Asian J. Exp. Sci., Vol. 20, No. 2, 2006, 261-268

Effect of VAM soil Containing *Glomus fasciculatum* on Growth of *Withania somnifera* Dun

S. Halder[1] and M.B.Ray[2] *

1. Department of Botany, Burdwan Raj College, Burdwan, India
2. Department of Botany, Burdwan University, Burdwan, India

Abstract : *Withania somnifera* is an important medicinal and endangered plant growing as weed. The present sets of experiment have been designed to establish *Glomus fasciculatum* with the root system of *W. somnifera* and to analyze the effectiveness of this mycorrhiza on vegetative growth and absorption of nutrients from the soil. Plants were grown in plastic pots under aseptic conditions. The VAM soil containing *Glomus fasciculatum* was applied once after 30 days of transplanting in one set of plants, and twice after 30 days and 75 days of transplanting in another set of experiment. The establishment of the fungus within the root in the form of vesicle was observed. Formation of vesicles within the root indicated the affinity of the fungus with the plant. Mycorrhizal treatment showed better vegetative growth in comparison to untreated plants. Significant increase in height, number of branches and number of leaves over non-treated plants were found. Further observation showed the increase of fresh weight and dry weight of stem, leaf and root. The mycorrhiza like *Glomus fasciculatum* also enhanced the levels of phosphorus in different parts of treated plants, but nitrogen levels were higher in different parts of the plant when VAM soil applied once such results pointed out that *Glomus fasciculatum* has an effective role as bio-fertilizer on *Withania somnifera*.

Key words : VAM, medicinal plant, growth, phosphorus, nitrogen, mycorrization.

Introduction :

Withania somnifera, Ashwagandha, is used in Indian traditional system of medicine since long ago. The pharmacological activity of the root is attributed to the presence of several alkaloids; withanolides is obtained from leaves. It is necessary to study the growth and development of the plant like *Withania somnifera* because general extinction of medicinal plant resources including *Withania somnifera* in the potential districts of West Bengal is a great problem. In Ayurveda, the drug is claimed to be effective in the treatment of rheumatic pain, inflammation of joints, nervous disorder and epilepsy.

W. somnifera is generally grown wildly in warmer parts of India especially in Madhya Pradesh (Atal and Schwarting, 1961). The cultivated varieties are sown during April/May (30º to 35º C). One biological tool is now being integrated into biotechnology is the development of commercial VAM inoculants for use in agriculture, horticulture and forestry. The role of VAM on the growth and phosphate nutrition of various plants has been studied extensively (Mosse, 1973; Gerdemann, 1975). Meyer and Linderman (1986) enumerated bacteria and surveyed their community structure in mycorrhizal and nonmycorrhizal plants. They observed no difference in the total numbers of culturable

* **Corresponding author :** Dr. M.B. Ray, Plant Physiology and Biochemistry Section, Department of Botany, Golapbag, University of Burdwan, Burdwan – 713 104, India.

bacteria, but noted specific bacteria present more frequently in the mycorrhizal rhizospheres. Mycorrhizal plants develop extensive root system as compared to nonmycorrhizal plants, which ensures the plant with increased availability of water and nutrient, thereby helping the plant for better growth and development (Carling *et al.*, 1978; Jalali and Thareja, 1985; Manjunath and Bagyaraj, 1986).

Plants require large amount of P, but available form is very low in the soil. Large amount of P is present in inorganic forms. Again, the availability of P in the organic forms e.g. phytate, is controlled by mineralization rates in soil. The primary advantage of mycorrhizal hyphae in P uptake is the ability of hyphae to extend beyond the P depletion zone of the roots (Jakobsen *et al.*, 1994., Jakobsen, 1995). Verma (1998) reported that mycorrhizal treated plants showed better growth than non-mycorrhizal plant at lower level of phosphate in the soil.

So, the present sets of experiment have been designed to establish *Glomus fasciculatum* with the root system of *W. somnifera* and to analyze the effectiveness of this mycorrhiza on vegetative growth and absorption of nutrients from the soil.

Materials and Methods :

The VAM soil containing *Glomus fasciculatum* was collected from the Department of Botany and Forestry, Vidyasagar University. Seeds of *Withania somnifera* were collected from the Department of Botany, Burdwan University. The seeds were sown on earthen pots containing sterilized soil. The initial level of P and N_2 of the soil were recorded; the P and N_2 levels were found 0.012% and 0.19%, respectively. The seedlings were transplanted in the plastic pots containing sterilized soil after four months. The soil was sterilized in autoclave under pressure 15 lbs. for 15 mins. Ten replicates were taken for each treatment.

In control set, VAM soil was not inoculated but it was applied once after 30 days of transplanting in one set of plant (VAM +) and twice after 30 and 75 days of transplanting in another set (VAM ++) of experiment. After eight months, the plants were carefully harvested including roots. To study the mycorrhizal association, the root tissue was macerated and stained with 0.5% tryptan blue (Muthukumar and Udaiyan, 2000). Macerated tissue was observed under microscope. The different growth parameters like height, number of branches and number of leaves per plant were noted. The fresh and dry weights of the different plant parts were recorded. Phosphorus and nitrogen were estimated by following the methods of Murphy and Riley (1962) and Vogel (1961), respectively. For statistical calculations we have taken the model and formulas according to Emerson and East (1913), Finley and Wilkinson (1963), Fisher and Yates (1938), Nelder (1960) and Panse and Sukhatma (1954).

Results :

It was observed that prominent vesicles of *Glomus fasciculatum* had formed within the root tissue (Plate I and II). It indicated the affinity of the fungus with the host, *Withania somnifera*.

Different growth parameters were studied at harvest. It was noted that plants were growing better when VAM was applied twice (VAM++) in comparison to applied once (VAM+). Significant increase

Plate I : Vesicle with intcrcellular hyphae of *Glomus fasciculatum* in the root of *Withania somnifera* (x 450).

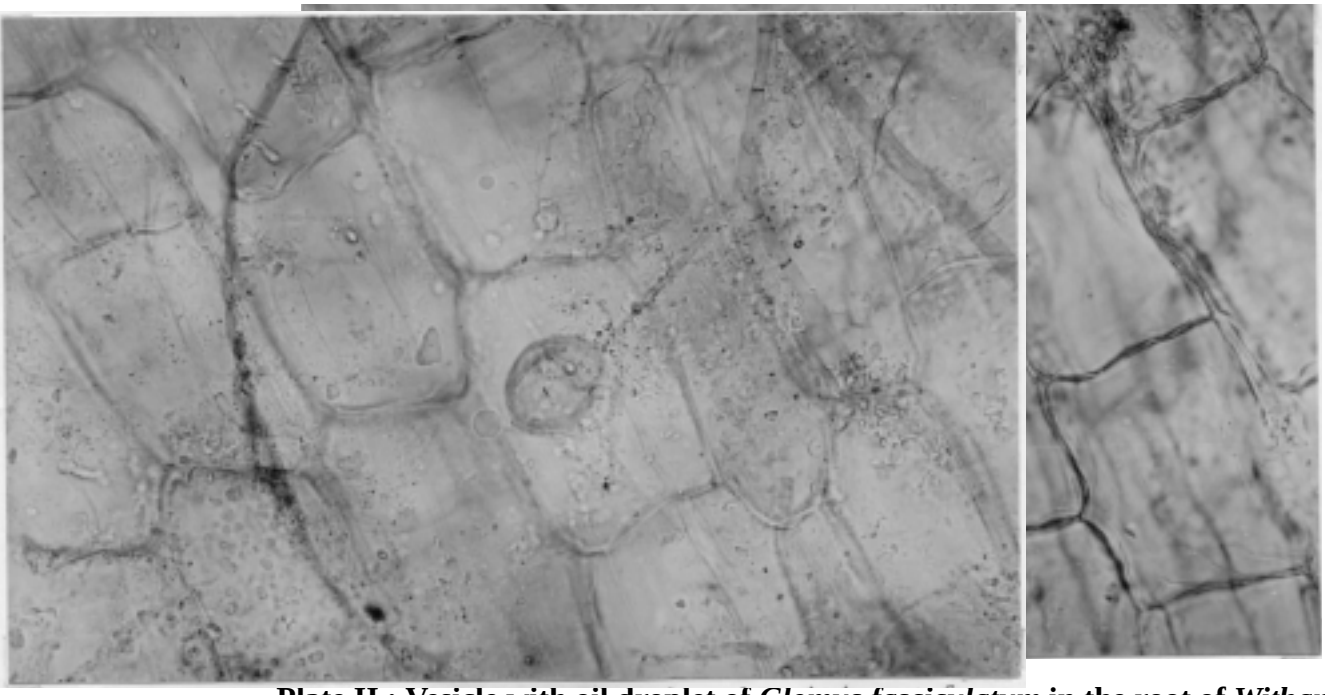

Plate II : Vesicle with oil droplet of *Glomus fasciculatum* in the root of *Withania somnifera* (x 450).

in height, leaf nos. and branch nos. of the plant were observed in VAM (++) treatment (Table 1). These parameters insignificantly increased in the plants of VAM (+) treatment. It indicated that this fungus showed influencing role on vegetative growth of the host plant. Takenaga *et al* (1998) reported that VAM fungi had promising role on the growth of the host plant.

Fresh weight and dry weight of stem, leaves and roots and root/shoot ratio were studied at harvest (Table 2). The fresh weights of stem, leaves and roots were significantly different among the treatments. Maximum fresh weights of stem, leaves and roots were observed in VAM (++). Dry weights of different parts of plant showed similar trend. Fresh weight of root/shoot ratio showed insignificant difference whereas dry weight had significant difference. More dry weight was observed in VAM (++) treatment.

The percentage of increase of height, leaves no. and branch no. of the plant were observed (Table 3). The result showed more vegetative growth in the plants of VAM (++) treatment compared to the plants of VAM (+) treatment.

Discussion :

VAM fungi enhance the root growth, expand the absorptive capacity of the root system for nutrient (Giovannetti, 1985; Hayman, 1982; Reid, 1984). The mycorrhizal plant showed significantly greater growth performance in leaf number, leaf area, diameter, root/shoot ratio as well as P uptake over non-mycorrhizal plant (Verma, 1998). VAM fungi ensure the plant with increased availability of water and nutrient, thereby helping the plant for better growth and development (Carling *et al.*,1978; Jaladi and Thureja, 1985; Manjunath and Bagyaraj, 1986). Fluctuation in mycoflora population was studied under low (25%), medium (50%) and waterlogged moisture conditions by treating rice (IR-36) with VAM + *Azolla* combinations and singly with VAM and *Azolla.* Variations were recorded as number of CFU gm-1 soil × 104 using rhizosphere soil. Fungal population was found to be increased and well maintained both in medium moisture condition and in VAM+*Azolla* combined treatment. This condition was found to be congenial for both mycorrhizal colonization and leaf phosphate accumulation in rice (Ghosh *et al*, 2004).

The study confirmed that *Glomus fasciculatum* infection and symbiosis occurred in *Withania somnifera*. This symbiotic phenomenon led to the promotion of growth of the plant. Our results indicated that *Glomus fasciculatum* inoculation was effective in increasing the height, nos. of leaves and nos. of branches per plant. Takenaga *et al* (1998) reported that VAM fungi increased the nos. of leaves and flowers. Similar report was presented by Matsubara and Sakurai (2000) who also reported that plant production was highly variable with or without mycorrhizae. Production of *Salsola kali* was often reduced when mycorrhizal fungi were present (Allen and Allen 1986) whereas *Artemisia tridentate* showed positive growth responses to mycorrhizae (Allen *et al.,* 1987).

Gill *et al* (2002) reported that biomass of the plant increased by mycorrhizal infection. Our result showed that root/shoot ratio in terms of dry weight increased in

Table 1 : Effect of VAM on vegetative growth of *Withania somnifera*.

Treatment	Height/plant (cm)	No. of branches/ plant	No. of leaves/ plant
Control	72 ±0.021	2.5 ±0.013	56 ±0.11
VAM(+)	77 ±0.165	4.5 ±0.114	58 ±0.23
VAM(++)	82 ±0.185	6.0 ±0.109	66 ±0.28
CD at 5% level	2.117	1.783	4.915

Table 2 : Effect of VAM on fresh and dry weights of different plant parts of *Withania somnifera*

Treatment	Stem		Leaves		Root		Root/shoot ratio	
	Fr.wt. (g)	Dry wt. (g)	Fr.wt. (g)	Dry wt. (g)	Fr.wt. (g)	Dry wt (g)	Fr.wt. (g)	Dry wt (g)
Control	79±0.02	12.5±0.012	22±0.014	3.8±0.01	25±0.01	4.1±0.012	0.24±0.014	0.25±0.01
VAM(+)	99±0.11	14.0±0.12	33±0.11	4.5±0.11	30±0.2	6.9±0.12	0.22±0.14	0.37±0.15
VAM(++)	111±0.2	14.9±0.19	47±0.22	6.3±0.2	36±0.21	8.7±0.22	0.22±0.21	0.41±0.26
CD at 5% level	0.1034	1.0539	0.1942	1.5391	0.6938	1.2413	1.2451	0.7934

Table 3 : Effect of VAM on percentage of increase of different growth parameters of *Withania somnifera*.over nontreated plant

Treatment	Height	No. of Branches	No. of leaves	Fr. Wt.			Dry wt.		
				Stem	Leaf	Root	Stem	Leaf	Root
VAM(+)	6.0	80	4.0	25	50	20	12	18	68
VAM(++)	13	140	17	40	113	44	19	55	112

VAM treated plant and more increase was noticed in the plants of VAM(++) treatment. From this result, it can be concluded that the shoot part has grown more compared to root due to mycorrhiza. Finlay (1985) observed that plants in insecticide treated plots had higher shoot mass. The increase was not due to change in P availability but due to reduction in root feeding insects. However, Abbott and Robson (1984), Powell (1984) and Menge(1981) reported that improved growth of the plant required mycorrhizal infection and reduced level of P in the soil.

In mycorrhizal treated plants, the P content in root, stem and leaf showed higher level than non-treated plants. Again more P content observed in shoot portion than root (Fig. 1). But N_2 content is higher in root, stem and leaf when VAM soil applied once (Fig. 2). The influence of mycorrhiza on P uptake by plant has reviewed for many years (Tinker, 1975). From our result, it is

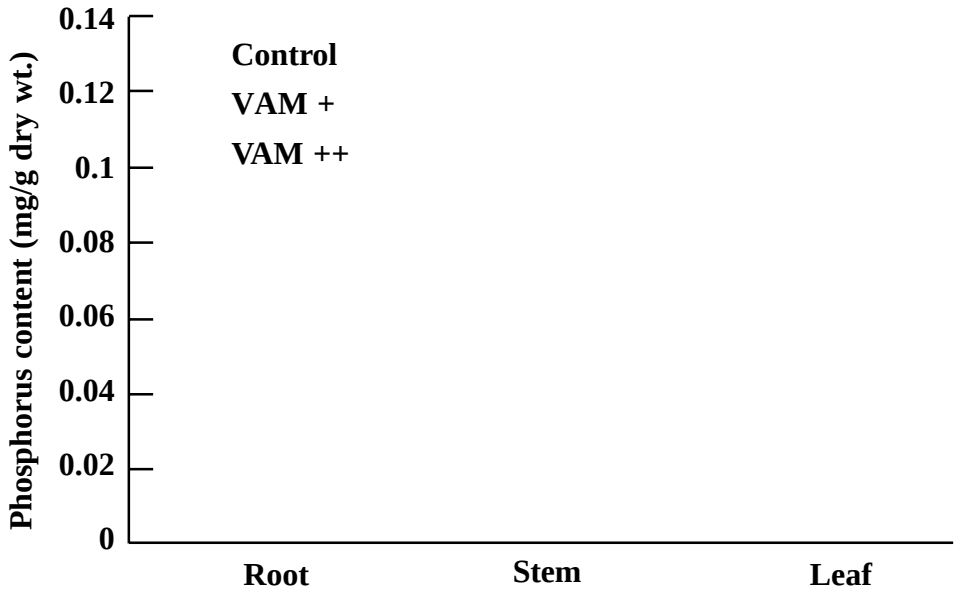

Fig. 1 : Effect of VAM on Phosphorus content (mg/g dry wt.) in different plant parts of *W. somnifera*

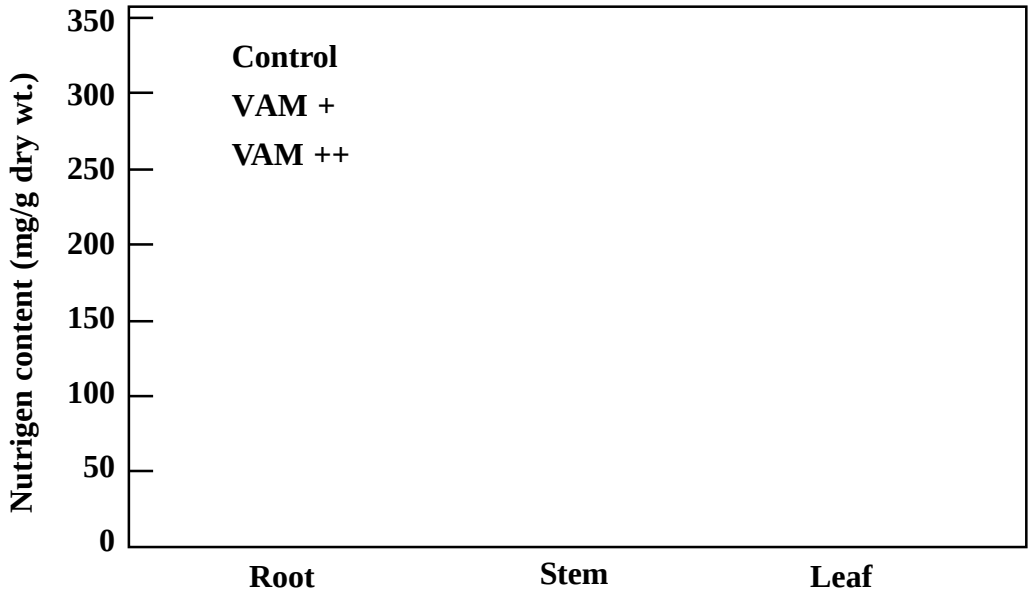

Fig. 1 : Effect of VAM on Nitrogen content (mg/g dry wt.) in different plant parts of *W. somnifera*

indicated that higher levels of P and N_2 in root, stem and leaf could be the result of the active role of mycorrhizal fungus.

Available form of P is poor in the soil. Mycorrhiza appears to convert the non-available form of P to available form. A more specific adaptation of hyphae could be a high surface phosphatase activity to mobilize organic P (George *et al.*, 1995). It is also reported that dry wt. of the shoot increased with the increase of P uptake when inoculated by *Glomus aggregatum, G. fasciculatum and G. mosseae* (Matsubara and Sakurai, 2000). Johansen *et al* (1994) investigated the influence of plant N_2 status on the hyphal N_2 transport to host plant in the association *Glomus intraradicus*, *Cucumis sativus*, *Glomus fasciculatum* inoculated in black pepper. It resulted in the increased tissue N, P and leaf nitrate reductase activity (Shivashanker and Iyer, 1998). Shreenivasa and Gaddagimani (1993) found better plant height and increased dry matter yield in chilli inoculated with *Glomus macrocarpum* with 50 Kg P_2O_5/Ha.

From our observation, it can be concluded that *Glomus fasciculatum* helps to increase the vegetative growth of *Withania somnifera* by drawing different elements particularly P and N_2 from the soil.

References :

Abbott L.K. and Robson A.D. (1984) : The effect of mycorrhizae on plant growth. In. *VA Mycorrhiza*. (Eds.) C.L. Powell and D.J. Bagyaraj, CRC Press. Boca Raton. FL, 113-130.

Allen E.B. and Allen M.F. (1986) : Water relations of xeric grasses in the field : Interactions of mycorrhizae and competition. *New Phytologist* **104,** 559-571.

Allen M.F., Allen E.B. and West N.E. (1987) : Influence of parasitic and mutualistic fungi on *Artemisia ridentate* during high precipitation years. *Bulletin of the Torrey Botanical Club* **114,** 272-279

Atal C.R. and Schwarting, A.E. (1961) : Intraspecific variability in *Withania somnifera*. I. A. Preliminary Survey. *Llyodia*. **25**, 78-88.

Carling D.E., Riehle W.G., Brown M.F. and Johnson D.R. (1978) : Effect of a vascular-arbuscular mycorrhizal fungus on nitrate reductase and nitrogenase activities in nodulating and non-nodulating legumes. *Phytopathology*. **68**, 1590.

Emerson R.A. and East E.M. (1913) : The inheritance of quantitative characters in maize. Bull. Agr. Exp. Sta. Nebraska. Res. Bull 2.

Finlay R.D. (1985) : Interactions between soil micro-arthopods and endomycorrhizal associations of higher plants. In. *Ecological interactions in soil*. (Eds) A.H. Fitter , D. Atkinson, D.J. Read and M.B.Usher. Special publication no. 4 of British Ecological Society. Blackwell. Scientific Publications. Oxford 319-331

Finley K.W. and Wilkinson G.M. (1963) : The analysis of adaptation in plant breeding programme. *Aust. J. Agric. Res.* **14.** 742-757.

Fisher R.A. and Yates F. (1938) : Statistical tables for biological, agricultural and medical research, 5 Aufl. Oliver and Boyd, Edinburgh.

George E., Marschner H. and Jakobsen I. (1995) : Crit. Rev. *Biotechnol.* **15,** 257-270

Gerdemann J. (1975) : Vesicular- arbuscular mycorrhizas. In.*The development and function of roots*. (Eds.) J.G. Torrey and D.T. Clarkson. Academic Press. London 579-591

Ghosh I., Chakrabarti, J., Kushari D. P. and Chatterjee, N. C. (2004): Integrated Effect of VAM and *Azolla* on Fluctuations in Fungal Population, Mycorrhization and Leaf Phosphates in Rice under Different Moisture Conditions. *Asian J. Exp. Sci.*, **18,** *27-35*

Gill T.S., Singh R.S. and Kaur J. (2002) : Comparison of four arbuscular mycorrhizal fungi for root colonization, spore population and plant growth responses in chickpea. *Indian Phytopath.* **55 (2)** 210-212.

Giovannetti M. (1985) : Seasonal variations of vesicular arbuscular mycorrhizas and endogonaceous spores in a maritime sand dune. *Trans. Br. Mycol. Soc.* **84**, 679.

Hayman D.S. (1982) : The physiology of vesicular endomycorrhizal symbiosis.*Can.J.Bot.* **6**, 944.

Jakobsen I. (1995) : In : *Mycorrhiza* (Eds.) A. Verma and B. Hock, Springer, Berlin, Heidelberg, New York, 297-324.

Jakobsen I., Joner E.J.and Larsen J. (1994) : Impact of arbuscular mycorrhizas on sustainable agricultural and natural ecosystem. In : *Gianinazzis*. (Eds.) H. Schiiepp. Birkhauser, Basel, 133-146.

Jalali B.L. and Thareja M.L. (1985) : Plant growth responses to VA mycorrhizal inoculation in soils incorporated with rock phosphate. *Indian Phytopath.* **38**, 306.

Johansen A., Jakobsen I. and Jensen E.S. (1994) : *Plant Soil*, **160**, 1-9.

Manjunath A. and Bagyaraj D.J. (1986) : Response of black gram, chickpea and mungbean to VAM inoculation in an unsterile soil.*Tropical Agriculture* **63,** 33

Matsubara Yoh-ichi and Sakurai Satoko (2000) : Effect of arbuscular mycorrhizal fungus inoculation on growth of Sandersonia. *J. of Soc. Of High Technol. In Agric.* **12,** 47-52.

Menge J.A. (1981) : Mycorrhiza agriculture technologies. In. *Back ground papers for innovative biological technologies for lesser developed countries.* (Eds.) US Congress. Office of Technology Assessment, US Government Printing Office. Washington DC. 383-424.

Meyer, J.R. and Linderman, R.G. (1986) : Selective influence on populations of rhizosphere or rhizoplane bacteria and actinomycetes by mucorrhizas formed by Glomus fasciculatum. Soil Biology and Biochemistry **18,** 191-196.

Mosse B. (1973) : Advances in the study of vesicular-arbuscular mycorrhizas IV In soil given additional phosphate. *New Phytol.* **72,** 127-136.

Murphy J. and Riley J. (1962) : A modified single solution method for the determination of phosphate in natural water. *Anal. Chem. Acta.***27,** 31

Muthukumar T. and Udaiyan K. (2000) : Vesicular arbuscular mycorrhizae in pteridophytes of western ghats, southern India. *Phytomorphology* **50(2),** 132-142.

Nelder J.A. (1960) : The estimation of variance components in certain types of experiment on quantitative genetics. *Biom. Genetics* (Ed.) O. Kempthorne, Pergaman Press. London.

Panse V.G. and Sukhatme P.V. (1954) : Statistical methods for agricultural workers. Published by ICAR

Powell C.L. (1984) : Field inoculation VA mycorrhizal fungi. In *VA Mycorrhiza.* (Eds) C.L. Powell and D.J. Bagyaraj , CRC Press. Boca Raton. FL 205-234.

Reid C.P.P. (1984) : Mycorrhizae : A root soil interface in plant nutrition. In. *Microbe-Plant interactions.* (Eds.)R.I.Todd and J.E. Gidens, ASA Special Pub. **47**, 29.

Shivashankar S., Iyer R. (1989) : Influence of vesicular arbuscular mycorrhiza on growth and nitrate reductase activity of black pepper. *Indian Phytopath.* **41,** 428-433.

Shreenivasa M.N., Gaddagimani N.B. (1993) : Mycorrhizal depending of chilli cultivars. *Zentralbi Mikrobiol.* **148,** 55-59.

Takenaga J., Shioya T. and Miyamoto H. (1998) : The effect of vesicular arbuscular mycorrhizal fungi and amount of fertilizer applied on the growth of cyclamen. *J. Japan Soc. Hort. Sci.* **67,** 451.

Tinker P.B. (1975) : In. *Endomycorrhizas* (Eds.) F.E. Sanders, B.Mosse and P.B. Tinker, Academic Press, London, 353-371.

Verma N.K. (1998) : Effect of VA mycorrhiza on the growth and P uptake in *Eupatorium adenophorum* Spring. (Asteraceae) grown in soil amended with soluble phosphate. *J. Natl. Bot. Soc.*, **52**, 41-45.

Vogel A.I. (1961) : In. A text book of quantitative inorganic analysis. Longman's Green and Co. Ltd., London, UK.