I.J.S.N., VOL. 3(1) 2012: 608-612 ISSN 2229 – 6441

INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

EFFECT OF INOCULATION WITH *AZOTOBACTER* AND PHOSPHATE SOLUBILISING MICROORGANISMS ON MULBERRY (MORUS SPP.)

Baqual, M. F. & Das, P. K.
[1]Températe Sericulture Research Institute, SKUAST-K, Mirgund (India)
[2]Central Sericultural Research and Training Institute,
Mysore, Karnataka, India

ABSTRACT
The dual-inoculation of mulberry with phosphate solubilizing microorganisms like *Bacillus megaterium*, *Aspergillus awamori* and nitrogen fixing bacteria *Azotobacter chroococcum* under varying levels and sources of nitrogen and phosphorus had significant beneficial effect upon fresh root biomass/sapling, total above ground biomass/sapling and leaf, stem and root nitrogen content of the saplings of mulberry varieties viz., V_1 (Victory-1) and S_{36} being grown in Southern India. Maximum fresh root biomass of 25.45 g per sapling was recorded with the variety V_1 followed by S_{36} (21.33 g / sapling). Significantly higher above ground biomass of 58.36 g per sapling was also recorded with the cuttings of V_1 followed by S_{36} (43.97 g / sapling). Among the sub plot effect (F_1-F_{10}), the treatment F_9 registered significantly higher nitrogen (2.56 %) content in leaf over control (F_{10}). The variety V_1 was found to be significantly higher in nitrogen content in stem over S_{36}. However maximum nitrogen in root was observed due to treatment F_8 among the sub plot effect.

KEY WORDS: Inoculation, Mulberry, *Azotobacter*, Phosphate solubilising bacteria.

INTRODUCTION

India has a unique distinction of producing all the four types of silk viz., Eri, Tassar, Muga and Mulberry Silk. Mulberry plant (Morus spp.) constitutes sole food for silkworm (*Bombyx mori* L.) rearing and the principal economic product for which mulberry is grown is its foliage. Different plant parts of mulberry have also different medicinal uses as well. Documents speak of the fact that 60% of the cost of cocoon production goes to mulberry (Sastry, 1983) and the success of silkworm cocoon crop is the direct outcome of quality mulberry leaf. Mulberry has a very high fertilizer requirement viz., 350: 140: 140 kg/ha/yr nitrogen, phosphorus and potassium which involve the major input cost. However about 60% of the farmers do not apply the fertilizer in their required quantity owing to their exorbitant cost and erratic availability which results in reduced leaf productivity. The beneficial effects of phosphate solubilising micro-organisms (*Bacillus megaterium, Aspergillus awamori*) and nitrogen fixing microbial inoculants (*Azotobacter)* on mulberry in curtailing fertilizer nitrogen and phosphorus with significant increase in leaf yield and quality are well documented (Yadav and Nagendra Kumar, 1993; Nagendra Kumar *et al*, 2003; Das *et al*, 1992; Katiyar *et al*, 1995; Sukumar *et al* 2001; 2005; Baqual *et al* 2005). However, the information on the combined use of these microbial inoculants and there effect on mulberry under nursery conditions is scanty. Keeping this in view the present pilot study was initiated to generate the information on the effect of dual inoculation of biofertilisers on mulberry under nursery conditions.

MATERIALS AND METHODS

A flat land free from stones and gravels was selected for the experimental study. The soil of the experimental site was red sandy loam (Alfisol), with soil pH of 8.21 obtained by using a digital electric pH meter (REMI make), EC 0.194 mhos/cm^2, organic carbon 0.640 %, available potassium 405 lbs/acre (Jackson, 1973), available nitrogen 0.0208 % (Jackson, 1973) and available phosphorus 12.78 % obtained by Olsen *et al* (1954) method. The experiment was laid in a split- split plot design with 40 treatments and 2 replications. The treatments comprised of 2 sources of phosphorus (single super phosphate S_1 and Rock phosphate S_2) as main plot effect, 10 levels of fertilizers including inoculation with phosphate solubilizing micro-organisms (*Bacillus megaterium* var-*phosphaticum*, *Aspergillus awamori, Aspergillus niger* and nitrogen fixing bacteria *Azotobacter*) as bio-fertilizers and no-inoculation (F_1 to F_{10}) as sub-plot effect and two mulberry varieties (Victory-1: V_1 and S_{36} : V_2) as sub-sub plot effect. The experimental nursery was divided in to two blocks of 40 plots each. Thus a total of eighty plots were made keeping sufficient space between each plot and block. The cuttings of two selected varieties S_{36} and V_1 (60 each) were planted in respective plots at a distance of 10 cm between cutting to cutting and 15 cm between row to row. The cuttings were selected from eight months matured shoots free from pest and diseases and each cutting of pencil thickness with 18 cm length and 3 - 4 active buds were prepared. The cuttings were planted straight in the nursery beds leaving only one top most bud exposed. Before planting the cuttings, farmyard manure was applied @ 5 kg / bed as common manure and mixed thoroughly by digging the soil. The planted nursery beds were then irrigated as and when required throughout the study period.

RESULTS

1) Effect on fresh root biomass per sapling.

Significant difference in fresh root biomass production of sapling was not recorded among the two sources of phosphorus as main plot effect (Table-1). Among the sub plot effect (F_1-F_{10}), maximum fresh root biomass of 25.16 g per sapling was recorded in the treatment F_{10} (recommended dose of phosphorus and nitrogen without inoculation as control) which was also at par with F_2, F_3, F_7, F_8 and F_9 treatments receiving reduced fertilizer doses and inoculation. Significant difference in the fresh root biomass per sapling was also observed due to sub-sub plot effect. Maximum fresh root biomass of 25.45 g per sapling was recorded with the variety V_1 followed by S_{36} (21.33 g / sapling). The difference was significant. However the interaction effect was found to be non-significant.

2) Effect on total above ground biomass per sapling

Statistically there was no significant difference among the two sources of phosphorus (S1 and S_2) as main plot effect on the production of total above ground biomass per sapling (Table-2). Among the sub plot effect (F_1-F_{10}), maximum above ground biomass of 69.36 g per sapling was produced in the treatment F_7 (1/2 of the recommended dose of phosphorus and full dose of nitrogen with inoculation). This was significantly higher and superior to all other fertilizer levels including control (F_{10}) but was at par with F_8. Significant difference in the above ground biomass per sapling was also observed among two mulberry varieties as sub-sub plot effect. Maximum above ground biomass of 58.36 g per sapling was recorded with the cuttings of V_1 followed by S_{36} (43.97 g / sapling). The difference was significant. The interaction effect was also found to be significant. Highest above ground biomass of 100.85 g per sapling was produced in V_1 due to interaction effect of inoculation and application of the recommended dose of phosphorus as RP and nitrogen ($F_9I_1S_2V_1$). This was significantly higher over all other treatments including control.

3) Effect on nitrogen content of leaf.

No significant difference in nitrogen content of leaf was observed due to main plot effect. However, significant differences were recorded in nitrogen content of leaf due to sub plot effect (Table:-3). Among the sub plot effect (F_1-F_{10}), the treatment F_9 registered significantly higher nitrogen (2.56 %) content in leaf over control (F_{10}). Non significant difference was recorded due to sub-sub plot effect (between varieties) in the nitrogen content of leaf.

4) Effect on nitrogen content of stem.

The difference in nitrogen content of stem was non significant due to main plot effect (Table-3). Significant difference in nitrogen content of stem was observed due to sub-sub plot (Varieties) effect. The variety V_1 was found to be significantly higher in nitrogen content in stem over S_{36}. Significant difference in nitrogen content of stem was not observed due to interaction effect.

5) Effect on nitrogen content of root.

No significant difference was observed in the nitrogen content of root due to main plot effect (Table: - 4). Significant difference in the nitrogen content of root was observed due to various fertilizer levels and source as sub plot effect. Among the sub plot effect, maximum nitrogen in root was observed due to treatment F_8. This was significantly higher as compared to F_3, F_4 and F_5 but was at par with all other fertilizer levels and sources. The sub-sub plot effect of two varieties on nitrogen content of roots was also found to be significant. Maximum nitrogen (3.16%) content in roots were recorded in the variety V_1 as compared to S36 which recorded (2.21%) of nitrogen. The interaction effect was also significant.

DISCUSSION

From the present study it is clear that the dual inoculation with phosphate solubilizing micro-organisms (*Bacillus megaterium* and *Aspergillus awamori*) and nitrogen fixing bacteria (*Azotobacter*) are highly efficient as supplementary agents for nitrogen and phosphatic fertilizers in mulberry cultivation. The use of these micro-organisms can as well bring down the cost of mulberry cultivation to a great extent by supplementing chemical fertilizers without any adverse effect on growth and yield of mulberry. Although the inoculation of these microbes aids in rhizosphere interaction but at the same time the plant microbe and microbe interaction result in increased biomass production. The enhanced yield is as a result of nutrient uptake in mulberry which in turn is the cumulative effect of synergism between various microbes (Kundu and Gaur, 1980, Balasubramanian, 1995., Sukumar *et al*, 1995). The present study also has indicated the increased nitrogen content in leaf, stem and root of the mulberry saplings in respect of inoculated treatments thus confirming the earlier studies of Das *et al*, 1994.

TABLE:-1. Effect of dual inoculation on fresh root biomass per sapling (g).

A.	Main plot effect (Sources of phosphorus)
S_1	23.77
S_2	23.02
B.	Sub plot effect (Fertilizer levels)
F_1	22.23
F_2	24.70
F_3	23.67
F_4	22.45
F_5	22.57
F_6	20.01
F_7	23.93
F_8	24.37
F_9	24.85
F_{10}	25.16
C.	Sub sub plot effect (Varieties)
V_1	25.45
V_2	21.33

S_1 = Single super phosphate
S_2 = Rock phosphate
V_1= Victory-1
V_2= S36

F_1= ½ of recommended dose of nitrogen and phosphorus

Cd at 5% for

A: NS

B: 1.71

C: 1.17

Ax B x C: NS

F_2= 3/4th of P and ½ of N.
F_3= Recommended dose of P and ½ of N.
F_4= ½ P and 3/4th of N.
F_5= 3/4th N and P.
F_6 = Recommended doze of P and 3/4th N.
F_7 = ½ P and recommended doze of N.
F_8 = 3/4th of P and recommended doze of N.
F_9 = Recommended doze of N and P.
F_{10}= Recommended doze of N and P(I_0)
I_1 =inoculation
I_0= No inoculation
NS= Non significant

TABLE:-2. Effect of dual inoculation on total above ground biomass per sapling (g)

A. Main plot effect
(Sources of phosphorus)

S_1	50.28
S_2	52.05

B. Sub plot effect
(Fertilizer levels)

F_1	34.37
F_2	49.78
F_3	54.16
F_4	42.67
F_5	39.80
F_6	36.13
F_7	69.36
F_8	68.25
F_9	58.62
F_{10}	58.10

INTERACTION EFFECT

Fertilizer Levels	**V_1 S_1**	**V_1 S_2**	**V_2 S_1**	**V_2 S_2**
F_1I_1	42.25	31.80	39.20	25.85
F_2I_1	43.95	75.35	40.00	39.85
F_3I_1	78.30	45.05	64.70	28.60
F_4I_1	67.00	24.80	52.85	26.05
F_5I_1	56.40	33.25	48.85	20.60
F_6I_1	44.65	37.00	40.05	22.85
F_7I_1	79.25	84.30	58.40	55.50
F_8I_1	57.80	93.95	49.90	71.35
F_9I_1	37.10	100.85	28.25	68.30
$F_{10}I_0$	* 39.55	94.55	37.25	61.05

C. Sub sub plot effect
(Varieties)

V_1	58.38
V_2	43.97

Cd at 5% for

A: NS

B: 3.53

C: 1.04

AxBxC: 4.68

S_1 = Single super phosphate
S_2 = Rock phosphate
V_1= Victory-1
V_2= S36
F_1= ½ of recommended dose of nitrogen and phosphorus
F_2= 3/4th of P and ½ of N.
F_3= Recommended doze of P and ½ of N.
F_4= ½ P and 3/4th of N.
F_5= 3/4th N and P.
F_6 = Recommended doze of P and 3/4th N.
F_7 = ½ P and recommended doze of N.
F_8 = 3/4th of P and recommended doze of N.
F_9 = Recommended doze of N and P.
F_{10}= Recommended doze of N and P(I_0)
I_1 =inoculation
I_0= No inoculation
NS= Non significant
* control

TABLE:- 3. Effect of dual inoculation on leaf and stem nitrogen % of saplings

Leaf nitrogen percentage

A. Main plot effect (Sources of phosphorus)

S_1	2.30
S_2	2.47

B. Sub plot effect (Fertilizer levels)

F_1	2.33
F_2	2.26
F_3	2.34
F_4	2.40
F_5	2.42
F_6	2.49
F_7	2.47
F_8	2.44
F_9	2.56
F_{10}	2.18

C. Sub sub plot effect (Varieties)

V_1	2.52
V_2	2.26

Cd at 5% for

A:	NS
B:	0.07
C:	NS
AxBxC:	NS

Stem nitrogen percentage

Main plot effect (Sources of phosphorus)

S_1	1.49
S_2	1.50

B Sub plot effect (Fertilizer levels)

F_1	1.50
F_2	1.49
F_3	1.50
F_4	1.50
F_5	1.50
F_6	1.48
F_7	1.51
F_8	1.51
F_9	1.50
F_{10}	1.50

C. Sub sub plot effect (Varieties)

V_1	1.55
V_2	1.45

Cd at 5% for

A:	NS
B:	NS
C:	0.01
AxBxC:	NS

TABLE:- 4. Effect of dual inoculation on root nitrogen % of saplings

A. Main plot effect (Sources of phosphorus)

S_1	2.70
S_2	2.67

B. Sub plot effect (Fertilizer levels)

F_1	2.70
F_2	2.68
F_3	2.65
F_4	2.63
F_5	2.64
F_6	2.71
F_7	2.70
F_8	2.76
F_9	2.69
F_{10}	2.72

INTERACTION EFFECT

Fertilizer levels	**V_1**		**V_2**	
	S_1	**S_2**	**S_1**	**S_2**
F_1I_1	3.17	3.19	2.20	2.23
F_2I_1	3.21	3.11	2.23	2.11
F_3I_1	3.26	3.05	2.22	2.05
F_4I_1	3.08	3.06	2.18	2.18
F_5I_1	3.18	3.13	2.15	2.08
F_6I_1	3.22	3.03	2.17	2.42
F_7I_1	3.24	3.14	2.20	2.22
F_8I_1	3.26	3.15	2.25	2.35
F_9I_1	3.20	3.17	2.16	2.23
$F_{10}I_0$	*3.16	3.22	2.26	2.25

C. Sub sub plot effect (Varieties)

V_1	3.16
V_2	2.21

S_1 = Single super phosphate
S_2 = Rock phosphate
V_1 = Victory-1
V_2 = S36

F_1 = ½ of recommended dose of nitrogen and phosphorus

Cd at 5% for

A: NS

B: 0.06

C: 0.02

AxBxC: 0.11

F_2= 3/4th of P and ½ of N.
F_3= Recommended doze of P and ½ of N.
F_4= ½ P and 3/4th of N.
F_5= 3/4th N and P.
F_6 = Recommended doze of P and 3/4th N.
F_7 = ½ P and recommended doze of N.
F_8 = 3/4th of P and recommended doze of N.
F_9 = Recommended doze of N and P.
F_{10}= Recommended doze of N and P(I_0)
I_1 =inoculation
I_0= No inoculation
NS= Non significant
*control

ACKNOWLEDGEMENTS
We, authors acknowledge the help and the facilities provided by Director CSR and TI, Mysore, Karnataka, India and we are also thankful to Madam Rekha, Statistician CSR and TI, for statistical analysis of data.

REFRENCES
Balasubramanian, A. (1995) Developing *Azospirrilum* and *Azotobacter* biofertilizers for mulberry. *Project Report, Tamil Nadu Agricultural University, Coimbatore.*

Baqual, M. F, Das, P.K and Katiyar, R. S. (2005) Effect of arbuscular mycorrhizal fungi and other microbial inoculants on chlorophyll content of mulberry (Morus spp.). *Mycorrhiza News* Vol. 17(3) 12-14.

Das P. K., Choudhury, P. C. Gosh, A, Katiyar, R. S., Mathur, V. B., Madhava Rao, A. R. and Datta, R. K. (1994) Studies on the effect of bacterial biofertilizer in irrigated mulberry (*Morus alba*). *Ind. J. Seric*, 33 (2), 170-173.

Das P. K., Gosh, A., Choudhuey., P. C., Katiyar, R. S. and Sengupta, K. (1992) Response of irrigated mulberry to *Azotobacter* and *Azospirrillum biofertilisers* under graded levels of nitrogen. In: Biofertiliser Technology Transfer. L. V. Gangawane (Ed.), Associated Publishing Co., New Delhi, 72-77 pp.

Jackson, M. L. (1973) In: Soil Chemical Analysis. Prentice Hall, New Delhi, India pp 326-328.

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.* 170: 331-337.

Kundu, B. S. and Gaur, A. C. (1980) Effect of nitrogen fixing and phosphate solubilising microganisms as single and composite inoculants on cotton. *Indian J. Microbiol*., 20, 225-229.

Nagendra Kumar, T.D, Sukumar, J. and Padma, S. D. (2003) Phosphate solubilising microbial inoculants in mulberry cultivation – A review. *Bull. Ind. Acad. Seri*., 7. 1-10

Olsen (1954) U. S. D. A. cir. 939. Quoted from : Jackson, M. C. 1973. *Soil Chemical Analysis*, Prentice Hall of India, Pvt. Ltd., New Delhi, India.

Sastry C. R. (1983) Mulberry Agrnomy, Pest and diseases Lead Paper, *National Seminar on Silk Research and Development*, Bangalore. India. March 10-13, pp 1-24.

Sukumar, J., Padma, S. D., Bongal, U. D. and Veeresh, M. (2205) Response of mulberry to co-inoculation with *Azotobacter chtoococum* and *Bacillus megaterium,* Proceedings of the National Seminar on Mulberry sericulture in India, KSSRDI, Bangalore – 62, 388-391.

Sukumar, J. Thimma Reddy, H., Padma, S. D., Bongale, U. D. (2001) Effect of combined inoculation of *Bacillus megaterium* and *Azospirrillum brasilense* on leaf yield and nutrient uptake in mulberry. *International Journal of Tropical Agriculture*, 19, 35-42.

Yadav, BRD and Nagendra Kumar, T. D. (1993) Response of mulberry (Morus alba L var Kanva-2) to inoculation with the nitrogen fixing bacterium, *Azospirrillum brasilense. Sericologia*, 33, 635-640.