http://www.pjbs.org

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 3 (4): 694-698, 2000

EM and VAM Technology in Pakistan IX: Effect of EM Application on Growth, Yield, Nodulation and VA Mycorrhizal Colonization in *Vigna radiata* (L) Wiczek

Arshad Javaid, Rukhsana Bajwa, Nusrat Rabbani and Malika Uzma
Department of Botany, University of the Punjab, Quaid-e-Azam Campus Lahore, Pakistan

Abstract: A variable response of crop growth, nodulation and VAM colonization to EM (effective microorganisms) application was observed when *Vigna radiate* (L) Wilczek was grown in farmyard manure (FYM) and *Trifolium* green manure (GM) amended soils with different histories of EM application. In soil 1 EM application was started six months prior to soil 2. Shoot growth was enhanced due to EM application in GM amended soil 1 but suppressed in soil 2 with either organic amendment. Root biomass was increased by EM application in FYM but reduced in GM amended soils, EM reduced pod dry biomass in soil 1 but enhanced in FYM amended soli 2. Irrespective of the soil type and organic amendment, nodulation was enhanced by EM application at vegetative growth stage. VAM colonization showed a positive response to EM application in both the soils. The response of crop growth was independent of the response of nodulation and VAM colonization to EM.

Key words: Effective microorganisms, VA mycorrhiza, Farmyard manure, Green manure, *Vigna radiate.*

Introduction

Of the various mycorrhizal fungi vesicular arbuscular mycorrhizae (VAM) is the largest group and is predominantly associated with agricultural crops. These fungi penetrate root cortical cells and form arbuscules that interface with the host cytoplasm. These fungi also interface directly with the surrounding soil by means of extraradical hyphae that extend into the soil and increases the potential of the root system for nutrient and water absorption, and contribute greatly to the improvement of the soil structure for better aeration and water percolation (Bethlenfalvay and Linderman, 1992). They also impart other benefits to plants including stimulation of growth regulating substances, increased rate of photosynthesis, osmotic adjustment under drought stress, enhancement of N_2-fixation by symbiotic or associative N_2-fixing bacteria, increased resistance to pests and tolerance to environmental stresses (Jeffries and Rhodes, 1987).

Other soil microorganisms may influence establishment of VAM fungi. Daniels and Trappe (1980) showed that spores of *Glomus epigaeus* did not germinate in autoclaved or gamma irradiated soils, but did in nonsterile soils. Azcon-Aguilar and Barea (1985) showed that bacterial contaminated spores of *Glomus mosseae* formed more VA mycorrhizae on roots of plants grown in sterile soil than surface disinfected spores. Bagyaraj and Menge (1978) studied the interaction between free living N_2-fixing bacterium *Azotobacter chroococcum* and mycorrhizal fungus *Glomus fasciculatum* in tomato and found a synergistic effect on plant growth. Mycorrhizal infection increased the *A. chroococcum* population in rhizosphere and *A. chroococcum* enhanced infection and spore production by the mycorrhizal fungus. Similar interactions have also been observed between *A. paspali* and VAM fungi in *paspalum* (Barea *et al*., 1973). However, soil microbes may also inhibit VAM fungi (Wilson *et al*., 1988; Ross, 1980).

Higa (1989) isolated some beneficial microorganisms collectively called as Effective Microorganisms (EM). EM consists of photosynthetic bacteria, *Azotobacter,* Streptomyces and *Lactobacillus* spp. that improve crop growth and yield by increasing photosynthesis, nitrogen fixation, controlling soil diseases and accelerating decomposition of lignin materials in the soil (Hussain *et al*., 1994). Some workers have studied the effect of EM application on VAM development and subsequent crop growth and yield in maize, pea, wheat, soybean, chickpea, *Trifolium* and sunflower (Bajwa and Jilani, 1994; Bajwa *et al*., 1997, 1998, 1999; Javaid *et al*., 1995, 1999), however, the results were controversial. The present study was, therefore, undertaken to investigate the effect of EM application on VAM development and subsequent crop growth and yield in *Vigna radiate.* Two types of manure viz. green manure and farmyard manure were used as organic amendments in two types of soil having different histories of EM applications.

Materials and Methods

Two types of soils were used in the pot experiment conducted during July-August 1998 at the Botany Department University of the Punjab, Quaid-e-Azam Campus Lahore. Soil 1 received the EM in November 1997 for the first time and *Brassica campestris* was grown as the first test crop at that time. In soil 2, EM application was started in May 1998 to be used in the present experiment. Two types of organic amendments viz. farmyard manure (FYM) and *Trifoium alexandrianum* green manure (GM) were used in the experiment. The amendments were mixed in the soils of respective pots at 10 percent volume of soil. In each soil type there were four treatments viz. FYM, FYM +EM, GM and GM +EM, In EM treated pots amendments were treated with a dilute solution of EM in water (1:1000) seven days before mixing in the pot soil (Anonymous, 1995).

Seeds of *Vigna radiate* were surface sterilized with 1.0 % sodium hypochlorite solution. Five seeds were sown in each pot, which were thinned to 2 seedlings on emergence. Each treatment was replicated thrice. Plants were irrigated with tap water whenever required. The EM treated pots also received 300 ml of dilute EM solution in water (1:1000) at fortnight intervals throughout the experimental period. Pots were kept in open in a wire house. Plants were harvested 40 and 60 days after sowing. On each harvest nodules were carefully separated from roots and counted. The fresh and dry weights of roots, shoots, pods and nodules were recorded. Data were analyzed statistically by applying t-test. A part of fresh roots were cut into 1-cm pieces. These root pieces were cleared and stained following the procedure of Phillips and Hayman (1970) for VAM infection study. The stained root pieces were examined under compound microscope. The percentage frequencies of occurrence of VAM structures (mycelium, arbuscules and vesicles) were recorded. The extent of VAM infection was estimated by slide length method (Giovannetti and Mosse, 1980).

Results

A variable response of shoot growth to EM was observed with respect to soil type, organic amendment and growth stage. In FYM amended soil 1 shoot length was reduced by EM application at 60 days growth stage (DGS) while shoot biomass showed an insignificant response at both the growth stages viz. 40 and 60 days after sowing. In GM amended soil 1 response of plant height to EM application was insignificant while shoot biomass was increased significantly at 60 DGS after an initial decline at early growth stage. In soil 2 a persistent negative impact of EM was recorded on shoot growth with either organic amendment

Table 1: Effect of EM application on VAM colonization in *Vigna radiata*

Soil types	Treatments	40 days after sowing: VAM infection (%) M	A	V	Extent of Infection (cm/100 cm of root)	60 days after sowing: VAM infection (%) M	A	V	Extent of Infection (cm/100 cm of root)
Soil 1	Farmyard manure	90	30	10	35.5	100	0	60	51
	Farmyard manure+EM	80	30	50	40	100	20	90	85
	Green manure	100	20	100	60	100	10	60	44
	Green manure+EM	70	10	80	70	90	0	90	50
Soil 2	Farmyard manure	70	30	60	40	90	20	50	13.5
	Farmyard manure+EM	50	40	40	30	50	0	40	17
	Green manure	20	0	20	10	80	0	50	20
	Green manure+EM	50	10	40	22	80	0	40	22

Soil 1 = EM application was started in November 1997. Soil 2 = EM application was started in May 1998.
M = mycelium, A = arbuscnles, V = vesicles

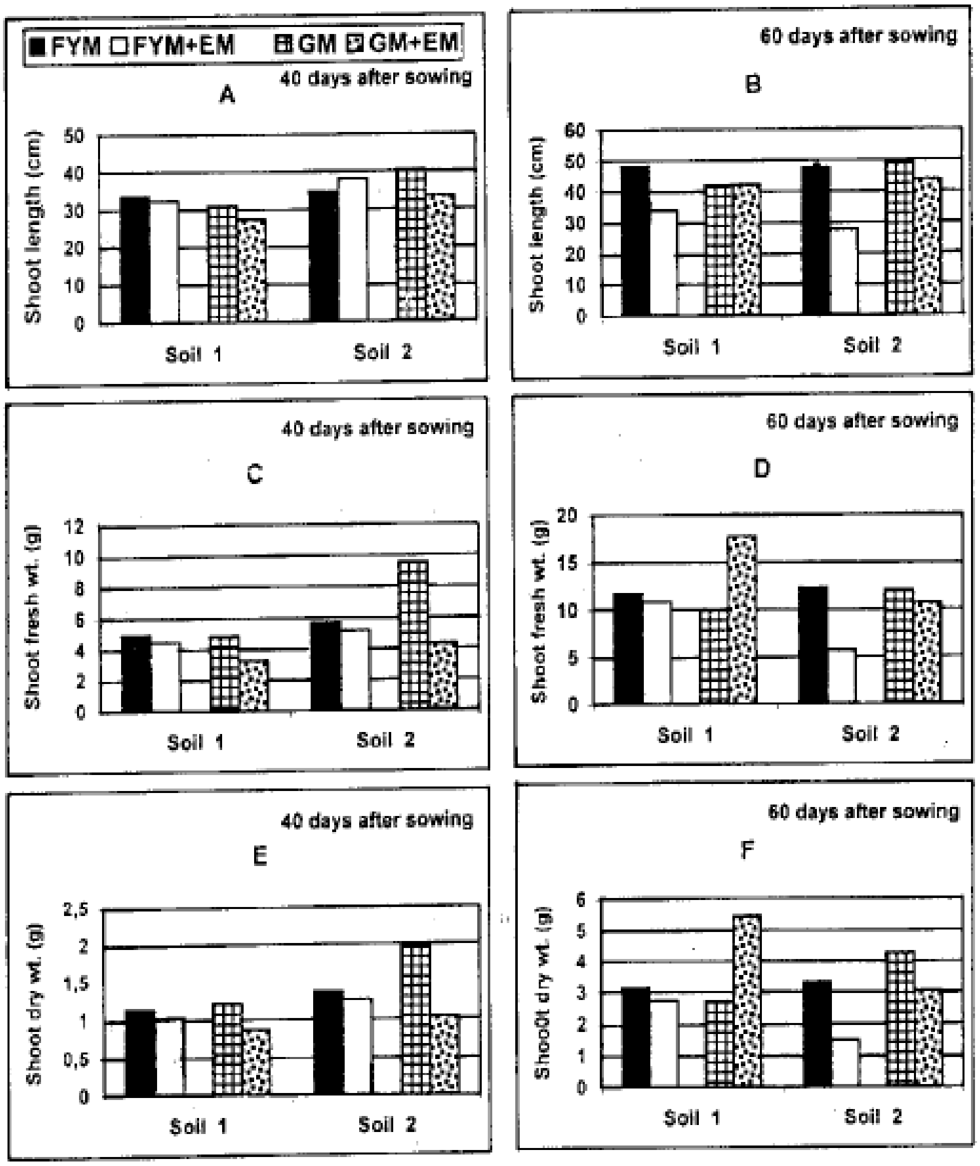

Fig. 1(A-F): Effect of EM appllications on shoot growth of *Vigna radiata* in two types of soils.
Soil 1: EM application started in November 1997 Soil 2: EM application started in May 1998
FYM: Farmyard manure GM: Green manure EM: Effective microorganisms

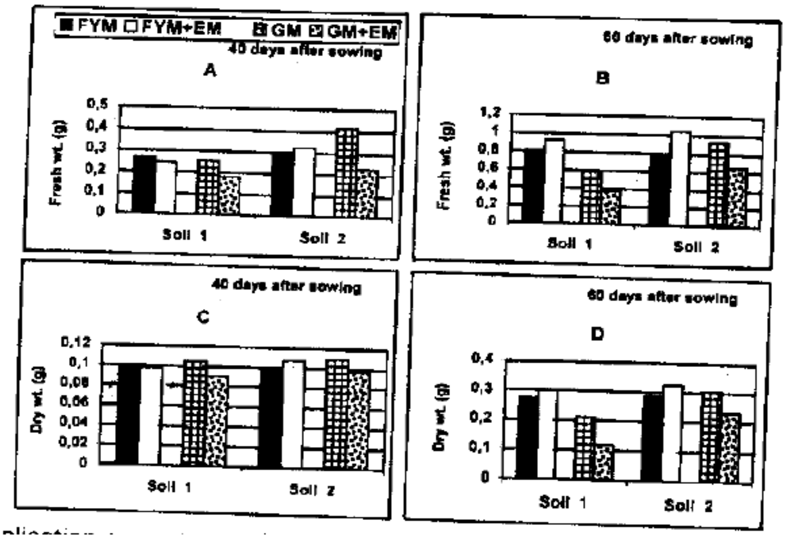

Fig. 2(A-D): Effect of EM application on root growth of Vigna radiata in two typed of soils
Soil 1: EM application started in November 1997 Soil 2: EM application started in May 1998
FYM: Farmyard manure GM: Green manure EM: Effective microorganisms

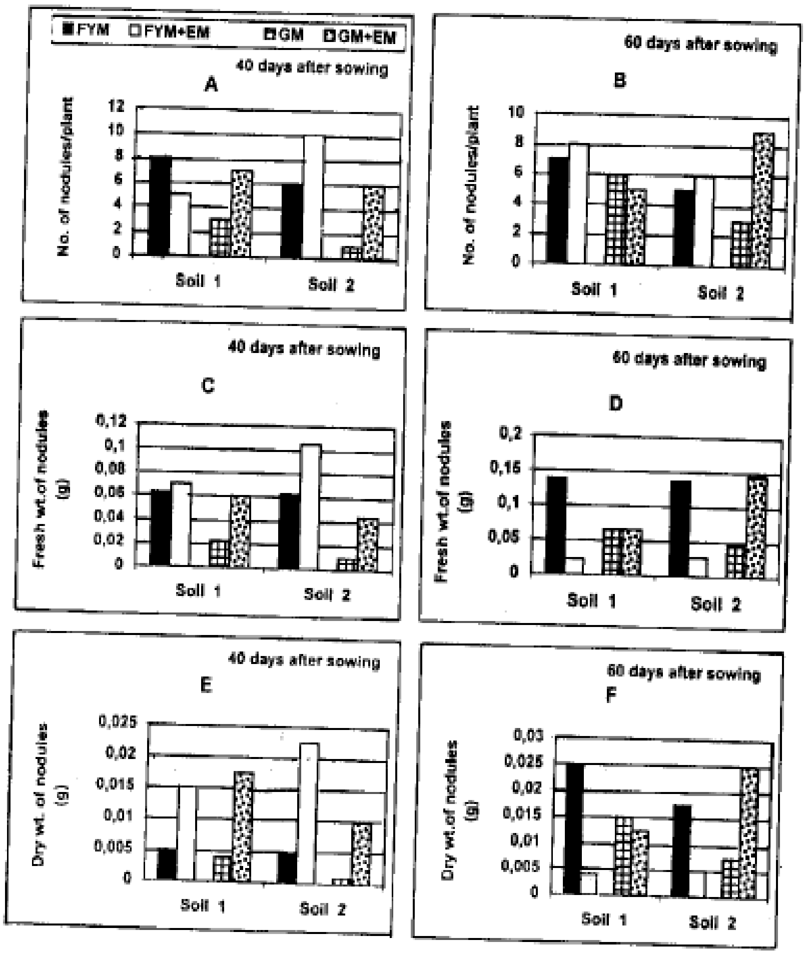

Fig. 3(A-D): Effect of EM application on nodule formation in Vigna radiata in two types of soils
Soil 1: EM application started in November 1997 Soil 2: EM application started in May 1998
FYM: Farmyard manure GM: Green manure EM: Effective microorganisms

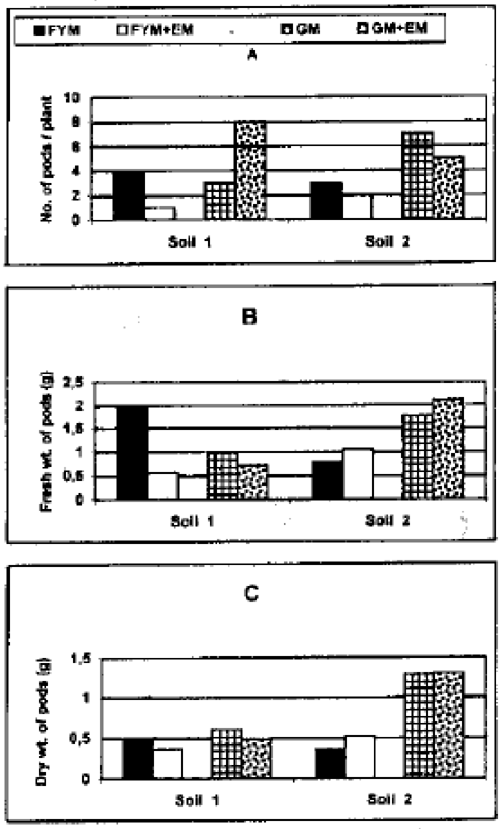

Fig. 4(A-C): Effect of EM application on pod yield in *Vigna radiate* in two types of soils
Soil 1: EM application started in November 1997
Soil 2: EM application started in May 1998
FYM: Farmyard manure GM: Green manure
EM: Effective microorganisms

(Fig. 1). Root biomass was increased by EM application in FYM but reduced in GM amended soils (Fig. 2).

Nodulation in terms of number and biomass was significantly enhanced by EM application in both the soils with either organic amendment at 40 DOS except nodule number in FYM amended soil 1. At 60 DOS a variable response of nodulation to EM was observed with respect to soil type and organic amendment. With FYM amendment nodule number was increased but biomass was reduced significantly ($P = 0.05$) due to EM application in both the soils. With GM amendment response to EM was insignificant in soil 1 while a significant increase in number and biomass of nodules was recorded in soil 2 (Fig. 3).

Pod yield in terms of number of pods per plant was suppressed by EM application in FYM amended but increased in GM amended soil 1. However, EM invariably reduced pod fresh and dry weights in soil 1 irrespective of the organic amendment. In soil 2 EM application decreased the pod number but increased fresh weight with either organic amendment and dry weight in FYM amended soil. Dry weight in GM amended soil was, however, remained unaffected by EM application (Fig. 4).

Variable response of VAM colonization to EM application was observed with respect to soil type, growth stage and organic amendment used. Generally EM favoured the VAM colonization in soil 1 at both the growth stages with either organic amendment. Effect was more pronounced in FYM amended soil at 60 days growth stage. In FYM amended soil 2 EM application adversely affected the VAM development at 40 DOS while a slight increase in extent of VAM infection was observed at later growth stage. In GM amended soil 2 EM application enhanced VAM development at 40 days growth stage while no remarkable effect was observed at later growth stage (Table 1).

Discussion

EM application reduced the shoot growth in soil 2 where EM application was started in May 1998, two months prior to mungbean cultivation while in soil 1 where EM application was started in November 1997, shoot growth showed a positive response to EM application when soil was amended with Trifolium GM. However, the shoot response to EM application in FYM amended soil 1 was slightly negative but insignificant. It reveals that growth response is dependent upon history of EM application as well as organic manure incorporated in the soil. Experiences of other workers (Lin, 1991; Panchaban, 1991; Bajwa *et al.*, 1999) revealed that the effect of EM on crop growth and yield was usually not evident or even negative in the first test crop. Generally the crop growth and yield tend to increase gradually as subsequent crops are grown (Higa, 1989).

Root's growth response to EM application was independent of history of EM application but dependent upon the type of organic amendment, A positive response to EM was observed in FYM amended soil while EM application adversely affected the root growth when GM was added to the soil, with either history of EM application.

EM enhanced nodulation at early growth stage irrespective of history of EM application and type of organic manure. However, at later growth stage EM reduced the size and biomass of the nodules in FYM amended soils. Earlier have reported increased nodulation in *Vigna radiate* and *Phaseolus vulgaris* due to EM application under agro-ecological conditions of Sri Lanka. Javaid *et al.* (1995) also reported similar increase in nodulation in pea. This enhanced nodulation could be due to the increased rhizobial population due to EM. However, in the present study any relationship between nodulation and crop vegetative or reproductive growth was entirely lacking.

The response of VAM colonization to EM was better in soil 1 with a parallel increase in shoot growth as compared to soil 2. Earlier Bajwa and Jilani (1994) and Javaid *et al.* (1999) have observed an enhanced VAM colonization and crop growth due to EM application in maize and sunflower respectively. Similar effects of EM on VAM colonization and crop growth have also been reported by Bajwa *et al.* (1998) in *Cicer arietinurn.* However, in contrast to these Javaid *et al.* 1995 reported that EM increases the growth and yield but suppress VAM colonization in pea. Recently Bajwa *et al.* (1999) observed enhanced VAM colonization but reduced crop growth due to EM application in *Trifolium alexandrianum*.

The earlier studies regarding the effects of EM application on crop growth, yield and VAM colonization were mainly confined to first test crop only. Although the present study represents the effect of EM on crop growth and yield, nodulation and. VAM colonization in two soils with different histories of EM applications, however, the difference in starting of EM application was of only six months. Therefore, further investigations of long term effectiveness of EM on crop productivity are required before any specific conclusion is drawn.

References

Anonymous, 1995. EM Application Manual for APNAN Countries. 1st Edn., Asia-Pacific Natural Agriculture Network, Bangkok, Thailand.

Azcon-Aguilar, C. and. J.M. Barea, 1985. Effect of soil micro-organisms on formation of vesicular-arbuscular mycorrhizas. Trans. Br. Mycol. Soc., 84: 536-537.

Bagyaraj, D.J. and J.A. Menge, 1978. Interaction between a VA mycorrhiza and *Azotobacter* and their effects on rhizosphere microflora and plant growth. New Phytol., 80: 567-573.

Bajwa, R. and S. Jilani, 1994. Mycorrhizal colonization and subsequent growth response to co-inoculation with VA mycorrhiza and EM in maize. Proceeding of the 2nd National Seminar on Nature Farming, September 18, 1994, University of Agriculture, Faisalabad, Pakistan, pp: 88-101.

Bajwa, R., A. Javaid and T. Akhtar, 1997. EM and VAM technology in Pakistan. III: Effect of co-inoculation of EM and VAM on growth of wheat. Sci. Int. (Lahore), 9: 311-314.

Bajwa, R., A. Javaid and B. Haneef, 1998. EM and VAM technology in Pakistan. Effect of co-inoculation of effective microorganisms (EM) and VA-mycorrhiza on plant growth and nutrient uptake in chickpea (*Cicer arietinum* L.). Pak. J. Phytopathol., 10: 48-52.

Bajwa, R., A. Javaid and N. Rabbani, 1999. 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. Pak. J. Biol. Sci., 2: 590-593.

Barea, J.M., M.E. Brown and B. Mosse, 1973. Association between VA mycorrhiza and *Azotobacter*. Rothamsted Experimental Station Annual Report, pp: 82.

Bethlenfalvay, G.J. and R.G. Linderman, 1992. Mycorrhizae in Sustainable Agriculture. American Society of Agronomy Inc., Wisconsin, USA., ISBN-13: 9780891181125, pp: 45.

Daniels, B.A. and J.M. Trappe, 1980. Factors affecting spore germination of the vesicular-arbuscular mycorrhizal fungus, *Glomus epigaeus*. Mycologia, 72: 457-471.

Giovannetti, M. and B. Mosse, 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol., 84: 489-500.

Higa, T., 1989. Studies on applications of microorganisms in nature farming. II-The practical application of Effective Microorganisms (EM). Proceedings of the IFOAM 7th International Scientific Conference, January 2-5, 1989, Ouagadougou, Burkina Faso, West Africa.

Hussain, T., R. Ahmad, G. Jilani and T. Higa, 1994. Applied EM technology. Nature Farming Research Center, University of Agriculture Faisalabad, Pakistan.

Javaid, A., R. Bajwa and I. Siddiqi, 1999. EM and VAM technology in Pakistan VI: Effect of EM (Effective Microorganisms) on VA mycorrhizal development and subsequent crop growth and yield in sunflower. Pak. J. Biol. Sci., 2: 586-589.

Javaid, A., R. Bajwa, N. Rabbani and Q. Ahmad, 1995. Growth, nodulation, nitrogen nutrition and VAM colonization of pea (*Pisum sativum* L.) in soil treated with EM. Acta Sci., 5: 1-6.

Jeffries, P. and L.H. Rhodes, 1987. Use of mycorrhizae in agriculture. Crit. Rev. Biotechnol., 5: 319-357.

Lin, L., 1991. Nature farming in Taiwan: Effect of EM on growth and yield of paddy rice. Proceedings of the 1st International Conference on Kyusei Nature Farming, October 17-21, 1989, Khon Kaen, Thailand, pp: 125-131.

Panchaban, S., 1991. Effect of EM on growth and yield of corn. Proceedings of the 1st International Conference on Kyusei Nature Farming, October 17-21, 1989, Khon Kaen, Thailand, pp: 132-139.

Phillips, J.M. and D.S. Hayman, 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc., 55: 158-161.

Ross, J.P., 1980. Effect of nontreated field soil on sporulation of vesicular-arbuscular mycorrhizal fungi associated with soybean. Phytopathology, 70: 1200-1205.

Wilson, G.W.T., B.A.D. Hetrick and D.G. Kitt, 1988. Suppression of mycorrhizal growth response of big bluestem by non-sterile soil. Mycologia, 80: 338-343.