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Reactions of VAM and *Azospirillum* Species on Wheat Growth

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Abstract

Axenic rhino base inocula of indigenous *Glomus macrocarpum* and *Azospirillum brasilense* were prepared to study the effect of their separate and combined inoculations on wheat growth. Inoculations either separate or in combined form of the inocula at 10, 20, 40 g/200 g in wheat vars. Blue Silver variety showed a significant increase (p<0.0011 in VAM infection (%), fresh weight, thousand grains weight (TGW) and dry weight (p<0.05) at the third dose (40 g/200 g soil) whereas the increase in plant height and grains number/head found non significant at the same dose as compared to the control treatments (00 g/200 g soil). The above inoculations in any form never showed adverse effect on wheat growth. In separate inoculations, the response of *G. macrocarpum* was found to be dominated over the *A. brasilense.* The combined form of the inocula always found highly effective for the aforesaid parameters in respect of the control treatment indicating a mutual beneficial interaction between them. Such reactions of the inocula on wheat growth are being reported for the first time from Sindh, Pakistan.

Introduction

Phosphorus (P) and Nitrogen (N) are the principal nutrients for plants, their optimum uptake lead toward the better growth and yield. Some effective soil micro-organisms like species of VAM and *Azospirillum* greatly enhance their mobilisation in crop plants (Baldani *et al*., 1983; Harley, 1989). VAM are symbiotic soil borne fungi, increase the absorptive area of host roots by means of their extra metrical mycelia and greatly enhance the mobilisation of "P" to the host plants which results in the increase of yield many folds (Abbott and Robson, 1984; Diederichs and Moawad, 1993). *Azospirillum* are rhizotrophic bacteria usually present as a free living in rhizospheric soil of cereals or in association with their roots. They efficiently fix and mobilise the atmospheric nitrogen especially in N-poor and micro aerobic soil (Day *et al*., 1975; Boddey *et al*., 1986). *Azospirillum* species also induce the mobilisation of various nutrients from soil to plants by the abundant root hairs formation which leads toward the better growth and yield (Martin and Glatzle, 1982; Kapulnik *et al*., 1987). Therefore, the species of *Azospirillum* could be utilised to enhance the uptake of biologically fixed nitrogen in wheat roots which lacking the root nodulating bacteria *(Rhirobium* spp.) as found in leguminous plants (Okon and Baker, 1987; Mertens and Hess, 1984). According to the some reports (Pacovsky, 1988; Subba Rao *et al*., 1985) growth and yield of crop plants particularly cereals could be enhanced effectively by increasing the uptake of "P" and "N" from soil after the combined inoculations of VAM and *Azospirillum* species. However, the studies on the interactions between VAM and *Azospirillum* species and their effect on plant growth have received very little attention in the world. In Pakistan, such dual inoculations have not ever been carried out to increase the growth and yield of wheat. This research work deals for the first time in Pakistan, the production of axenic rhizobase inocula of indigenous *G. macrocarpum* Tulasne and Tulasne and *A. brasilense* (wild type), their separate and combined inoculations to wheat var. Blue Silver in pot condition.

Materials and Methods

Production of axenic rhizobase VAM inocula: The spores of VAM fungi were extracted from the rhizospheric soil samples (100 g) of wheat, growing in field condition by the sucrose centrifugation method. Of which *G. macrsocarpum.* Tulasne and Tulasne was selected and identified by following Schenck and Perez (1990) and Morton and Benny (1990). Its viability was confirmed by vital stain method (Menge and Timmer, 1982). A single viable and surface disinfected (Menge, 1984) spore of *G. macrsocarpum.* Tulasne and Tulasne was aseptically placed in close proximity with fine feeder roots of 6 days old seedlings of wheat, grown on water agar slant at 1 seedling/slant. At the site of spore placement 0.5 ml of 01 percent aqueous carboxymethyl cellulose solution (BOH-Bngland, viscosity 1500 f 400 centipoise at 20°C) was added as an adhesive (Hepper, 1981; Ferguson and Woodhead, 1982). Ten days old VAM infected wheat seedlings were aseptically transplanted from slants to surface disinfected {with $(Na\ OCl)_2$ V/V} clay pots at 3 seedlings/pot of 15 cm height with 12 and 8 cms top and bottom diams respectively containing 2 kg steam sterilised (under 1:1 kg/cm^2 at 121°C for 2 separate 1-hours periods) sandy clay loam soil (true density 2.66 glcc, pore space 43 per cent and pH level 7.2) supplemented with vermiculite at 1.5 g /100 g soil. The transplanted pots were placed in screen house under natural condition along with 3 sets of control series by complete randomised design method. The seedlings were watered with sterilised distilled water and during early 3 months of growth 1/2 strength of Hoagland solution (Hoagland and Arnon, 1950) excluding the "P" ingredient was also added to the each soil pot at 250 ml I pot at 6 days interval (Menge, 1984). On maturity, the plants were harvested from the soil level and substrata of the pots were collected as mother culture. If found necessary the culture was further multiplied separately by inoculating the same spores in pots. The 80 to 90 percent VAM infected roots and root lets, present in the mother culture were sieved out (using a sieve mesh of 350 µm pore size) as axenic rhizobase VAM inocula with adhered soil particles, hyphae and vermiculites. The inocula was preserved at 5±2°C in polyethylene bags. Production of axenic rhizobase inocula of *A. brasilense: A. brasilense* (wild type) was isolated by serial dilution plate method (Warcup, 1955) on BLCR selective medium (Bashan and Levanony, 1985) from the same rhizospheric soil sample that was used to extract the VAM spores. Its identification was based on morphology (motile, showing undulating movement, straight to curve rods with slightly pointed ends), colonial characters on BLCR medium (dark pink round colony of 0.5 to 1 mm in diameter with protruding ridges, showing gram negative reaction) and on diagnostic biochemical tests (Tarrand *et al*., 1978). An aqueous suspension was made from the bacterial culture containing $2.3 \pm 1.25 \times 10^7$ colony forming unit (cfu)/ml, counted on Petroff-Hausser counting chamber by using the formula given below.

No. of cells in large sq. x Dilution factor x percent Dilution x Factor for large sq. (1.25×10^6). The suspension was poured aseptically in 100 ml sterile conical flask containing freshly harvested 0.5 cm long chopped root fragments (free from infection and contamination, obtained from 60 days old wheat plants grown in strictly aseptic condition) at 5 ml/100 g root fragments. The inoculated flasks were incubated at 30±2°C for 72 hours for multiplication of the bacterium on root fragments. Thereafter population of the bacterium was recounted ($3.8 \pm 1.25 \times 10^9$ cfu/g root fragments). The colonised root fragments was the axenic rhizobase inocula of *A. brasilense* (wild type).

Inoculation of the Rhizohase Inocula in Soil Pots: A series of well washed and surface disinfested backed clay pots (20 cm height with 25 and 10 cm top and bottom diam respectively) each containing 4 kg steam sterilised sandy clay loam Soil with aforementioned properties were inoculated separately and in combined form (1:1) with 3 doses of inocula (at 10 g, 20 g and 40 g/200 pot soil). The ratio of pot soil-inocula was maintained as 1:20, 1:10 and 1:05 g respectively. The surface disinfected seeds of wheat var. Blue Silver were sown in the inoculated pots at 3 seeds/pot (Menge and Timmer, 1982). Each treatment was replicated 3 time. The inoculated pots with control treatments (00 g/200 g soil) were placed in screen house under natural condition by completely randomised design method. Seeds were allowed to germinate and grown up to the stage of maturity. The growing plants were watered regularly with sterilised distilled water.

Results

The effect of separate and combined inoculation of *G. macracarpum* Tulasne and Tulasne and *A. brasilense* (wild type) in the form of axenic rhizobase base inocula in wheat variety. Blue Silver was recorded with the parameters of VAM infection (%), fresh and dry weights, plant height and yield (in terms of grains number/head and TGW). The separate and combined inoculation of the aforesaid inocula with three different doses (10 g, 20 g, 40 g/200 g soil) showed a progressive response on increasing the doses, therefore, the highest response was always found at third dose (Table 1). The first dose of either separate or combined inoculation never showed significant response, however, the response of second and third doses differed non-significantly (Table 1 and 2). On separate inoculation, the response of *G. macrocarpum* Tulasne and Tulasne was found to be dominated over the response of *A. brasilense* as compared to the control series (00 g/200 g soil) (Table 1). The combined inoculation always showed better response than the separate inoculation. The detailed study and analysis of individual parameters are given below.

VAM infection percentage: On separate inoculation by *G. macracarpum* Tulasne and Tulasne 60.3 percent VAM infection was observed at third dose (40 g1200 g soil) in the root of wheat plant whereas no VAM infection was observed on inoculation by *A. brasilense* (wild type) at the same dose. The combined inoculation showed more VAM infection at all the three doses as compared to the individual inoculation indicating the beneficial interactions between them (Table 1). The Analysis of variance (ANOVA) of the data (Table 2) showed that the 3 doses and different types of inocula were found to be differed significantly ($p<0.001$). A significant interaction was found between doses and inocula ($p<0.001$) in respect to the VAM infection percentage.

Fresh weight: On separate inoculation of *G. macrocarpum* Tulasne and Tulasne fresh weight of wheat plant was increased by 13.17 and 10.70 percent by *A. brasilense* (wild type) at the third dose as compared to the control treatment whereas 20.19 percent increase was observed by the combined inoculation at the same dose (Table 1). ANOVA of the data (Table 2) showed that the doses of the inocula were differed significantly ($p<0.001$) whereas 3 different types of inocula were also found to be significantly different at $p<0.01$. The interaction between doses and inocula was found non significant (Table 2).

Dry weight: Seprate inoculation of *G. macrocarpum* Tulasne and Tulasne showed 24.69 percent increase in dry weight of wheat plant whereas 20.74 percent increase by *A. brasilense* (wild type) at the third dose (40 g/200 g soil) as compared to the control treatment (Table 1). On combined inoculation the dry weight was increased up to 37.19 per cent at the same dose (Table 1). The statistical analysis of the data (ANOVA) given in Table 2 showed that the 3 different doses of the inocula were differed significantly ($p<0.05$) whereas the different types of inocula were not found significantly different. The interaction between doses and inocula was found non significant (Table 2).

Plant height: Either separate or combined inoculation of the aforesaid inocula showed non-significant increase in plant height at all the three doses as compared to the control series (Table 2). On separate inoculation the plant height was increased up to 7.90 percent by the *G. macrocarpum* Tulasne and Tulasne and 5.25 percent increase by *A. brasilense* (wild type) whereas 9.22 percent increase was observed by the combined inoculation at the third dose (Table 1). The analysis of the data (Table 2) showed that the different doses of the different types of inocula were not significantly different. The interaction between doses and inocula was non significant.

Grains number/head: The number of grains/head of wheat plant were found to be increased non-significantly at all the three doses either on separate or combined inoculation. On separate inoculation, number of grains/head increased up to 32.78 percent by *G. macrocarpum* Tulasne and Tulasne and 25 percent by *A. brasilense* (wild type) at third dose. The combined inoculation at the same dose showed 39.52 percent increase (Table 1). The ANOVA of the data (Table 2) showed that the 3 different doses of the different inocula were not differed significantly. A non-significant interaction was found between doses and inocula (Table 2).

Thousand grain weight-TGW (g): The wheat plant showed significantly positive response in respect of TGW on either separate or

Table 1: Effect of VAM and *Azospirillum* spp. on growth and yield of wheat

Doses (g/200 g soil)	*Glomus macrocarpum*		*Azospirillum brasilense*		*A. braslense + G. macrocarpum*	
		% increase		% increase		% increase
			VAM Infection 1%)			
00	0.0	0.0	0.0	0.0	0.0	0.0
10	38.8	38.8	0.0	-	39.8	39.8
20	58.2	58.2	0.0	-	60.2	60.2
40	60.3	60.3	0.0	-	63.0	63.0
			Fresh weight (g)			
00	180.6	-	181.2	-	181.2	-
10	183.4	1.55	182.3	0.60	190.8	5.29
20	200.8	11.18	198.8	9.71	214.7	18.48
40	204.4	13.17	200.6	10.70	217.8	20.19
			Dry weight (g)			
00	32.4	-	32.3	-	32.8	-
10	36.6	12.96	34.8	7.73	40.3	22.86
20	39.2	20.98	38.2	18.26	44.8	36.58
40	40.4	24.69	39.0	20.74	47.0	37.19
			Plant height (cm)			
00	93.6	-	93.3	-	93.2	-
10	96.2	2.77	95.8	2.67	97.8	4.93
20	98.8	5.55	97.3	4.28	101.2	8.58
40	101.3	7.90	98.2	5.25	102.0	9.22
			Grains No./Head			
00	30.2	-	30.8	-	29.6	-
10	32.6	7.94	31.2	1.29	34.0	14.86
20	38.2	26.49	36.4	18.18	40.2	35.81
40	40.1	32.78	38.5	25.0	41.3	39.52
			1000-grains weight (9)			
00	30.3	-	29.8	-	29.7	-
10	32.2	6.27	31.4	5.36	33.8	14.57
20	36.8	21.45	33.8	13.42	45.5	54.23
40	37.2	21.45	35.8	20.13	46.2	56.61

Numerical values in each column are the mean of 6 replicate samples $LSD_{0.05}$ (Dose) = 4.38, $LSD_{0.05}$ (Inocula) = 3.79

Table 2: Analysis of variance (ANOVA) on separate and combined inoculation of *VAM* and *Azospirilum* spp, on wheat growth

S.O.V	df	Sum of square	Mean square	F	Probability level
			VAM infection		
Doses	3	10430.46	3476.82	312.82	<0.001
Inocula	2	14011.69	7005.84	630.35	< 0.001
Doses vs inocula	6	5283.22	880.53	79.22	< 0.001
Error	24	266.74	11.11		
Total	35	29992.11			
			Fresh weight		
Doses	3	4736.61	1578.87	40.87	<0.001
Inocula	2	693.37	346.68	8.97	<0.01
Doses vs inocula	6	310.15	51.69	1.33	NS
Error	24	926.94	38.62		
Total	35	6667.07			
			Dry weight		
Doses	3	456.74	152.24	3.05	< 0.05
Macula	2	144.65	72.32	1.44	NS
Doses vs inocula	6	40.48	6.74	0.13	NS
Error	24	1197.58	49.89		
Total	35	1839.45			

			Plant height		
Doses	3	1251.76	417.25	1.24	NS
Inocula	2	264.32	132.16	0.39	NS
Doses vs inocula	6	1261.82	210.30	0.62	NS
Error	24	8057.44	335.72		
Total	35	10835.34			
			Grains number/head		
Doses	3	585.67	195.22	1.38	NS
Inocula	2	139.32	69.61	0.65	NS
Doses vs inocula	6	166.40	27.73	0.26	NS
Error	24	2553.75	106.40		
Total	35	3445.05			
			1000-grains weight		
Doses	3	514.74	171.58	8.45	< 0.001
Inocula	2	205.05	102.52	5.05	<0.05
Doses vs inocula	6	280.93	46.83	2.30	NS
Error	24	487.19	20.29		
Total	35	1487.91			

combined inoculation of *G. macrocarpum* Tulasne and Tulasne and *A. brasilense* (wild type). On separate inoculation by *G. macrocarpum* Tulasne and Tulasne TGW was found to be increased up to 21.45 and 20.13 percent by *A. brasilense* (wild type) at the third dose. The result of combined inoculation showed up to 56.61 percent increase in TGW at the same dose as compared to the control series (Table 1). The ANOVA of the data (Table 2) showed that the 3 different doses of the different inocula were differed significantly (p<0.001). The different types of inocula were also found significantly different (p<0.01). The interaction between doses and macula was found nonsignificant (Table 2).

Discussion

Effective soil microorganisms (ESM) are now being explored in agriculture with especial interest to increase the growth and yield of crop plants (Nandi, 1990; Giller and Day, 1985). Researches on VAM fungi and *Azospirillum* spp. separately in stimulation of plant growth and suitable methods of achieving their potential benefits in agriculture are being conducted on a world wide scale (Avivi and Feldman, 1982; Bagyaraj, 1991; Diederichs and Moawad, 1993; Hall, 1988; Harley, 1989; Millet *et al*., 1984; Okon, 1982; Pacovsky *et al*. 1985; Bashan and Levanony, 1990). *VAM-Azospirillum* biotechnology is a nascent agricultural strategy for boosting up the growth and yield of crops particularly cereals which lack the root nodulating bacteria. Barea *et al*. (1983) suggested that VAM and *Azospirillum* species together provide a means by which cereal plants lacking symbiotic N-fixers (Rhizobium species) could compensate the nutrient deficiency. However, studies on interactions between VAM and *Azospirillum* spp. and their combined effects on plants are relatively very few in the world. Our results on either separate or combined inoculation of the inocula with three different doses (10 g, 20 g, 40 g/200 g soil) showed a progressive response of wheat var. Blue Silver in respect of the increase in VAM infection per cent (only in the presence of VAM fungus), fresh and dry weights, plant height, grains number/head and TGW (Table 1). The first dose of either separate or combined inoculation never showed significant response, however, the response of second and third doses differed non-significantly. On separate inoculation, the response of *G. macrocarpum* Tulasne and Tulasne was found to be dominated over the response of *A. bras* ilense as compared to the control treatments (00 g/200 g soil) (Table 1). The combined inoculation of the aforesaid inocula showed always better response than their separate inoculations. Tewari and Boyetchko (1987), Wood (1987) and Menge (1983) worked on possible commercial use of VAM fungi as an alternative to the "P"-fertilizers and their possible application in agriculture. *Azospirillum* inoculation has now been considered a substitute for N-fertilizers (Lau-Wong, 1987; Mertens and Hess, 1984). The enhancement of growth and yield of crop plants due to VAM fungi have already been reported by many workers (Jakobsen, 1983; Yocom and Boosalis, 1991) which corroborate our findings. Our results showed better response at third dose (40 g/200 g soil) (Table 1). Clapperton and Reid (1992) stated that there is a correlation between the inoculum density of VAM fungi and soil nutrient availability which affect plant growth. Sieverding (1991) evaluated various responses of crop plants after VAM inoculation by correlation analysis and concluded that one response was independent to the another that also supports our result. The results of inoculation by *G. macrocarpum* Tulasne and Tulasne showed 32.78 percent increase in grains number/head and 21.45 percent increase in TGW of wheat var. Blue Silver. Whereas Saif and Khan (1977) reported 290 per cent increase in grains yield of barley on inoculation with VAM fungi which has not been considered to be a realistic (Powell, 1984) since lacking of the statistical analysis to the data. We have obtained 24.69 percent increase in dry weight of wheat var. Blue Silver by the same inoculation at same dose which greatly differed from the results of Khan (1972, 1975). The Khan's results have already been challenged by Powell and Bagyaraj (1984) on account of lacking of the statistical analysis of the data. However, each parameter of our result has been analysed individually to evaluate the status of it significant level. Most studies of the *Azospirillum-plant* association have been conducted on cereals and grasses. The response of cereals like increases in fresh and dry weights, N content in shoots and grains, total number of tillers and ears, grains number/head, increase in grains weight, higher germination rate on inoculation with *Azospirillum* strains have already been reported by many workers (Bashan 1986; Cohen *et al*., 1980; Mertens and Hess, 1984; Pacovsky *et al*., 1985) which lend support to our findings (Table 1). Our investigation showed that the separate inoculation of *A. brasilense* at 40 g/200 g soil increased the yield of wheat var. Blue Silver in term of grains number/head (25.00%) and TGW (20.13%). The result of Kapulnik *et al*. (1987) showed 10 to 30 percent increase in the total yield of crop plants which confirm our findings. Okon (1985) obtained on average 65 percent increase in yield to all field experiments after the inoculation of *Azospirillum* sp. Smith *at al.* (1984) observed 75 percent increases in yield to summer cereals and 50 percent in spring wheat due to *Azospirillum* inoculation. Our dual inoculation of *G. macrocarpum* Tulasne and Tulasne and *A. brasilense* (wild type) in wheat var. Blue Silver produced highly significant response which clearly indicate mutual beneficial interactions between them (Table 1). The similar results were also obtained by Pacovsky *at al.* (1985) and stated that VAM and *Azospirillum* species together produce an additive effect on plant which confirmed our findings. Bashan and Levanony (1985) and Subba Rao *et al*. (1985) described the synergistic effects of *Azospirillum* and VAM fungi on combined inoculation in barley which resulted the increase in growth along with the enhanced uptake of "N" and "P" in the inoculated plants. This dual inoculation could completely replace the application of "N" and "P" fertilizers (Barea *et al*., 1983; Pacovsky, 1988; Pacovsky *et al*., 1985). Zuberer (1990) pointed out that wheat and corn are usually provided a small portion of the biologically fixed Nitrogen below the estimated need (10 to 15 %). Barea *et al.* (1983) suggested that combined inoculation of VAM and *Azospirillum* increase the uptake of nitrogen greater than the estimated needs. Nevertheless, experimental studies on VAM-*Azospirillum* have not received much attention in world and not at all in Pakistan. Therefore, extensive researches are needed for achieving their possible potential benefits in agriculture. However, from our result it can be concluded that the VAM fungi along with *Azospirillum* species in the form of fertilizer could be utilised to enhance the growth and yield of wheat. This information would be of great significance for the production of wheat in Sindh Pakistan. Since this technology is potentially more useful biological means of assuring plant production with minimum input of agro-chemicals and does not entail any environmental pollution and soil degradation. However, it needs further investigation on its possible application in field condition and commercial utilization in Pakistan.

References

Abbott, L.K. and A.D. Robson, 1984. The Effect of VA Mycorrhizae on Plant Growth. In: VA Mycorrhiza, Powell, C.L. and D.J. Bagyaraj (Eds.). CRC Press, Boca Raton, FL., USA., ISBN-13: 9780849356940, pp: 113-130.

Avivi, Y. and M. Feldman, 1982. The response of wheat to bacteria of the genus *Azospirillum*. Isr. J. Bot., 31: 237-245.

Bagyaraj, D.J., 1991. Utilization and commercialisation of mycorrhizal fungi. Mycorrhiza News, 3: 4-5.

Baldani, V.L., J.I. Baldani and J. Dobereiner, 1983. Effects of *Azospirillum* inoculation on root infection and nitrogen incorporation in wheat. Can. J. Mirobiol., 29: 924-929.

Barea, J.M., A.F. Bonis and J. Olivares, 1983. Interactions between *Azospirillum* and VA mycorrhiza and their effects on growth and nutrition of maize and ryegrass. Soil Biol. Biochem., 15: 705-709.

Bashan, Y. and H. Levanony, 1985. An improved selection technique and medium for the isolation and enumeration of *Azospirillum brasilense*. Can. J. Microbiol., 31: 947-952.

Bashan, Y. and H. Levanony, 1990. Current status of *Azospirillum* inoculation technology: *Azospirillum* as a challenge for agriculture. Can. J. Microbiol., 36: 591-608.

Bashan, Y., 1986. Alginate beads as synthetic inoculant carriers for slow release of bacteria that affect plant growth. Applied Environ. Microbiol., 51: 1089-1098.

Boddey, R.M., V.L. Baldani, J.I. Baldani and J. Dobereiner, 1986. Effect of inoculation of *Azospirillum* spp. on nitrogen accumulation by field-grown wheat. Plant Soil, 95: 109-121.

Clapperton, M.J. and D.M. Reid, 1992. A relationship between plant growth and increasing VA mycorrhizal inoculum density. New Phytol., 120: 227-234.

Cohen, E., Y. Okon, J. Kigel, I. Nur and Y. Henis, 1980. Increase in dry weight and total nitrogen content in *Zea mays* and *Setaria italica* associated with nitrogen-fixing *Azospirillum* spp. Plant Physiol., 66: 746-749.

Day, J.M., M.C.P. Neves and J. Dobereiner, 1975. Nitrogenase activity on the roots of tropical forage grasses. Soil Biol. Biochem., 7: 107-112.

Diederichs, C. and A.M. Moawad, 1993. The potential of VA mycorrhizae for plant nutrition in the tropics. Angewandte Botanik, 67: 91-96.

Ferguson, J.J. and S.H. Woodhead, 1982. Production of Endomycorrhizal Inoculum: A. Increase and Maintenance of Vesicular-Arbuscular Mycorrhizal Fungi. In: Methods and Principles of Mycorrhizal Research, Schenck, N.C. (Ed.). American Phytopathological Society, St. Paul, MN., USA., ISBN-13: 9780890540466, pp: 47-54.

Giller, K.E. and J.M. Day, 1985. Nitrogen Fixation in the Rhizosphere: Significance in Natural and Agricultural Systems. In: Ecological Interactions in Soil: Plants, Microbes and Animals, Fitter, A.H. (Ed.). Blackwell Publ., Oxford, UK., ISBN-13: 978-0632013869, pp: 127-147.

Hall, I.R., 1988. Potential for Exploiting Vesicular-Arbuscular Mycorrhizas in Agriculture. In: Biotechnology in Agriculture (Advances in Biotechnological Processes), Mizrahi, A. (Ed.). A.R. Liss Publ., New York, USA., ISBN-13: 978-0845132081, pp: 141-174.

Harley, J.L., 1989. The significance of mycorrhiza. Mycol. Res., 92: 129-139.

Hepper, C.M., 1981. Techniques for studying the infection of plants by vesicular-arbuscular mycorrhizal fungi under axenic conditions. New Phytol., 88: 641-647.

Hoagland, D.R. and D.I. Arnon, 1950. The water-culture method for growing plants without soil. California Experiment Station Circular No. 347, University of California, Berkeley, CA., USA., pp: 1-32.

Jakobsen, I., 1983. Vesicular-arbuscular mycorrhiza in field-grown crops II. Effect of inoculation on growth and nutrient uptake in barley at two phosphorus levels in fumigated soil. New Phytol., 94: 595-604.

Kapulnik, Y., Y. Okon and Y. Henis, 1987. Yield response of spring wheat cultivars (*Triticum aestivum* and *T. turgidum*) to inoculation with *Azospirillum brasilense* under field conditions. Biol. Fertil. Soils, 4: 27-35.

Khan, A.G., 1972. The effect of vesicular-arbuscular mycorrhizal associations on growth of cereals. New Phytol., 71: 613-619.

Khan, A.G., 1975. The effect of vesicular arbuscular mycorrhizal associations on growth of cereals. II. Effects on wheat growth. Ann. Applied Biol., 80: 27-36.

Lau-Wong, M.M., 1987. Field testing of the effectiveness of bacterial fertilizer in Nepal. Agric. Ecosyst. Environ., 19: 145-153.

Martin, P. and A. Glatzle, 1982. Mutual Influences of *Azospirillum* spp. and Grass Seedlings. In: *Azospirillum*: Genetics, Physiology, Ecology, Klingmuller, W. (Ed.). Birkhauser Verlag, Basel, Switzerland, ISBN-13: 9783764313302, pp: 108-120.

Menge, J.A. and L.W. Timmer, 1982. Procedure for Inoculation of Plants with Vesicular-arbuscular Mycorrhizae in Laboratory, Greenhouse and Field. In: Methods and Principles of Mycorrhizal Research, Schenck, N.C. (Ed.). American Phytopathological Society, St. Paul, MN., USA., ISBN-10: 0890540462, pp: 59-68.

Menge, J.A., 1983. Utilization of vesicular-arbuscular mycorrhizal fungi in agriculture. Can. J. Bot., 61: 1015-1024.

Menge, J.A., 1984. Inoculum Production. In: VA Mycorrhiza, Powell, C.L. and D.J. Bagyaraj (Eds.). CRC Press Inc., Boca Raton, FL., USA., ISBN-13: 9780849356940, pp: 187-203.

Mertens, T. and D. Hess, 1984. Yield increases in spring wheat (*Triticum aestivum* L.) inoculated with *Azospirillum lipoferum* under greenhouse and field conditions of a temperate region. Plant Soil, 82: 87-99.

Millet, E., Y. Avivi and M. Feldman, 1984. Yield response of various wheat genotypes to inoculation with *Azospirillum brasilense*. Plant Soil, 80: 261-266.

Morton, J.B. and G.L. Benny, 1990. Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): A new order, Glomales, two new suborders, Glomineae and Gigasporineae and two new families, Acaulosporaceae and Gigasporaceae, with an emendation of Glomaceae. Mycotaxon, 37: 471-491.

Nandi, P.N., 1990. Microbes and Ecosystem. In: Economic Plants and Microbes, Purkayastha, R.P. (Ed.). Today and Tomorrow Printers and Publishers, New Delhi, India, ISBN-13: 9788170193715, pp: 121-122.

Okon, Y. and R. Baker, 1987. Microbial inoculants as crop-yield enhancers. Crit. Rev. Biotechnol., 6: 61-85.

Okon, Y., 1982. *Azospirillum*: Physiological properties, mode of association with roots and its application for the benefit of cereal and forage grass crops. Isr. J. Bot., 31: 214-220.

Okon, Y., 1985. *Azospirillum* as a potential inoculant for agriculture. Trends Biotechnol., 3: 223-228.

Pacovsky, R.S., 1988. Influence of inoculation with *Azospirillum brasilense* and *Glomus fasciculatum* on sorghum nutrition. Plant Soil, 110: 283-287.

Pacovsky, R.S., E.A. Paul and G.J. Bethlenfalvay, 1985. Nutrition of sorghum plants fertilized with nitrogen or inoculated with *Azospirillum brasilense*. Plant Soil, 85: 145-148.

Powell, C.L. and D.J. Bagyaraj, 1984. VA Mycorrhiza. CRC Press, Boca Raton, FL., USA., ISBN-13: 9780849356940, Pages: 234.

Powell, C.L., 1984. Field Inoculation with VA Mycorrhizal Fungi. In: VA Mycorrhiza, Powell, C.L. and D.J. Bagyaraj (Eds.). CRC Press Inc., Boca Raton, FL., USA., ISBN-13: 9780849356940, pp: 205-222.

Saif, S. and A.G. Khan, 1977. The effect of vesicular-arbuscular mycorrhizal associations on growth of cereals. III. Effects on barley growth. Plant Soil, 47: 17-26.

Schenck, N.C. and Y. Perez, 1990. Manual for the Identification of VA Mycorrhizal Fungi. 3rd Edn., Synergistic Publications, Gainesville, FL., ISBN-13: 9780962598036, Pages: 286.

Sieverding, E., 1991. Vesicular-Arbuscular Mycorrhiza Management in Tropical Agrosystems. Deutsche Gesellschaft fur Techrische Zusammenarbeit (GTZ) GmbH, Germany, ISBN-13: 9783880854628, Pages: 371.

Smith, R.L., S.C. Schank, J.R. Milam and A.A. Baltensperger, 1984. Responses of *Sorghum* and *Pennisetum* species to the N_2-fixing bacterium *Azospirillum brasilense*. Applied Environ. Microbiol., 47: 1331-1336.

Subba Rao, N.S., K.V.B.R. Tilak and C.S. Singh, 1985. Synergistic effect of vesicular-arbuscular mycorrhizas and *Azospirillum brasilense* on the growth of barley in pots. Soil Biol. Biochem., 17: 119-121.

Tarrand, J.J., N.R. Krieg and J. Dobereiner, 1978. A taxonomic study of the *Spirillum lipoferum* group, with descriptions of a new genus, *Azospirillum* gen. nov. and two species, *Azospirillum lipoferum* (Beijerinck) comb. nov. and *Azospirillum brasilense* sp. nov. Can. J. Microbiol., 24: 967-980.

Tewari, J.P. and S.M. Boyetchko, 1987. The biological fertilizers. Agric. For. Bull., 10: 33-34.

Warcup, J.H., 1955. On the origin of colonies of fungi developing on soil dilution plates. Trans. Br. Mycol. Soc., 38: 298-301.

Wood, T., 1987. Commercial Production of VA Mycorrhiza Inoculum: Axenic versus Non-axenic Techniques. In: Mycorrhizae in the Next Decade: Practical Applications and Research Priorities, Sylvia, D.M., L.L.L. Hung and J.H. Graham (Eds.). Institute of Food and Agricultural Sciences, University of Florida, USA.

Yocom, D.H. and M.G. Boosalis, 1991. Mycorrhizal Fungi Increase Yields of Winter Wheat. In: The Rhizosphere and Plant Growth, Keister, D.L. and P.B. Cregan (Eds.). Kluwer Academic Publishers, Netherlands, ISBN: 978-94-010-5473-7, pp: 382.

Zuberer, D.A., 1990. Soil and Rhizosphere Aspects of N_2-Fixing Plant-Microbe Associations. In: The Rhizosphere, Lynch, J.M. (Ed.). John Wiley and Sons, New York, USA., ISBN-13: 9780471925484, pp: 317-353.