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EFFECT OF CO-INOCULATION OF MICROBIAL CONSORTIUM ON MULBERRY LEAF YIELD AND SILKWORM COCOON PRODUCTION

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Abstract: Significant beneficial effect was recorded on mulberry leaf yield, growth and yield parameters of bivoltine silkworm double hybrid reared on two mulberry varieties viz., Victory-1 and MR-2 due to inoculation with microbial consortium containing nitrogen fixing bacteria Azotobacter and Azospirillum, phosphate solubilizing & mobilizing microorganism besides EMs even after curtailing nitrogenous and phosphatic chemical fertilizers to the extent of 25-75% of recommended dose. The rearing performance with the variety V-1 exhibited highest matured larval weight in the treatment T10 and this was significantly superior over T1. Highest effective rate of rearing (ERR), higher cocoon and shell weight were recorded in the treatment T6, T10 and T2. The treatment T7 recorded the highest shell ratio (24.35%). The rearing performance with the variety MR-2 exhibited highest matured larval weight, effective rate of rearing by number and weight in the treatment T10. The single cocoon weight and single shell weight were found to be the highest in the treatment T6 followed by T10, whereas higher shell ratio was observed in the treatment T8. Significant improvement in various cocoon characters of mulberry silkworm was observed as a result of feeding leaf from microorganisms inoculated plots over the plot receiving full dose of inorganic fertilizers and no inoculation.
Keywords: Rearing, effective microorganisms, microbial consortium, effective rate of rearing, cocoon weight, shell ratio.

Introduction

The mulberry leaf, since is utilized for silkworm rearing, the quality of leaf ultimately influences the productivity as also the quality of silkworm cocoons. The continuous production of mulberry leaves for a long time without proper care of the soil results in gradual reduction in leaf yield and quality which can be overcome through proper soil fertility management. Mulberry leaf production in tropics is largely dependent on the application of chemical fertilizers which are mostly not available to plants due to leaching and fixation in the soil (Jayaraj *et al*., 2006). Hence, it is highly essential to search for the alternative and cheaper renewable source of fertilizers of biological origin for sustainable sericulture. Of late, a liquid concentrate containing a consortium of such beneficial microbes

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have become commercially available called as "EM" i.e., Effective microorganisms which is a consortium of beneficial microbes and acts as soil conditioner as well as microbial inoculants produced by Maple Orgtech (India) Ltd, EM Research organization, Japan and commercially available as Maple EM-1 was procured from local market. It is produced from cultures of over 80 strains of beneficial microorganisms, which are collected from the natural environment of India. Over 90 countries are using this technology successfully today. EM mainly consists of Lactic acid bacteria $1x10^5$ (*Lactobacillus casei)*, Photosynthetic bacteria ($1x10^5$), *Rhodopseudomonas palustris* ($1x10^1$), Yeast $1x10^2$ (S*accharomyces cerevisiae*) and filamentous fungi. The EM acts as soil conditioner as well as microbial inoculant helpful to maintain sustainable farming practices. Hence the present investigation was carried out to study the effect of EMs in combination with biofertilizers in mulberry with the objectives of studying effect of EMs and biofertilizers on silkworm rearing, cocoon production and quality.

Materials and methods

The experimental works were carried out in farmer's field at Gobichettipalayam, Erode district, Tamil Nadu during 2013- 2015. The experiment was laid out in well established mulberry garden with two elite mulberry varieties (Victory-1 and MR2) in Randomized Block design (RBD) comprising of 12 treatments inclusive of control and three replications. The treatments consisted of co-inoculation with *Azospirillum* ($1x10^8$), *Azotobacter* ($1x10^8$), Phosphate solubilizing bacteria ($1x10^8$), Vesicular Arbuscular Mycorrhiza (VAM) @ 25 spores/g of dry soil, and a commercially available consortium of beneficial microorganisms called as EM. The treatment details are as follows.

T0: Absolute control / No inoculation

T1: Full dose of fertilizer (300: 120: 120 Kg NPK/ha/yr) as standard control

T2: *Azospirillum* + (75% N +full dose of P&K /ha/yr) +EM

T3: *Azotobacter* + (50% N+ full dose of P&K/ha/yr) +EM

T4: Phosphate solubilizing bacteria + (75% P + full dose of N&K/ha/yr) +EM

T5: VAM+ (50% P + full dose of N&K/ha/yr) +EM

T6: *Azospirillum* + PSB + (75% N&P + full dose of K/ha/yr) +EM

T7: *Azospirillum* + VAM + (75% N + 50% P + Full dose of K/ha/yr) +EM

T8: *Azotobacter* +PSB + (50% N +75% P + full dose of K /ha/yr) +EM

T9: *Azotobacter* + VAM+ (50% N +50% P + full dose of K /ha/yr) +EM

T10: *Azospirillum* +PSB+VAM + (75% N+25% P + full dose of K /ha/yr) +EM

T11: *Azotobacter* +PSB+VAM + (50% N+25% P + full dose of K/ha/yr) +EM

The leaf yield was assessed by harvesting and weighing the leaves from all the plants available in each net plot of size 405 sq.ft. The total weight of leaf from net plot was then converted to yield per hectare and expressed in kilograms. It was recorded for two consecutive years. The silkworm rearings were conducted following the package of practices described by Krishnaswamy (1978) and modified by Dandin *et al.* (2005). A total of 5 rearings were conducted with bivoltine silkworm (*Bombyx mori* L.) double hybrid (CSR6xCSR26) x (CSR2xCSR27) for both the mulberry varieties. Feeding of the silkworms with the leaves obtained from the different treatment plots of the field experiment were commenced from first instar onwards. One disease free laying (DFL) of silkworm hybrid mentioned above was brushed for each treatment and replicated thrice. After third moult, 200 numbers of larvae were counted and retained in separate rearing trays for rearing up to spinning and cocoon formation. Data on larval weight, effective rate of rearing (ERR) per 10,000 larvae by number and weight, single cocoon weight, single shell weight and shell percentage were recorded as mentioned below. Finally, the mean of all the five trials on various rearing parameters mentioned above were calculated and analyzed statistically.

Larval weight

Ten matured larvae were selected randomly and weighed on fifth day of fifth instar and weight expressed in grams.

Effective rate of rearing (ERR) by number

The ERR by number is the survival percentage of the larvae. Larval survivability was worked out for total number of cocoons harvested out of 200 larvae kept after final counting. This was then converted to ERR by number on the basis of 10,000 larvae brushed and expressed as percentage.

Effective rate of rearing (ERR) by weight

The ERR by weight indicates the cocoon yield. The cocoons harvested from 200 larvae after completion of spinning were weighed. The yield was estimated for 10,000 larvae brushed and expressed as kg /10,000 larvae.

Single cocoon weight

After harvesting, ten cocoons from each treatment were randomly picked up and weighed individually and average weight was calculated and expressed as gram per cocoon.

Single shell weight The same cocoons which were collected for recording the cocoon weight were cut open and pupae removed. Then each empty shell was weighed individually and average weight was worked out and expressed in gram.

Shell Ratio (%)

After recording the shell weight and cocoon weight the shell percentage was determined by using the formula given below:

$$\text{Shell Ratio} = \frac{\text{Shell Weight}}{\text{Cocoon weight}} \times 100$$

Results and Discussion

First year

Significant differences among treatments were observed in variety V-1 recording the highest leaf yield of 10915 kg/ha/crop in the treatment T1 receiving full dose of recommended chemical fertilizers followed by T10 (10351 kg/ha/crop) due to the application of *Azospirillum*+PSB+VAM + (75% N+25% P+ full dose of K /ha/yr) +EM. This was significantly higher against all other treatments (Fig-1). The lowest leaf yield was recorded in T0 (7391 kg/ha/crop).

The highest leaf yield of (7047 kg/ha/crop) was recorded due to the application of *Azospirillum*+PSB+VAM + (75% N+25% P+ full dose of K /ha/yr) +EM (T10) in case of variety MR2. Next best leaf yield (6915 kg/ha/crop) was observed in the treatment T1 receiving full dose of chemical fertilizers which was statistically on par with the treatment T11 receiving *Azotobacter*+PSB+VAM + 50% N+25% P+ full dose of K/ha/yr +EM (Fig-1). The lowest leaf yield (5020 kg/ha/crop) was observed in T0.

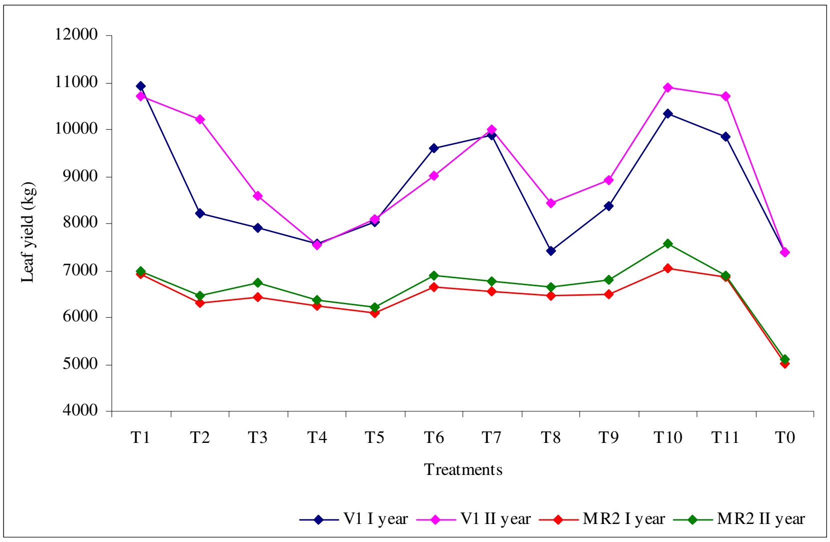

Fig.1. Effect of co inoculation of microbial consortium on mulberry leaf yield / ha / crop

Second year

During the second year, in the variety V1 highest leaf yield (10884 kg/ha/crop) was obtained in T10 receiving *Azospirillum*+PSB+VAM + (75% N+25% P+ full dose of K /ha/yr) +EM followed by T11 (10718 kg/ha/crop) receiving *Azotobacter*+PSB+VAM + (50% N+25% P+ full dose of K/ha/yr) +EM. However the later was on par with T1 (10713 kg/ha/crop) receiving full dose of chemical fertilizers (300: 120: 120 kg NPK/ha/yr) only. The lowest leaf yield (7387 kg/ha/crop) was observed in uninoculated control (T0). Similarly in variety MR2 the highest leaf yield (7569 kg/ha/crop) was recorded in T10 which was significantly superior over all other treatments (Fig-1)). However the differences in the leaf yield between the treatments T6 and T11 were not significant. The significant reduction in leaf yield (5106 kg/ha/crop) was recorded in uninoculated control (T0).

Rearing performance with variety V-1

Larval weight

The results revealed significant differences among all the treatments studied (Table 1). The larval weight recorded due to the treatment T10 (53.80g) was significantly superior over other treatments including T1 receiving full dose of chemical fertilizers (50.03 g). The differences in larval weight recorded due to the treatment T1, T2 and T8 were non significant. The minimum larval weight (40.10g) was observed in T0 the uninoculated control.

Effective rate of rearing (ERR) by number

The rearing results (Table 1) revealed highest ERR by number (9817) recorded due to the application of *Azospirillum*+PSB + (75% N&P + full dose of K/ha/yr) +EM (T6) followed by T10 (9633) and both of these were significantly superior over other treatments. The results obtained due to the treatments T2 (9500) and T7 (9483) were at par with each other. The lowest ERR by number (8583) was obtained in T0.

Table 1: Rearing parameters of silkworm influenced by feeding mulberry leaves harvested from different treatments of microbial consortium (V1 variety)

Treatment	Wt. of 10 matured larvae (g)	Effective rate of rearing /10000 larvae		Single cocoon wt.(g)	Single shell wt. (g)	Shell Ratio (%)
		By no.	By wt. (kg)			
T1	50.03	9150	18.27	1.950	0.445	22.84
T2	50.80	9500	19.97	2.101	0.492	23.41
T3	46.10	9117	18.08	1.992	0.454	22.87
T4	46.91	9050	16.97	1.871	0.425	22.72
T5	46.65	8950	17.44	1.953	0.455	23.36
T6	52.30	9817	22.85	2.372	0.565	23.82
T7	49.10	9483	19.20	2.013	0.491	24.35
T8	49.82	9233	18.25	1.940	0.451	23.23
T9	47.30	9200	20.26	2.010	0.476	23.66

T10	53.80	9633	22.11	2.295	0.521	22.77
T11	48.30	9017	18.18	1.872	0.420	22.44
T0	40.10	8583	15.84	1.800	0.382	21.29
SED±	0.62	35.828	0.230	0.022	0.003	0.216
CD @ 5%	1.28	74.303	0.477	0.045	0.006	0.447
CV%	1.56	0.476	1.486	1.310	0.852	1.146

Effective rate of rearing (ERR) by weight

The ERR by weight recorded due to T6 (22.85 kg) was significantly higher compared to all other treatments and this was followed by T10 (22.11 kg) and T9 (20.26 kg). Lowest value was recorded in T0 (15.84 kg). However the values of the ERR by weight recorded due to the treatments T9,T2,T1,T11 and T3 were statistically on par with each other (Table 1).

Single cocoon weight (g)

The data on single cocoon weight (Table 1) revealed significant differences among different treatments. It was highest due to the treatment T6 (2.372 g) followed by T10 (2.295 g) and T2 (2.101 g) which were significantly superior over all other treatments. The cocoon weight observed in T7 (2.013 g) was statistically at par with T9 (2.010 g) and T3 (1.992 g). The lowest cocoon weight (1.800 g) was found in uninoculated control (T0).

Single shell weight (g)

The highest single shell weight (0.565 g) was recorded due to treatment T6 which was at par with the treatment T10 (0.521 g) but differed significantly from all other treatments (Table 1). However the single shell weight recorded due to the treatment T2 was at par with T7. The lowest single shell weight (0.382 g) was observed in T0.

Shell Ratio

The data on shell ratio as influenced by certain EM and different levels of N and P presented in Table - 1 was observed to differ significantly. The treatment T7 recorded the highest SR% (24.35%) which was significantly superior over rest of the treatments. The SR % recorded due to the treatment T6 (23.82%) was statistically at par with T9, T2, T5 and T8. The lowest SR% (21.29%) was found in T0.

Rearing performance with variety MR2

Larval weight

The data on larval weight revealed significant differences among the treatments. The treatment combination T10 exhibited highest matured larval weight (51.32 g) which was significantly superior over other treatment (Table 2). The larval weight (49.05 g) observed in

T2 was statistically at par with T6 and T8. The lowest larval weight (40.09 g) was found in T0.

Effective rate of rearing (ERR) by number

The ERR by number recorded due to the treatment T10 was highest (9717) and statistically at par with T7, T6, T3 and T2. The lowest ERR by number (8183) was observed in T0 (Table2).

Effective rate of rearing (ERR) by weight

The ERR by weight recorded due to the treatment T10 was significantly highest (20.72 kg) and at par with T6 (19.91 kg) but significantly higher against all other treatments (Table 2). Moreover the ERR by weight recorded due to the treatment T2 was at par with T7 T8, T1 and T3. The lowest ERR by weight was recorded in T0 (15.13 kg) the uninoculated control.

Table 2: Rearing parameters of silkworm influenced by feeding mulberry leaves harvested from different treatments of microbial consortium (MR2 variety)

Treatment	Wt. of 10 matured larvae (g)	Effective rate of rearing /10000 larvae		Single cocoon wt.(g)	Single shell wt. (g)	Shell Ratio (%)
		By no.	By wt. (kg)			
T1	45.50	9317	17.89	1.901	0.433	22.81
T2	49.05	9417	19.23	2.003	0.457	22.85
T3	40.30	9450	17.73	1.867	0.434	23.36
T4	43.50	9333	16.91	1.792	0.429	23.90
T5	42.00	9050	16.38	1.830	0.428	23.39
T6	48.90	9500	19.91	2.107	0.498	23.63
T7	46.30	9550	18.98	1.942	0.464	23.94
T8	48.20	9050	18.00	1.961	0.475	24.25
T9	44.20	8867	16.98	1.884	0.434	23.05
T10	51.32	9717	20.72	2.003	0.472	23.61
T11	47.10	8483	16.14	1.803	0.410	22.73
T0	40.09	8183	15.13	1.670	0.365	21.84
SED±	0.41	129.62	0.21	0.013	0.004	0.304
CD @ 5%	0.85	268.81	0.43	0.027	0.009	0.629
CV%	1.10	1.73	1.41	0.831	1.223	1.598

Single cocoon weight (g)

The data recorded on single cocoon weight revealed significant differences among the treatments (Table 2). The highest single cocoon weight (2.107g) was recorded in T6 followed by T10 (2.003 g). However the treatment T10 was at par with T2, T8 and T7. The lowest single cocoon weight (1.670 g) was observed in T0 the uninoculated control.

Single shell weight (g)

The single shell weight recorded due to the treatment T6 (0.498 g) was significantly superior over the rest of the treatments including T1 (0.433 g) receiving full dose of chemical fertilizers. However single shell weight recorded due to T8 was statistically at par with T10, T7, T2 whereas that of T9 was at par with T3,T1,T4 and T5 (Table 2). Lowest single shell weight (0.365 g) was observed in T0.

Shell Ratio

The highest shell ratio (24.25%) was observed in treatment T8 which was statistically at par with T7, T4, T6 and T10. The uninoculated control treatment (T0) exhibited the lowest (21.84%) shell ratio (Table 2).

Leaf yield/ha/crop were significantly higher in the present study due to the combined application of biofertilizers and EM particularly in the case of treatment T10. Similar findings were observed due to inoculation of *Azospirillum* in mulberry at reduced levels of N (Yadav and Kumar, 1993). Not only the diazotroph *Azospirillum* but also the phosphate solubilizing organisms such as phosphobacteria and VA mycorrhiza are of importance in mulberry cultivation. The combined influence of *Azospirillum*+PSB+VAM is mainly due to the enhanced nutrient assimilation as well as growth hormone production and the compatability of the organisms among themselves (Balasubramanian, 1995). Similar results were reported by Jha and Mathur (1993) in *Pennisetum glaucum* inoculated with *Azospirillum*.

There was a clear indication of improvement in various cocoon characters of silkworm as a result of feeding mulberry leaves from the biofertilizer and EM inoculated plants. The combined effect of biofertilizers and EM along with reduced doses of N and P and full dose of K had a significant impact on growth parameters of silkworm. There was marked increase in the larval weight of silkworm (53.80 g / 10 larvae) in the treatment T10 receiving *Azospirillum*+PSB+VAM + (75% N+25% P+ full dose of K /ha/yr) +EM followed by the treatment receiving full dose of recommended chemical fertilizer. This is attributed to the increased nitrogen, phosphorus and potassium contents in the treated leaves. This was also evident from the high leaf moisture in treated leaves which in turn has resulted in improved quality of leaf suitable for silkworm rearing (Baqual, 2003). The role of quality mulberry leaf in production and increased quantity of quality cocoons has been well established (Krishnaswami, 1978; Datta, 1992; Ravikumar, 1988). Katiyar *et al*. (1995) reported increased mulberry plant growth and improved moulting percentage of silkworm larvae through VA- mycorrhizal inoculation and reduced ‘P’ dose through single super phosphate application. Das *et al*. (1993) also observed improvement of larval weight due to application of *Azotobacter* along with 150 kg of N/ha/yr.

Other rearing parameters including economic characters were however not affected even after 50% reduction in chemical nitrogen fertilizer. The ERR by number and weight recorded due to the treatment T6-*Azospirillum*+PSB + (75% N&P + full dose of K/ha/yr) +EM and T10-*Azospirillum*+PSB+VAM + (75% N+25% P+ full dose of K /ha/yr) +EM were superior over control. The higher ERR by number and weight is mainly due to the better leaf quality in terms of higher N, P and K contents. This is in accordance with the findings of Fathima *et al.* (1995) and Rama Rao *et al.* (2007) who observed improvement in cocoon yield when *G.mosseae* treated leaves were fed to the silkworm.

Additionally it is reported that synthesis of other nutrients, vitamins, amino acids, hormones by nitrogen fixing microorganisms helped to enhance the growth by increasing lateral root formation of the host plants (Yoav Bashan and Gina Holguin, 1997) with the result that leaf quality and yield was improved and due to this reason improvement in silkworm growth and cocoon characters were observed (Sannappa *et al.,* 2005 and Rama Rao *et al*., 2007).

There was a clear indication of improvement in various cocoon characters like single cocoon weight, single shell weight and silk ratio % of silkworms because of feeding leaf from the inoculants treated plot. This is attributed to the increase in N, P and K content in leaf due to microbial applications. The increased leaf nutrient content in turn could contribute for better economic characters of silkworms as has been observed in the present study. Similarly the importance of nutritive care for young age silkworm rearing and its influence on cocoon crop production is also reported (Chaluvachari, 1995). Since the young age silkworm needs more amount of sugar and protein in mulberry leaf for their better survivability in late age, the increased protein and other essential nutrients in mulberry leaf due to combined application of microbial consortium further underlines the importance of the use of eco-friendly and beneficial microorganisms as bio-inoculants for better yields in mulberry (Baqual and Das, 2006).

References

[1] Balasubramanian, A. (1995) Developing *Azospirillum* and *Azotobacter* biofertilizers for mulberry. *Project Report*, TNAU, Coimbatore. p.73.

[2] Baqual, M.F. (2003) Response of mulberry to co- inoculation with phosphate solubilizing microorganisms, nitrogen fixing bacteria and VA- mycorrhiza under varying levels and sources of phosphorus and nitrogen. Ph.D., Thesis in Applied Botany, University of Mysore, Mysore, p. 120.

[3] Baqual, M.F. and Das, P.K. (2006) Influence of biofertilizers on macronutrient uptake by the mulberry plant and its impact on silkworm bioassay. *Caspian J. Env. Sci*., **4**(2): 98-109.

[4] Chaluvachari, (1995) Leaf quality evaluation of few selected mulberry varieties. Ph.D., Thesis, Bangalore University, Bangalore, p. 220.

[5] Dandin, S.B., Jayaswal, J. and Giridhar, K. (2005) Hand Book of Sericulture Technologies, CSB, Bangalore, p.284.

[6] Das, P.K., Choudhury, P.C., Gupta, Y.K., Ghosh, A. and Datta, R.K. (1993) Effect of different levels of nitrogen, phosphorus and spacings on the growth, yield and nutritional quality of mulberry under rainfed condition. *Indian J. Seric*., **32**(20): 201-207.

[7] Datta, R.K. (1992) Guidelines for bivoltine rearing. Central Silk Board, Bangalore, India, p. 36.

[8] Fathima, P.S, Das, P.K., Katiyar, R.S., Himantharaj, M.T. and Pallavi, S.N. (1995). Effect of vesicular arbuscular mycorrihizal inoculation in mulberry under different levels and sources of phosphorous on silkworm rearing performance. In: Current Technology Seminar on Mulberry Silkworm Breeding Genetics, Molecular biology and Agronomy, Sept, 20-22, p.43.

[9] Jayaraj, S., Dandin, S.B., Veeraiah, T.M., Qadri, S.M.H and Krishna Rao, J.V. (2006) Soil health management for sustainable sericulture. Presented in National Seminar on Soil Health and Water Management for Sustainable sericulture, 27-28th September, RSRS, Kodathi, Bangalore, pp. 1-13.

[10] Jha, D. and Mathur, R.S. (1993) Combined effect of nitrogenous fertilizers and *Azospirillum brasilense* on the yield of nitrogen uptake by pearl millet. *International J. Trop. Agric*., **11**: 31-35.

[11] Katiyar, R.S., Das, P.K., Choudhury, P.C., Ghosh, A., Singh, G.B. and Datta, R.K. (1995) Response of irrigated mulberry (*Morus alba* L.) to VA-mycorrhizal inoculation under graded doses of phosphorus. *Plant and Soil*, **17**: 331-337.

[12] Krishnaswami, S. (1978) New technology of silkworm rearing, CSRTI, Mysore, (India) *Bulletin*, 2: 1-24.

[13] Ramarao, D.M., Kothandaramaiah, J., Reddy, M.P., Katiyar, R.S. and Rahamathulla, V.K. (2007) Effect of VAM fungi and bacterial biofertilizers on mulberry leaf quality and silkworm cocoon characters under semi-arid conditions. *Caspian J. Env. Sci*., **5**(2): 111-117.

[14] Ravikumar, C. (1988) Western Ghats as a bivoltine region-prospects, challenges and strategies for its development. *Indian Silk*, **26** (9): 39-54.

[15] Sannappa, B., Doreswamy, C., Ramakrishna Naika, Govindan, R. and Jagadish, K.S. (2005). Influence of sources of organic manures applied to S-36 mulberry on rearing performance of silkworm (PMxCSR2). In: Progress of Research in Organic Sericulture and Seri-Byproduct Utilization, Seri Scientific Publishers, Bangalore, p. 231.

[16] Yoav Bashan and Gina Holguin. (1997) *Azospirillum*-plant relationships: Environmental and physiological advances. *Can. J. Microbiol*., **43**(2): 103-121.

[17] Yadav, B.R.D. and Kumar, T.D. (1993) Response of mulberry (*Morus indica* var Kanva-2) to inoculation with the nitrogen-fixing bacterium, *Azospirillum brasilense*. *Sericologia*, **33**(4): 635-640.