants and rows were maintained for the experiment. The treatments were: T_1 (recommended rate, that is, 100:50:50 kg/ha/year NPK and 12 MT/ha/year FYM), T_2 (75% of recommended NP from inorganic fertilizers, 25% of recommended NP from *A. awamori* and *Azotobacter* sp., 12 MT/ha/year FYM and 50 kg/ha/year K), T_3 (50% of recommended NP from inorganic fertilizers, 50% of recommended NP from *A. awamori* and *Azotobacter* sp., 12 MT/ha/year FYM and 50 kg/ha/year K), T_4 (75% of recommended NP from inorganic fertilizers; 25% of recommended NP from *A. awamori*, *Azotobacter* sp. and *T. hazianum;* 12 MT/ha/year FYM and 50 kg/ha/year K), T_5 (50% of recommended NP from inorganic fertilizers; 50% of recommended NP from *A. awamori*, *Azotobacter* sp. and *T. hazianum;* 12 MT/ha/year FYM and 50 kg/ha/year K), T_6 (recommended NPK from FYM), T_7 (Recommended NPK from inorganic fertilizers) and T_8 (Control, that is, no application of fertilizer).

Cultural practices were followed as per the package of practices for rain-fed mulberry as described by Dandin et al. (2003). Bottom pruning was carried out during the last week of June, 2006 and 2007 just at the onset of main rainy season. After one and two weeks of bottom pruning the inorganic fertilizers, bio-inoculants and FYM were applied as per the treatment details. Observations on fresh leaf yield and moisture percentage were recorded by harvesting the fresh leaves from five randomly selected and tagged plants from each plot 45 and 60 days after bottom pruning for two seasons. Leaves were harvested individually plant wise, weighed and then mean leaf yield per plant was determined. But observation on other quality variables viz., chlorophyll, crude protein, total soluble protein, total soluble sugars and NPK content of the leaf were made on the second crop 60 days after bottom pruning.

Leaf quality variables viz., moisture, chlorophyll (a), chlorophyll (b) and total chlorophyll, NPK, crude protein, total soluble proteins and total soluble sugar contents of the leaf were each estimated through analysis. Moisture percentage was determined by A.O.A.C. (1970), chlorophyll content by Hiscox and Israelstam (1979) and

Table 2. Fresh leaf yield responses of M_5 mulberry to integrated application of bio-inoculants, FYM and Inorganic fertilizers under rain-fed conditions

Treatments**	Fresh leaf yield/plant (g)			
	Crop I		Crop II	
	45DAP***	60DAP	45DAP	60DAP
T_1	403.60	538.13	718.74	867.57
T_2	397.00	529.33	526.00	605.70
T_3	373.60	498.13	421.33	452.71
T_4	397.80	530.40	614.07	795.20
T_5	390.80	521.07	503.92	581.60
T_6	360.20	480.27	364.20	497.10
T_7	373.20	497.60	323.07	377.77
T_8	257.00	342.67	226.77	280.05
F-test	*	*	*	*
SEm±	10.37	13.82	57.912	78.24
$LSD_{5\%}$	31.45	41.93	175.68	237.34
CV (%)	4.86	4.84	21.70	25.23

*Significant at 5% level of significance; ***DAP-Days after pruning, **T_1- Standard check (RNPK + RFYM), T_2- 75 % NP through IF and 25% through *A. awamori* and *Azotobacter* sp. + RFYM + RK T_3 – 50% NP through IF and 50% NP through *A. awamori* and *Azotobacter* sp. + RFYM + RK, T_4 - T_2 + *T. harzianum,* T_5 – T_3 + *T. harzianum,* T_6 – RNPK from FYM, T_7 – RNPK only from IF, T_8 –control.

Arnon (1949), total soluble protein was estimated as per the procedure of Lowry et al. (1951), crude protein content by multiplying the percentage of nitrogen in the leaf by a factor 6.25, total soluble sugar by colorimetric method (Dubios et al., 1956) and NPK percentage using standard procedures. Data were analyzed using ANOVA as described by Cochran and Cox (2000). Least significant difference (LSD) test were used to differentiate statistical mean differences at 5% level of significance whenever the means were found significant for pair wise comparison of the treatments.

RESULTS AND DISCUSSION

Fresh leaf yield per plant (g/plant)

The treatments were significantly ($P<0.05$) different from each other with respect to the fresh leaf yield of M_5 mulberry (Table 2). In the first and second crops, T_1 has recorded maximum fresh leaf yield per plant (403.60 and 538.13; 718.74 and 867.57 g/plant), which was consistently and statistically on par with T_4 (397.80 and 530.40; 614.07 and 795.20 g/plant) 45th and 60th days after pruning, respectively. Minimum fresh leaf yield (257.00 and 342.67; 226.77 and 280.05 g/plant) was recorded from the control 45th and 60th days after pruning (Table 1). The increased fresh leaf yield of M_5 mulberry from T_1 and T_4 may be due to more number of shoots, higher plant height and more number of leaves per plant. Further, the combined application of FYM might have helped in slow and steady release of nutrients in addition to supply of important macro- and micro-nutrients besides efficient supply of N and P by nitrogen fixing and phosphorus solubilizing bio-inoculants utilized. Thus, NP application can be curtailed to the tune of 25% by supplementing with bio-inoculants without adverse effect on fresh leaf yield of M_5 mulberry under rain-fed condition. Katiyar et al. (1995) in their earlier studies on the effect of dual inoculation of *A. chroococum* and *G. mosseae* with 50% recommended dosage of N and P fertilizers have reported that, the yield of mulberry was on par with uninoculated control receiving full dosage of N and P fertilizers. Das et al. (1994) in their studies have also reported that, there was no reduction in leaf yield in K_2 variety of mulberry when 50% nitrogen fertilizer was compensated with 20 kg *Azotobacter* sp./ha/year. Sreeramulu et al. (2004) also reported a saving of 25% N and P chemical fertilizers application without affecting leaf yield of mulberry when *A. brasilense* and *B. megaterium* were inoculated.

Moisture content (%)

The moisture content of M_5 mulberry leaves was significant ($P<0.05$) due to the influence of the different treatments in both the crops 45th and 60th days after pruning (Table 3). Among the different treatments, in both crops, the leaves harvested from T_1 plot recorded maximum moisture percentage (71.89 and 70.90; 71.50 and 68.90), respectively 45th and 60th days after pruning. This treatment was statistically similar with T_4 (71.08 and

Table 3. Moisture, chlorophyll and crude protein contents of M_5 mulberry as influenced by integrated application of bio-inoculants, FYM and inorganic fertilizers under rain-fed condition.

Treatments**	Moisture (%)				Chlorophyll (mg/g)		
	Crop I		Crop II				
	45DAP***	60DAP	45DAP	60DAP	Chl.'a'	Chl.'b'	Total chl.
T_1	71.89	70.90	71.50	68.90	1.45	0.69	2.14
T_2	71.00	69.96	70.00	67.84	1.42	0.64	2.06
T_3	69.85	69.15	67.00	66.95	1.36	0.60	1.96
T_4	71.08	70.13	70.75	68.13	1.44	0.68	2.13
T_5	69.64	69.25	69.00	67.39	1.41	0.63	2.04
T_6	67.99	67.09	68.00	66.26	1.36	0.63	1.99
T_7	69.52	68.61	66.56	66.05	1.30	0.58	1.87
T_8	65.56	64.92	62.56	62.82	1.24	0.48	1.71
F-test	*	*	*	*	*	*	*
SEm±	0.32	0.47	0.571	1.328	0.016	0.010	0.020
$LSD_{5\%}$	0.98	1.44	1.731	4.029	0.048	0.031	0.062
CV (%)	0.81	1.19	1.450	3.444	1.984	2.871	1.768

*Significant at 5% level of significance; ***DAP-Days after pruning, **T_1- Standard check (RNPK + RFYM), T_2- 75% NP through IF and 25% through *A. awamori* and *Azotobacter* sp. + RFYM + RK T_3 – 50% NP through IF and 50% NP through *A. awamori* and *Azotobacter* sp. + RFYM + RK, T_4 - T_2 + *T. harzianum,* T_5 – T_3 + *T. harzianum,* T_6 – RNPK from FYM, T_7 – RNPK only from IF, T_8 –control.

70.13%; 70.75 and 68.13%) and T_2 (71.00 and 69.96%; 70.00 and 67.84%) consistently. Minimum moisture content (65.56 and 64.92%; 62.56 and 62.82%) was registered from T_8. Increased leaf moisture percentage may be due to the greater ability of the combined usage of the bio-inoculants, FYM and IF to support better plant performance by fixing more nitrogen and making available the bound phosphorous thereby improving water absorbing capacity of the roots which in turn has been translocated to the leaf. Better moisture percentage of mulberry leaf as a result of bio-inoculants application to mulberry plant was reported by Patil et al. (2001).

Chlorophyll content (mg/g)

Maximum chlorophyll (a), (b) and total chlorophyll content of 1.45, 0.69 and 2.14 mg/g, respectively were recorded from leaves of mulberry plants that received T_1 which was followed and on par with T_4 (1.44, 0.68 and 2.13 mg/g) whereas minimum content of all the chlorophyll were registered from T_8 (1.24, 0.48 and 1.71 mg/g) after 60 days of pruning (Table 3). The positive influence of bio-inoculants, FYM and IF on the chlorophyll content was in conformity with the findings of Sunil (2005), who observed that, higher chlorophyll content in mulberry leaves was due to addition of organic manures, biofertilizers and inorganic fertilizer. The findings of Singhal et al. (2000), who also reported that application of nitrogen helped in harnessing more solar energy through chlorophyll synthesis corroborates the present findings. Shivakumar et al. (2000) revealed that the organic manure (FYM) supplemented with varied levels of inorganic fertilizers caused increment in chlorophyll content of leaves in mulberry.

Crude protein and total soluble protein content (mg/g)

The crude protein and total soluble protein content were significantly ($P<0.05$) more in T_1 (18.21 and 8.82 mg/g, respectively) 60 days after pruning (Table 4). The next best treatment was T_4 (17.71 and 8.47 mg/g). The lowest of the same were observed in T_8 (15.61 and 5.23 mg/g) (Tables 3 and 4). Katiyar et al. (1995) and Umesh (1999) reported similar leaf chemical constituent in mulberry when biofertilizers were inoculated with reduced chemical fertilizers. Curtailment of 50% N application without affecting mulberry leaf quality by supplementing mulberry through 20 kg of *Azotobacter* has been reported by Das et al. (1994).

Total soluble sugar (mg/g)

Total soluble sugar was significantly ($P<0.05$) different among the different treatments (Table 4). The highest total soluble sugar content was found in the plot that received treatment T_1 (7.95 mg/g), which was statistically on par with T_4 (7.72 mg/g). The lowest total soluble sugar content of 5.32 mg/g was recorded in T_8. Similar findings were reported earlier by many scientists, notably (Shankar et al., 2000; Das et al., 1994) stated quality variables of mulberry were not adversely affected when

Table 4. Total soluble protein, total soluble sugar and NPK contents of M_5 mulberry as influenced by integrated application of bio-inoculants, FYM and inorganic fertilizers under rain-fed conditions

Treatments**	Crude protein (mg/g)	Total soluble protein (mg/g)	Total soluble sugar (mg/g)	N (%)	P (%)	K (%)
T_1	18.21	8.82	7.95	2.98	1.72	1.33
T_2	17.23	8.09	7.11	2.86	1.60	1.31
T_3	16.77	6.69	6.36	2.68	1.40	1.30
T_4	17.71	8.47	7.72	2.90	1.68	1.33
T_5	17.12	7.98	7.14	2.74	1.52	1.30
T_6	16.61	6.99	6.85	2.66	1.47	1.30
T_7	16.25	5.79	5.60	2.67	1.35	1.22
T_8	15.61	5.23	5.32	2.62	0.95	0.91
F-test	*	*	*	*	*	*
SEm±	0.150	0.464	0.155	0.038	0.069	0.025
$LSD_{5\%}$	0.456	1.408	0.471	0.114	0.209	0.075
CV (%)	1.536	11.076	3.978	2.364	8.178	3.426

*Significant at 5 % level of significance; **T_1- Standard check (RNPK + RFYM), T_2- 75% NP through IF and 25% through *A. awamori* and *Azotobacter* sp. + RFYM + RK T_3 – 50% NP through IF and 50% NP through *A. awamori* and *Azotobacter* sp. + RFYM + RK, T_4 - T_2 + *T. harzianum,* T_5 – T_3 + *T. harzianum,* T_6 – RNPK from FYM, T_7 – RNPK only from IF, T_8 – control.

biofertilizers were used with reduced dosages of chemical fertilizers. Further, Mamatha and Bagyaraj (1999) stated the use of vesicular-arbuscular mycorrhizal (VAM) fungus, *G. fasciculatum* and mycorrhiza helper bacteria, *B. coagulans* in mulberry can reduce the use of IF by 50% without affecting both the leaf yield and quality variables.

Nitrogen-phosphorus-potassium (NPK) percentage of mulberry leaf

Statistically significant (P<0.05) differences with respect to the NPK content of M_5 mulberry leaf was recorded among the different treatments (Table 4). Maximum leaf NPK of 2.98, 1.72 and 1.33%, respectively were recorded from T_1, which was on par with T_4 (2.90, 1.68 and 1.33%). On the contrary, T_8 had the lowest leaf NPK of 2.62, 0.95 and 0.91%. The improvement in the leaf NPK percentage in mulberry leaves may be due to better fixation of ‘N’ by *Azotobacter*; mobilization and solubilization of P by *A. awamori* and *T. harzianum* in turn leading to better availability and absorption of the nutrients to plant system and its translocation to leaves. The improvement in K content of the leaf may be due to adequate availability of potassium through FYM and IF, particularly the recommended K in all the treatments except the control, and the synergistic effect of the inputs utilized.

The present results are in accordance with the findings of Das et al. (1994), who reported higher total nitrogen content in mulberry leaf when mulberry plant is inoculated with *Azotobacter*. Further, Krishnababu (1994) reported that there is an increase in NPK content of mulberry plant applied with integrated chemical and bio-fertilizers. Moreover, Sukumar et al. (2001) concluded better efficiency in N and P uptake by mulberry when inoculated with *B. megaterium* and *A. chroococum* along with the recommended dosage of fertilizers. Increased uptake of NPK by mulberry as a result of integration of biofertilizers with different organic manures and inorganic fertilizers has been reported by Murali et al. (2006).

Conclusion

In conclusion, 25% application of inorganic fertilizers (NP) to M_5 mulberry under rain-fed conditions can be reduced without affecting mulberry fresh leaf yield and quality variables by supplementing mulberry garden with integrated use of inorganic fertilizers, farm yard manure and bio-inoculants.

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