EVALUATION OF QUALITY OF MYCORRHIZAL MULBERRY LEAVES THROUGH S!LKWORM REARING

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The quality and quantity of mulberry leaves var. S1 associated with three strains of VA mycorrhiza separately with graded levels of phosphorus (180, 90, 60, and 30 Kg P/ha/yr) were studied at CSR&TI, Berhampore (W.B.) for 2 years (1996-97 & 1997-98). Two years average data revealed the overall improvement in leaf yield, leaf moisture (%) and P uptake by leaves in all the VAM-treated mulberry plants (Var. S1) particularly associated with Glomus mosseae_at 30Kg P/ha/yr which statistically found to be at par with control (180 Kg P/ha/yr without VAM) and other treatments. The silkworm rearing result with Multi x Bi (CB₅ x P₅) hybrid indicated the overall improvement in larval weight, single shell weight, S.R.%, absolute silk content in mycorrhizal leaves of any of the strains under reduced doses of P when fed to silkworms, while ERR, filament length, denier and non-breakable filament length were found to be at par with control. It was further observed that Glomus etunicatum with 50% reduced dose of P during favourable season and G. fasciculatum with 67% reduced dose of P during unfavourrable season performed better result in respect of important economic characters of rearing and reeling.

INTRODUCTION

In the present decade Vesicular arbuscular mycorrhiza (VAM) is widely applied as biofertilizer in different crop plants as also in mulberry due to having the capacity of phosphate mobilisation and shortening of P diffusion pathways resulted more diffusion as well as absorption of H₂Po₄ in plant-available form thus reduced the requirement of P fertilizer, improved the quality and quantity of leaf particularly in mulberry, solefood of silkworm, Bombyx mori L. (Nye & Tinker, 1977; Bagyaraj & Sreeramulu, 1982; Bolan,1991; Katiyar et al., 1995). For quality assessment of mulberry leaf, silkworm rearing (bio-assay) is generally conducted in view of studying the improvement in rearing and reeling parameters over control (Sarkar & Fujita, 1994; Das et al., 1995; Fathima et al., 1996; Setua et al., 1998). The objective of the present study was to assess the quality and quantity of mulberry leaves obtained from different VAM-inoculated mulberry plants at reduced doses of P fertilizer through silkworm rearing (bio-assay).

MATERIALS AND METHODS

In nursery, cuttings of S1, a popular mulberry variety, were planted at 15 x 10 cm² spacing after 2 times heat sterilization of nursery soi1. Three strains of VAM *i.e. Glomus etunicatum* (GE), *G. fasciculatum* (GF) and *G. mosseae* (GM) @ 200 kg/ha were applied separately in furrows at the root zone of cuttings after 30 days of plantation. The inoculum contains 100 spores/5 g rhizosphere soil as measured by wet sieving and decanting technique (Gerdemann & Nicolson, 1963). After studying the colonization in roots (Phillips & Hayman, 1970), mycorrhizal plants were transplanted in the field after 4 months of age and package of practices for maintenance were followed as per recommendation for irrigated garden (Ullal & Narasimhanna, 1987). N 336 kg, K 112 kg/ha/yr alongwith P at three reduced levels (30, 60 & 90 kg/ha/yr) were applied in different treatments. The plants without VAM and with N 336, P 180 and K 112 kg/ha/yr were treated as control.

The experiment was laid out in randomised block design (RBD) in three replications. Phosphorus uptake by leaves was studied by following the method of Jackson (1973). Data on leaf yield, leaf moisture percent and phosphorus uptake by leaves were obtained during February, May, August and November seasons for two years. The overall mean of two years in respect to each treatment and control alongwith critical difference (C.D.) value (P <= 0.05) have been calculated. Bioassay was conducted during favourable (February) and unfavourable (April-May) seasons with CB5 x P5 (Multi x bi) hybrids, fed with the leaves obtained from mycorrhizal (with three reduced levels of P) and non-mycorrhizal plants (P 180 kg/ha/yr) for two years. Data of two years average of two seasons in respect of important rearing and reeling parameters alongwith C.D. values have been calculated.

RESULTS AND DISCUSSION

It was overall observed from Table I that the leaf yield, leaf moisture % and phosphorus uptake by leaves were were found to be statistically similar in any of the treatments irrespective of strains of VAM with reduced doses of P and control. No deterioration was observed in any of the parameters by the influence of VAM.

Out of ten treatments, mycorrhizal plants associated with GE at 60 kg P/ha/yr registered highest leaf yield, while GM at most economic dose of P (30 kg/ha/yr) contributed maximum leaf yield (Table I). It was further observed that there was no change of leaf yield under reduced doses of P fertilizer with the influence of VAM over control.

Leaf moisture content and phosphorus uptake by leaves are the important criteria for maintaining palatability to silkworms which were also found to be maximum in GM with 30 Kg P/ha/yr though it was statistically at par with other treatments and control (Table I). Reduced doses of P under the influence of any of the three strains of VAM did not show any deterioration on these two aspects over control.

Table I: Effect of VAM-inoculated saplings on leaf yield, leaf moisture (%) and phosphorus
uptake by leaves in mulberry (2 years pooled data).

Treatment	Leaf yield	Leaf moisture	Phosphorus uptake
(Kg P/ha/yr	(Kg/ha/yr)	(%)	by leaves (mg/g)
180 (Control)	22023	74.0	3.78
90 with GE	22333	73.26	3.64
90 with GF	22717	73.89	3.77
90 with GM	23782	74.29	3.66
60 with GE	23976	74.02	3.64
60 with GF	23013	73.89	3.75
60 with GM	22647	74.13	3.81
30 with GE	22147	74.01	3.90
30 with GF	22049	74.04	3.82
30 with GM	23526	74.61	3.91
C.D. at 5%	NS	NS	NS

Table II: Effect of leaves obtained from VAM-inoculated mulberry plants on rearing and reeling parameters (avg. of 2 years).

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33.73 1.707	17.38	8500	14.50	2522	813	563	2.40
39.45 1.728	17.95	9250	15.75	2829	829	757	2.58
	16.76	8333	14.39	2412	807	591	2.54
1.743	16.46	0006	15.71	2586	698	699	2.47
1.621	17.52	8416	13.64	2391	817	448	2.36
P30 + VAM-3 (T10) 38.23 1.645 0.291	17.71	8000	13.13	2326	831	570	2.41
C.D. at 5% 0.522 NS NS	0.331	700	1.48	263	NS	NS	NS

The probable reason for improvement in leaf yield, leaf moisture percent and phosphorus uptake by leaves with low doses of phosphorus might be due to more solubilization of colloid form of phosphorus with the contact of organic and hydroxy acid and more absorption of H_2Po_4 by profused enhancement of mycelia of VAM due to shortening of P diffusion pathways (Hatting et al., 1973; Nye & Tinker, 1977). The result confirmed the findings of Rhodes & Gerdemann (1975) in onion, Iqbal & Qureshi (1977) in sunflower and Ambika et al. (1994) in mulberry.

As regards the rearing performances conducted both in favourable (February) and unfavourable (April-May) seasons, it was overall observed that by feeding the leaves to the silkworms obtained from any of the three mycorrhizal plants, the important rearing and reeling parameters were remained on par in any of the treatments with the influence of VAM under reduced doses of P and control except weight of matured larvae, SR % and absolute silk content which were found to be significant over control (Table II).

The rearing data of February indicated that the SR %, absolute silk content, ERR (No.) and ERR (wt.) were found to be maximum over other treatments and control by the influence of GE under 90 Kg P/ha/yr (T2). Single cocoon weight and filament length were found to be maximum in T8 while single shell weight was found to be significant in this crop and maximum was obtained in T6. Rearing results during April-May revealed the best performance on single shell wt., SR %, absolute silk content, non-breakable filament length, ERR (No.) and ERR (wt.) made by the influence of GF under P60 Kg/ha/yr (T6) over other treatments and control. Further it was also observed that ERR (No.) and ERR (wt.) were also found significant among the treatments. Highest single cocoon wt. and filament length were found in T3 and T8, respectively. Weight of 10 matured larvae was maximum in T10 during February and in T2 during April-May crop. The result corroborated the findings of Fathima *et al.* (1996).

It is thus inferred from the study that the VAM-inoculated mycorrhizal saplings irrespective of strains overall poduced the similar quality and quantity of mulberry leaves under irrigated, alluvial soil conditions with reduced doses of phosphatic fertilizer (reduction level: 50-80%), especially at 30 kg P/ha/yr (economic level) in comparison with the control containing full dose of P fertilizer (180 kg/ha/yr) without VAM inoculation. Rearing the silkworms (Multi x B1) with those leaves produced from VAM-inoculated saplings under reduced doses of P also showed either similar or significant improvement in respect of important rearing and reeling parameters compared to control. No significant deterioration in any of the important rearing and reeling parameters was observed by feeding the leaves obtained from VAM-inoculated saplings. Hence, it is suggested that the application of VAM is very much useful and beneficial to quality and quantity mulberry leaf production as also to rearing and reeling aspects towards the curtailment on the use of chemical fertilizer, particularly phosphatic fetilizer substituted partially by VAM biofertilizer. Further it can also help to reduce the cost of cultivation of mulberry by saving 50-80% P fertilizer and could also prevent diseases as also environmental pollution by this sort of eco-friendly approach.

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REFERENCES

AMBIKA, P.K., DAS, P.K., KATIYAR, R.S. & CHOUDHURY, P.C. 1994. The influence of vesicular-arbuscular mycorrhizal association on growth, yield and nutrient uptake in some mulberry genotypes

- (Morus sp.). Indian J. Seric. 33(2): 166 169.
- BAGYARAJ, D.J. & SREERAMULU, K.R. 1982. Pre-inoculation with VA mycorrhiza improves growth and yield of chilli transplanted in the field and saves phosphatic fertilizer. *Plant Soil.* 69: 375 381.
- BOLAN, N.S. 1991. A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. *Plant Soil.* 134: 189 207.
- DAS, P.K., KATIYAR, R.S., HANUMANTHAGOWDA, M., FATHIMA, P.S. & CHOUDHURY, P.C. 1995. Effect of Vesicular Arbuscular Mycorrhizal inoculation on growth and development of mulberry (*Morus* sp.) saplings. *Indian J. Seric.* 34(1):15 17.
- FATHIMA, P.S., DAS, P.K., KATIYAR, R.S., HIMANTHARAJ, M.T. & PALLAVI, S.N. 1996. Effect of Vesicular Arbuscular Mycorrhizal inoculation in mulberry under different levels and sources of phosphorus on silkworm growth, cocoon yield and quality. *Indian J. Seric.* 35(2): 99 103.
- GERDEMANN, J.W. & NICOLSON, T.H. 1963. Spores of mycorrhizal *Endogone* species extracted from soil by wet-sieving and decanting. *Trans. Brit. Mycol. Soc.* 46: 235 244.
- HATTINGH, M.J., GRAY, L.E. & GERDEMANN, J.W. 1973. Uptake and translocation of 32p labelled phosphates to onion roots by endomycorrhizal fungi. *Soil Science*. 116: 383 387.
- IQBAL, S.H. & QURESHI, K.S. 1977. The effect of vesicultar-arbuscular mycorrhizal associations on growth of sunflower (*Helianthus annus* L.) under field conditions. *Biologia*. 23(2): 189 196.
- JACKSON, M.L. 1973. Soil chemical analysis. Prentice-Hall India (Pvt.) Ltd., New Delhi, India. pp. 326 -338.
- KATIYAR, R.S., DAS, P.K., CHOUDHURY, P.C., GHOSH, A., SINGH, G.B. & DATTA, R.K. 1995. Response of irrigated mulberry (*Morus alba* L.) to VA-mycorrhizal inoculation under doses of phosphorus. *Plant Soil.* 170: 331 337.
- NYE, T.H. & TINKER, P.B. 1977. Solute movement in the soil root system. Blackwell Scientific Publication, Oxford.
- PHILLIPS,, J.M. & HAYMAN, D.S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Brit. Mycol. Soc.* 55(1): 158 160.
- RHODES, L.H. & GERDEMANN, J.W. 1975. Phosphate uptake zones of mycorrhizal and non-mycorrhizal onions. *New Phytol.* 75: 555 561.
- SARKAR, A. & FUJITA, H. 1994. Better technique for nutritive evaluation of mulberry leaves for silkworm, Bombyx mori L. Indian J. Seric. 33(1): 19 - 22.
- SETUA, M., DAS, C., MANDAL, B.K., GHOSH, D.C. & SEN, S.K. 1998. Influence of growth regulators on leaf protein and some economic characters of silkworm, *Bombyx mori. Uttar Pradesh J. Zool.* 18(1): 13 18.
- ULLAL, S.R. & NARASIMHANNA, M.N. 1987. Mulberry cultivation. In: *Handbook of Practical Sericulture* (Sampath, J. Ed.). Central Silk Boarad, Bangalore. pp. 7 32.