Effect of Bio-inoculants Applied to M_5 Mulberry Under Rain-fed Condition on Growth and Cocoon Traits Performance of Silkworm, *Bombyxmori* L.

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ABSTRACT

Nutrition plays a pivotal role in sericulture. It improves the growth, development, health, feed consumption and conversion of silkworm thereby improving the commercial traits. Silkworm, *Bombyx Mori* L., is a monophagous insect that drives almost all required nutrients for its growth and development from mulberry leaf. Application of the required nutrient in the required amount to mulberry plant is, therefore, very essential for the successful silkworm growth and cocoon production. The present investigation was carried out at the department of sericulture, GKVK, UAS, Bangalore, India in 2007 with an objective to determine the effect of three bio-inoculants application to M_5 mulberry plant on silkworm (PM x CSR_2) growth, development and coocoon traits. The feeding experiment was laid-out in Completely Randomized Design (CRD)with eight treatments replicated three times. The result revealed that the larval growth variables and cocoon traits were significantly better when developing worms were fed on mulberry leaves raised by applying the recommended doses of nutrients. However, the recommended rate of chemical fertilizers application was found either the same or on par with 25 per cent reduced Nitrogen and Phospherous (NP) application when supplemented with bio-inoculants (*Azotobacter*sp., *Aspergillus awamori* and *Trichodermaharzianum*). This indicate that 25 per cent reduction of NP application does not adversely affect larval growth and cocoon traits when supplemented with the above three microbes.

Key words: Bio-inoculants, Cocoon traits, Growth variables, M_5 mulberry, Rain-fed.

1. INTRODUCTION

Mulberry, *Morus alba* L., is a sole food plant for silkworm, *Bombyx mori* L. Good quality leaf production in mulberry is highly dependent on supply of various inputs especially nitrogen and phosphorus fertilizers (Nasreen et al., 1999). Singhal et al. (1999) opined that quality of mulberry leaf fed to silkworms is the most important factor that influences successful cocoon production by mulberry silkworm. Use of inorganic fertilizers has increased mulberry yield leading to better silkworm productivity (Bose and Majumder, 1999). But it has been realized that, in the past, this was achieved at the expense of soil health. Moreover, some portions of the nutrients applied to the soil are still bound to be unused as they are not available to the plant. This increased the cost of mulberry leaf production. In addition, continuous supplementation of chemical fertilizers to mulberry is hazardous to environment. Further, with the application of inorganic fertilizers alone, particularly in unbalanced manner, problems such as diminishing soil productivity and multiple nutrient deficiencies appears (Krishna and Bongale, 2001). The deficiency of essential nutrients in the soil has been found to cause nutritional, anatomical and histological disorders in mulberry (Shankar, 1990). In effect, complementing inorganic nutrients with bio-inoculants and Farm Yard Manure (FYM) is a cost effective means to achieve the desired ends by overcoming the problems of soil degradation and poor leaf production in mulberry sericulture.

Bio-inoculants are carrier based preparations containing beneficial micro-organisms in a viable form intended for soil or seed application and designed to improve the soil fertility and help the plant growth by increasing their number and biological activity in the rhizosphere (Rao, 1998). Many nitrogen fixing microorganisms are known to secrete plant growth promoting substances like Indole acetic acid (IAA), Gibberellic acid (GA) and Vitamins besides fixation of atmospheric nitrogen (Fallick et al., 1989; El-ruan et al., 1973). Usage of N fixers and P solubilizers is known to improve the quality of mulberry leaf besides saving the application of 'N' and 'P' chemical fertilizers to certain extent under irrigated mulberry (Katiyar et al., 1996). According to Goswami (1997) nitrogen fixing bio-inoculants can add 20 to 400 kg N per hectare where as P solubilizing bio-inoculants can solubilize 20-30 kg P_2O_5 per hectare under optimum conditions depending up on the crop and agro-ecological conditions. Further he stated, these crop benefiting bio-inoculants are known to secrete plant growth promoting substances and vitamins needed for improved crop growth and are also known to produce certain antibiotic substances, which help in suppressing the incidence of several root borne pathogens as well as foliar diseases.

Umesh (1999) reported that conjunctive use of biofertilizers (*Azotobacter*) at the rate of 10 kg/ha with 50 per cent recommended N has a positive effect on cocoon production and silk parameters. Venkataramana et al. (2001) studied the integrated effect of triacontanol (a naturally occurring plant growth promoting substance) and *Azotobacter* bio-fertilizer (800 g mixed with 40 kg of FYM) on silkworm cocoon yield. Accordingly, 7.3 kg more cocoons per 100 Disease Free Laying (DFL) with a profit of Rs.10 (0.2 US$) per kg of cocoon was obtained over the control. Integrated organic manures packages of practices which included *Azotobacter*, VAM, seriphos, vermicompost and green manure application to V-1 mulberry and in turn feeding the leaves to CSR_2 and CSR_4 bivoltine silkworm breeds resulted to improved rearing and grainage parameters of silkworms similar to the standard fertilizers application (Jagadeesh et al., 2005).

According to Sannappa et al. (2005) and Raje Gowda (1996) application of organic fertilizers to mulberry had a significant influence on cocoon yield, shell ratio, silk productivity and single cocoon filament length.

However, information on the effect of bio-inoculants on rain-fed mulberry growth and its subsequent influence on silkworm growth and cocoon production are scanty. Thus it was deemed necessary to investigate the effect of bio-inoculants application to rain-fed M_5 mulberry variety on growth, development and cocoon traits of mulberry silkworm (PM x CSR_2),*B. mori* L.

2. MATERIAL AND METHODS

Influence of bio-inoculants (*Azotobacter* sp. @ 20kg/ha/year, *Aspergillusawamori* @ 25 kg/ha/year and *Trichodermaharzianum* @ 20 kg/ha/year), FYM @ 12 MT/ha/year and inorganic fertilizers (IF) (N, P and K @100:50:50 kg/ha/year and reduced doses plus the above bio-inoculants) applied to M_5 mulberry on growth, development and cocoon traits of silkworm *B. mori* (PM x CSR_2) was studied at Gandhi Krishi Vignana Kendra (GKVK), University of Agricultural Sciences (UAS), Bangalore (B) in the Department of Sericulture premises. The bio-inoculants were obtained from bio-fertilizers scheme, Department of Agricultural Microbiology, UAS (B), GKVK. The treatment details are indicated in table 1.

Table 1. Details of the treatments used for the experiment.

Notation	***Treatment details***
T_1	Standard check: R. NPK* + R. FYM**
T_2	75% NP through chemical fertilizers + 25% NP through *Aspergillus awamori* and *Azotobacter* sp. + R. FYM + R. K***
T_3	50% NP through chemical fertilizers + 50% NP through *A. awamori* and *Azotobacter* sp. + R. FYM + R. K
T_4	75% NP through chemical fertilizers + 25% NP through *A. awamori* , *Azotobacter* sp. and *Trichoderma harzianum* + R. FYM + R. K
T_5	50% NP through chemical fertilizers + 50% NP through *A. awamori* , *Azotobacter* sp. and *T. harzianum* + R. FYM + R. K
T_6	R. NPK only through FYM
T_7	R. NPK only through chemical fertilizers
T_8	Control: No application of any fertilizer

<u>Key</u>: *R. NPK: Recommended nitrogen, phosphorus and potash (100:50:50kg/ha/yr).
**R. FYM: Recommended farm yard manure (12 MT/ha/yr).
***R. K: Recommended Potash (50kg/ha/yr).

Mulberry leaves raised by application of the above treatments were fed to silkworm, *B. mori*, larvae four times a day (6:30 A.M., 11:30 A.M., 3:30 P.M. and 8:30 P.M.) for two rearings (crop I & II). The larvae were provided with chopped leaf during young stage (I to III instars) and whole leaf during late-age (IV & V instars) of their growth. Bed cleaning was done according to recommended schedule (once during I-instar before first moult; twice during II-instar after first & before second moults; thrice during III-instar after second moult, middle of third instar & before 3rd moult; and every day during IV & V instars) (Dandin et al., 2003). As the larvae grew, the required spacing was appropriately provided. In order to control the incidence of diseases, vijetha (recommended bed disinfectant) was dusted at the rate of 5.5 kg/100 disease free layings (dfls) after bed cleaning and 30 minutes before feeding resumes as per the recommendation given by Dandin et al. (2003). One dfl was maintained for one treatment per replication; three replications were used for each treatment. Observations on silkworm growth variables and cocoon traits were recorded following standard procedures as follows:-

2.1. Larval weight (g/10)

Ten larvae were randomly selected from each treatment replication wise at the end of each instar and their weight was recorded using electronic balance. The average larval weight was recorded. Besides, on the fifth day of fifth instar, average weight of ten larvae from each treatment replication wise was also computed and recorded.

2.2. Larval duration

Larval duration starting from hatching of the eggs up to 90 per cent of worms attain spinning was recorded as total larval duration. Each instars' larval duration was also recorded.

2.3. Effective rate of rearing (ERR) (%)

ERR was calculated using the formula:

ERR (%) = (No. cocoons harvested/ Number of larvae brushed) x 100

2.4. Mean cocoon weight (g/10)

After five days of spinning, ten cocoons were harvested from each replication treatment wise and mean cocoon weight was recorded using electronic balance.

2.5. Shell weight (g/10)

Ten cocoons which were used to obtain cocoon weight were cut open and pupae along with the exuviae were removed. Then shell weights was recorded and mean shell weight was computed.

2.6. Shell ratio

The shell ratio was calculated by using the formula:

Shell ratio (%) = (Weight of cocoon shell/ Weight of whole cocoon) x 100

2.7. Silk filament length (m)

After sorting, the cocoons were stifled by using hot air oven at 90^{o}C for 20 min. The stifled cocoons were deflossed by hand and used for reeling to record post cocoon parameters. Three cocoons from each replication were taken and reeled individually on a single cocoon reeler (epprouvette). The total number of revolution was recorded and converted in to meter by using the formula:

L = R x 1.125

where, 'L' is total length of the filament in meter per cocoon

'R' is number of revolutions recorded by epprouvette

'1.125' is circumference of epprouvette

The average filament length of three cocoons of each replication was calculated and recorded.

2.8. Denier

Denier was computed using the formula:

Denier = (Silk filament weight (g)/ Silk filament length (m)) x 9000 (constant value)

2.9. Silk Productivity (SP)

SP was computed using the formula:

SP (cg/day) = Shell weight (centigram)/ Fifth instar larval duration (days)

All data recorded were analyzed statistically for the test of significance using Fisher's method of "Analysis of variance". The level of significance of "F- test" was at five per cent (Cochran and Cox, 2000).

3. RESULTS

The study revealed that application of bio-inoculants, FYM and inorganic fertilizers in combination, to the soil and in turn feeding of mulberry leaves produced from such garden to silkworms (PM x CSR_2), exhibited significantly different results on growth (moulting and larval duration, feeding period, larval weight and Effective Rate of Rearing (ERR)) and other cocoon traits (cocoon weight, shell weight, shell ratio, filament length, denier and silk productivity) (α = 5%). Minimum of 90.19 and 91.34 h (moulting duration), 9.14 and 9.07 days (fifth instar larval duration), 26.52 and 25.61days (total larval duration) and 22.71 and 21.81 days (feeding period) was recorded from the standard check in the first and second crops, respectively which was found on par with T_4 (90.71 and 91.73 h; 9.17 and 9.50 days; 26.55 and 26.19 days; and 22.76 and 22.37 days, respectively) (Table 2).

Table 2. Influence of feeding mulberry leaves raised by applying bio-inoculants on rearing parameters of *B. mori* (PM x CSR_2).

Treatments	***Total moulting duration (h)***		***Fifth instar larval duration (days)***		***Total larval duration (days)***		***Feeding period (days)***		***ERR (%)***	
	Crop I	***Crop II***	***Crop I***	***Crop II***	***Crop I***	***Crop II***	***Crop I***	***Crop II***	***Crop I***	***Crop II***
T_1	90.19	91.34	9.14	9.07	26.52	25.61	22.71	21.81	92.40	98.00
T_2	91.18	92.01	9.20	9.54	26.68	26.26	22.88	22.43	90.29	96.98
T_3	92.02	92.93	9.22	9.71	26.77	26.77	22.95	22.90	87.42	96.58
T_4	90.71	91.73	9.17	9.50	26.55	26.19	22.76	22.37	91.30	97.71
T_5	91.74	92.74	9.21	9.58	26.69	26.32	22.91	22.46	88.09	96.81
T_6	93.11	94.18	9.25	9.79	26.84	26.84	22.96	22.91	86.51	96.12
T_7	92.84	95.84	9.24	9.83	26.82	27.08	22.95	23.09	86.61	95.95
T_8	95.02	96.32	9.26	10.71	28.14	28.27	24.51	24.26	81.08	83.14
F-test	*	*	*	*	*	*	*	*	*	*
SEm±	0.23	0.95	0.024	0.018	0.042	0.134	0.136	0.134	0.462	0.331
LSD @ $_{5\%}$	0.70	2.89	0.074	0.053	0.129	0.405	0.413	0.407	1.401	1.004
CV (%)	0.44	1.77	0.458	0.311	0.274	0.868	1.022	1.020	0.909	0.602

Key: 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 & 50% NP through *A. awamori* and *Azotobacter* sp. + RFYM+RK
T_4 - T_2 + *Trichodermaharzianum,*
T_5 - T_3 + *Trichodermaharzianum,*
T_6 - RNPK from FYM,
T_7 - RNPK only from IF,
T_8 - control.

Table 3. Influence of feedingM_5 mulberry raised through application of bio-inoculants on larval weight (g/10) of silkworm, B. mori (PM x CSR_2)

Treatments	***Instars***										***Mature larval weight***	
	I		***II***		***III***		***IV***		***V (5th day)***			
	Crop I	***Crop II***	***Crop I***	***Crop II***	***Crop I***	***Crop II***	***Crop I***	***Crop II***	***Crop I***	***Crop II***	***Crop I***	***Crop II***
T_1	0.0163	0.017	0.209	0.21	0.9700	1.17	5.0733	5.69	21.4000	31.69	22.83	27.87
T_2	0.0157	0.016	0.206	0.21	0.9593	1.14	4.7233	5.34	20.2867	29.89	20.70	27.51
T_3	0.0147	0.015	0.203	0.20	0.9430	1.10	4.3467	5.00	19.3300	28.68	20.30	26.59
T_4	0.0163	0.017	0.208	0.21	0.9653	1.15	4.8033	5.36	20.4700	31.51	21.71	27.83
T_5	0.0147	0.015	0.205	0.21	0.9527	1.13	4.5100	5.28	19.4400	29.30	20.54	26.62
T_6	0.0143	0.014	0.202	0.19	0.9340	1.06	4.1167	4.88	17.8400	26.87	19.29	25.76
T_7	0.0147	0.014	0.202	0.19	0.9347	1.05	4.2400	4.88	18.9800	26.55	19.50	25.69
T_8	0.0143	0.014	0.198	0.18	0.8777	1.01	3.9333	4.31	15.8200	23.44	15.58	22.18
F-test	*	*	*	*	*	*	*	*	*	*	*	*
SEm±	0.0002	0.0007	0.0007	0.008	0.0131	0.039	0.0378	0.247	0.2639	0.545	0.506	0.607

LSD @$_{5\%}$	0.0007	0.0020	0.0021	0.024	0.0396	0.117	0.1147	0.749	0.8004	1.653	1.536	1.842
CV (%)	2.5494	7.398	0.5837	6.673	2.4029	6.068	1.4656	8.395	2.3809	3.313	4.373	4.006

On the other hand, maximum of 92.40 and 98.00 per cent (ERR) (Table 1), 0.0163 and 0.017 g/10 (I instar larval weight), 0.209 and 0.210 g/10 (II instar larval weight), 0.970 and 1.170 g/10 (III instar larval weight), 5.073 and 5.690 g/10 (IV instar larval weight), 21.40 and 31.69 g/10 (V instar 5th day larval weight), 22.83 and 27.87 g/10 (mature larval weight) were observed in the standard check from the two rearings (crop I & II), respectively. This treatment was either the same or on par with T_4 (Table 3).

Table 4. Influence of feedingM_5 mulberry cultivated by the application of bio-inoculants on silkworm, B. mori (PM x CSR_2) cocoon variables.

Treatments	***Cocoon yield (g/dfl)***		***Single cocoon weight (g)***		***Single shell weight (g)***		***Cocoon Shell Ratio (%)***	
	Crop I	***Crop II***	***Crop I***	***Crop II***	***Crop I***	***Crop II***	***Crop I***	***Crop II***
T_1	640.88	691.11	1.57	1.67	0.30	0.37	19.11	22.72
T_2	577.33	626.55	1.52	1.59	0.27	0.35	17.76	22.06
T_3	520.89	569.12	1.42	1.51	0.24	0.33	16.90	21.68
T_4	638.32	684.91	1.57	1.62	0.30	0.36	19.11	22.43
T_5	548.56	570.83	1.46	1.57	0.26	0.34	17.81	21.66
T_6	511.16	563.35	1.36	1.48	0.23	0.30	16.91	20.45
T_7	514.03	545.21	1.41	1.43	0.24	0.29	17.02	20.28
T_8	437.10	469.05	1.29	1.39	0.20	0.25	15.50	17.95
F-test	*	*	*	*	*	*	*	*
SEm±	6.97	4.798	0.013	0.0096	0.008	0.0058	0.495	0.331
LSD @ $_{5\%}$	21.15	14.555	0.038	0.0291	0.025	0.0175	1.502	1.004
CV (%)	2.20	1.409	1.49	1.0852	5.647	3.0762	4.917	2.709

Table 5. Post cocoon parameters of silkworm, *B. mori* as influenced by feeding of M_5mulberry raised through the application of bio-inoculants.

Treatments	***Single filament length (m)***		***Denier***		***Silk productivity (cg/day)***	
	Crop I	***Crop II***	***Crop I***	***Crop II***	***Crop I***	***Crop II***
T_1	870.51	924.03	2.17	2.14	3.28	4.08
T_2	839.16	911.13	2.36	2.47	2.93	3.67
T_3	783.83	859.05	2.76	2.72	2.60	3.40
T_4	868.68	922.53	2.28	2.30	3.27	3.79
T_5	807.81	900.61	2.56	2.50	2.82	3.55
T_6	752.48	857.25	2.87	2.83	2.49	3.06
T_7	780.14	815.23	2.88	3.20	2.60	2.95
T_8	711.90	735.55	3.29	3.67	2.16	2.33
F-test	*	*	*	*	*	*

SEm±	6.88	12.33	0.06	0.08	0.09	0.06
LSD @ $_{5\%}$	20.87	37.39	0.174	0.238	0.277	0.175
CV (%)	1.49	2.47	3.780	4.994	5.734	2.982

The direct influence of bio-inoculants application along with organic manures and chemical fertilizers has significant influence on cocoon and post cocoon variables of PM x CSR_2 silkworm breed both in the first and second crops. Maximum cocoon yield (640.88 & 691.11 g/dfl), single cocoon weight (1.57 & 1.67 g), shell weight (0.30 & 0.37 g), cocoon shell ratio (19.11 & %) (Table 4), single filament length (870.51 & 924.03 m)and silk productivity (3.28 & 4.08 cg/day)(Table 4) were registered in the batch of worms reared on mulberry leaves from standard check, which was statistically non-significantly different from25 per cent reduced NP and bio-inoculants supplemented treatment (T_4). The lowest of all the above growth and cocoon traits were obtained from control batch (Table 1, 2, 3 & 4). On the other hand, minimum filament denier of 2.17 and 2.14 was registered in the batch of worms reared by feeding of mulberry leaves raised by the application of RNPK and RFYM (T_1), which was on par with T_4 (2.28 and 2.30). Maximum filament denier of 3.29 and 3.67 was recorded from the control (Table 5).

4. DISCUSSION

The present study revealed that application of bio-inoculants, FYM and IF in combination, to the soil of mulberry garden and in turn feeding of mulberry produced from such garden to silkworm, *B. mori* (PM x CSR_2 breed) exhibited significant results on growth (moulting duration, larval duration, feeding period, larval weight and ERR), cocoon (cocoon yield & weight, shell weight, &cocoon shell ratio) and post cocoon (single filament length, filament denier & silk productivity) variables. However, minimum moulting duration, fifth instar larval duration, total larval duration, feeding period, and denier was recorded from the standard check in the first and second crops, respectively which was found on par with 25 per cent reduced NP application supplemented with bio-inoculants. On the other hand, maximum ERR, larval weights, cocoon yield, single cocoon weight, cocoon shell ratio, single filament length and silk productivity were registered from the standard check fed batches of silkworms both in crop I and II, respectively. This treatment was either the same or on par with T_4 (bio-inoculants at 20 kg/ha of *Azotobacter* + 25 kg/ha of *A. awamori* + 20 kg/ha of *T. harzianum* + 75 per cent recommended NP each through chemical fertilizer with full recommended dose of FYM and K). These results are in close conformity with the findings of Umesh (1999) who opined the conjunctive use of

Azotobacter and half the recommended dose of N had positive influence on cocoon and silk productivity in mulberry silkworm under irrigated condition. An increase of 7.3 kg/100dfl in cocoon yield was reported by Venkataramana et al. (2001) when triacontanol and *Azotobacter* were integrated in mulberry cultivation. Further, integrated organic manures packages of practices which included *Azotobacter*, VAM, seriphos, vermicompost and green manure in V-1 mulberry cultivation didn't negatively affected the rearing and grainage parameters of CSR_2 and CSR_4 bivoltine silkworm breeds (Jagadeesh et al., 2005).

According to Sannappa et al. (2005) application of FYM @ 20 MT/ha/yr + *Azotobacter* @ 20kg/ha/yr + NPK @ 300: 120: 120kg/ha/yr to mulberry resulted in higher cocoon yield (291.6g/200 larvae), shell ratio (19.96%), silk productivity (3.503 cg/day) and cocoon filament length (666.0 m). Raje Gowda (1996) reported that, mean cocoon and shell weight, shell percentage and filament length as well as other economic parameters of silkworm, *B. mori* are significantly better when inorganic fertilizers are integrated with bio-fertilizers (*A. awamori* and *Aspergillus* sp.) than inorganic fertilizers alone.

Favorably the beneficial effect of bio-inoculants can be envisaged from the present findings, under rain-fed condition, which clearly demonstrate the realization of silkworm growth, development and cocoon improvement on par with the standard check. Thus supplementation of bio-inoculants along with FYM and IF to mulberry can result to the required ultimate cocoon productivity without significant sacrifices. Therefore, NP can be reduced to the tune of 25 per cent without affecting the silkworm, *B. mori*, growth, development and cocoon productivity. This may be due to the fact that, the inoculation of bio-inoculants had resulted in availing the macro-and micronutrients to mulberry root thereby maintaining the quality of mulberry leaves which provided all the required nutrients to the larvae, hence better growth and cocoon productivity.

5. CONCLUSION

Silkworm, *B. mori*, larval growth and cocoon traits, both quantitatively and qualitatively, are not significantly affected when chemical fertilizers (Nitrogen and Phospherous (NP)) application to mulberry plant is reduced by 25 per cent when the same is supplemented with bio-inoculants (*Azotobacter*sp., *Aspergillusawamori* and *Trichodermaharzianum* at 20, 25 and 20 kg/ha/year, respectively). Thus in view of the current increase in price of chemical fertilizers and environmental concerns on the negative impact of such chemical fertilizers, sericulturist can use these microbials to maintain the productivity of mulberry garden thereby silk cocoon.

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