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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences, 2 (2): 590-593, 1999 Research Article

EM and VAM Technology in Pakistan
VII: Effect of Organic Amendments and Effective Microorganisms (EM) on VA Mycorrhiza, Nodulation and Crop Growth in *Trifolium alexandrianum* L.

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Abstract
A pot experiment was conducted to observe the effect of effective microorganisms (EM) on vesicular arbuscular mycorrhizal (VAM) colonization, nodulation and crop growth in *Trifolium alexandrianum* L., in soils amended with farmyard manure (FYM) and green manure (GM). EM significantly enhanced mycorrhizal colonization in 60-day old plants in both types of soil. EM failed to induce any remarkable change in nodulation at this growth stage while it exhibited an inhibitory effect on root and shoot biomass production. After 80 days growth, EM treated plants exhibited no significant variation in mycorrhizal colonization than non-treated plants. EM application caused a significant reduction in nodule number while increased the size and biomass of nodules in both types of soils. EM application supported root growth in FYM amended soil, however, shoot growth was adversely affected at this growth stage. In GM amended soil EM inhibited both the root and shoot growth. EM failed to induce any relationship between VAM colonization, nodulation and plant growth.

Introduction
Most herbaceous legumes of the family Papillionaceae are symbiotic with both nodule forming Rhizobium and VA mycorrhizal fungi (Bethlenfalvay and Linderman, 1992). Generally, colonization of root by VA mycorrhizal fungi favours nodulation by *Rhizobium* (Smith *et al*., 1979) and increases the N_2-fixation activity of the nodules (Pacovsky *et al*., 1986; Javaid *et al*., 1994). Other soil microorganisms may influence establishment and development of VA mycorrhizal fungi, in turn (Ho and Trappe, 1979; Brown and Carr, 1979; Krone *et al*., 1987). Higa *et al*. (1984) developed a culture of 80 species of coexisting beneficial microorganisms and called them effective microorganisms (EM). It mainly contains photosynthetic bacteria, *Azotobacter*, *Streptomyces* and *Lactobacillus* spp., which increase crop growth and yield by increasing photosynthesis, nitrogen fixation, controlling soil-borne pathogens and accelerating decomposition of lignin material in the soil (Hussain *et al*., 1994). Higa and Wididana (1991) reported that in most cases the number of bacteria, fungi and actinomycetes increased in soil after treatment with EM. Sangakkara and Higa (1994) observed that EM application increased the rhizobial population of the soil. Some workers studied the effect of EM application on VAM development and subsequent crop growth in maize, soybean, wheat, chickpea and pea (Bajwa and Jilani, 1994; Bajwa *et al*., 1995, 1997, 1998; Javaid *et al*., 1995). However, they observed different effects of EM applications in different crops. The present study was, therefore, undertaken to investigate the effect of EM application on VAM colonization, nodulation and subsequent crop growth in *Trifolium alexandrianum*. It is an important leguminous fodder crop cultivated during winter on a large scale in Pakistan.

Materials and Methods
Farmyard manure (FYM) and green manure (GM) were moistened with a dilute suspension of EM (1:500), packed in air tight polythene bags and left for 7 days in a dark room. These EM treated organic materials were mixed in pot soil at 10% of total soil volume. Non-EM treated organic materials were similarly mixed in pot soil to be used as control. Seeds of *T. alexandrianum* were surface sterilized with 1% sodium hypochloride and sown in all the pots. Each treatment was replicated thrice with 2 plants each. Pots with EM treated organic materials were further supplemented with dilute suspension of EM (1:1000) twice a month until 15 days prior to final harvest.

Plants were harvested 60 and 80 days after sowing. On each harvesting date plants were carefully uprooted and washed thoroughly. Nodules were separated horn roots and counted. Fresh and dry weights of root, shoot and nodules were recorded. A subsample of fresh roots was cleared and stained by staining procedure of Phillips and Hayman (1970). Extent of mycorrhizal infection was measured by slide length method (Giovannetti and Mosse, 1980). Arbuscular and vesicular infections were quantified by counting these structures per cm of root length.

Results and Discussion
The effect of EM application on root and shoot growth of *Trifolium* in soils incorporated with farmyard manure (FYM) and green manure (GM) is represented in Fig. 1. EM treated plants exhibited a reduction in both root and shoot biomass production in both type of soils, 60 days after sowing (DAS). However, 80 DAS root growth in FYM amended soil showed a positive response to EM application while in GM amended soil a significant reduction in root growth was observed. Shoot growth was significantly suppressed in EM treated plants irrespective of the organic amendment

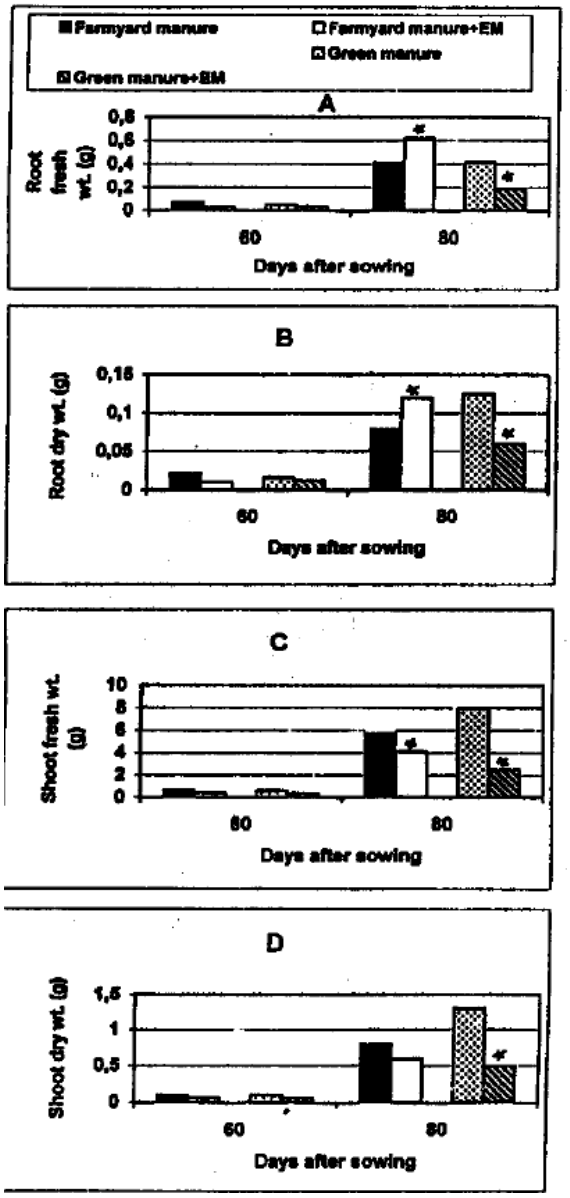

Fig. 1 (A-D): Effect of EM and organic amendments on root and shoot growth of *Trifolium alexandrianum*.
*Differ significantly as determined by t-test.

incorporated in the soil. Growth suppression due to EM was more evident in GM than in FYM amended soil. Similar expeitments about the effects of EM on crop growth conducted by other workers (Bajwa *et al*., 1997; Higa, 1989; Lin, 1991; Panchaban, 1991) revealed that the effect of EM was usually not evident or even adverse in the first test crop. Generally the crop yields tend to increase gradually as subsequent crops are grown (Higa, 1989; Arakawa, 1991; Imai and Higa, 1994). However, in contrast to these Minami and Higa (1994) observed an increase in the yield of rice during the first year application of EM.

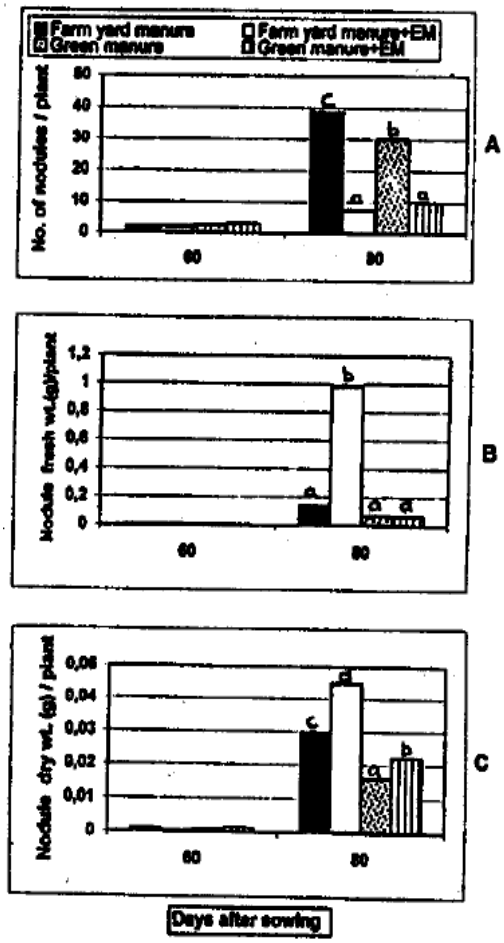

Fig. 2: Effect of EM and organic amendments on nodulation of *Trifolium alexandrianum*. Bars with different letters at their tops show significant differences.

Impact of FYM, GM and EM on nodulation of 60 and 80 days growth stages is presented in Fig. 2. No remarkable difference was evident in nodulation 60 DAS between FYM and GM amended soils. However, 80 DAS, FYM amended soil induced better nodulation than GM amended one. EM application with either amendment resulted in reduction of nodule number but increased the size of the nodules. Consequently fresh and dry biomass of the nodules increased significantly. Greater increase was observed in FYM amended soil. Sangakkara and Higa (1994) have reported that addition of EM plus organic materials increased nodulation of *Phaseolus vulgaris* and

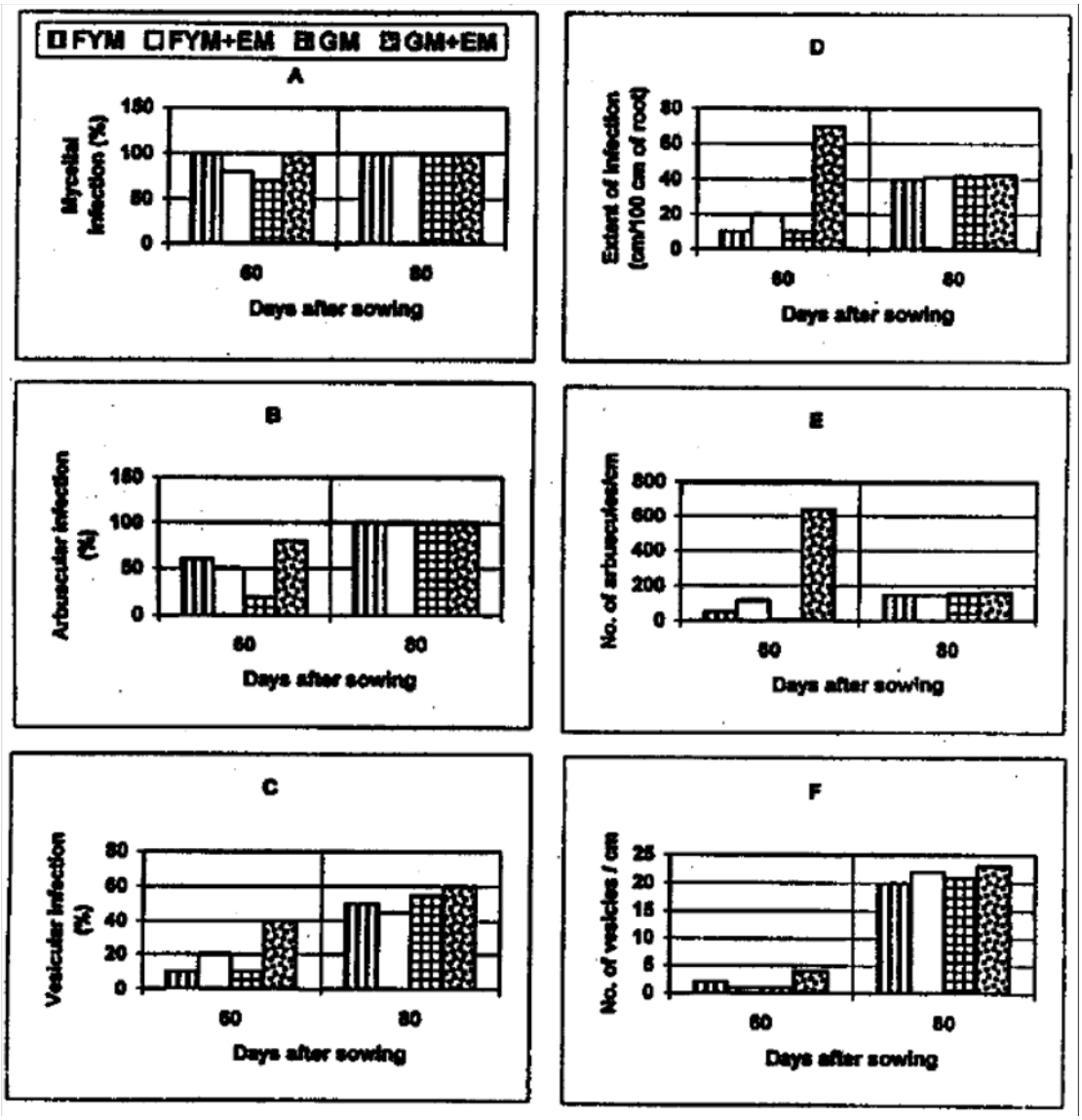

Fig. 3: Effect of EM on VA mycorrhizal development in *Trifolium alexandrianum*

Vigna radiata. Moreover, they observed that application of EM to *Gliridicia* leaves as organic amendment produced a greater response than when applied to coir dust.

Mycorrhizal development was markedly enhanced by EM application up to 60 days growth in both types of soils. Effect of EM was more pronounced in GM than in FYM amended soil, However, in 80-day old plants EM failed to induce any remarkable change in mycorrhizal development (Fig. 3). Any parallel relation between mycorrhizal colonization and crop growth was entirely lacking. In contrast to this, Bajwa and Jilani (1994) have reported an increased VAM colonization by EM application with a parallel increase in maize growth. Furthermore, Javaid *et al*. (1995) observed a marked suppression in VAM colonization but enhanced nodulation, growth and yield in pea due to EM application. It seems probable that an interaction develops between VAM and EM, which is different for different crops and soils. The advantage or disadvantage of EM application to test crop, particularly during the first year of EM application probably depends upon the nature of these interactions. Therefore, before drawing any specific conclusion regarding the nature of these interactions and their benefits to plants, further investigation of both short term and long term effectiveness of EM application is needed.

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