Journal of Agricultural Technology 2011 Vol. 7(4): 983-991
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

AM fungi and *Azotobacter chroococcum* affecting yield, nutrient uptake and cost efficacy of *Chlorophytum borivillianum* in Indian Arid Region

Solanki, A. S.[1*], Kumar, V.[2] and Sharma, S.[3]

[1]Plant Wealth Sector, Department of Soil and Water Research, Public Authority of Agricultural Affairs & Fish Resources, Kuwait, [2]Microbiology Section, Department of Soil and Water Research, Public Authority of Agricultural Affairs & Fish Resources, Kuwait, [3]Department of Applied Mechanics and Biotechnology, MLN National Institute of Technology, Allahabad, India.

Solanki, A. S., Kumar, V. and Sharma, S. (2011). AM fungi and *Azotobacter chroococcum* affecting yield, nutrient uptake and cost efficacy of *Chlorophytum borivillianum* in Indian Arid Region. Journal of Agricultural Technology 7(4):983-991.

The yield, nutrient uptake and cost efficacy of *Chlorophytum. borivillianum* were studied using dual inoculation with AM fungi (*Glomus fasciculatum*) and *Azotobacter chroococcum*. The experiment was conducted for two consecutive years with two levels of chemical fertilizers as F1(N-P-K : 24-24-32 kg ha^{-1}) and F2 (N-P-K : 30-30-40 kg ha^{-1}). Inoculation of bioinoculants encouraged results; the fresh tuber weight (53.12qha^{-1}), number of tubers $bunch^{-1}$ (13.19), tuber length (9.59cm) and tuber diameter (6.68mm) showed the highest with treatment T12 (F2 + both bioinoculants) followed by T9 (F1 + both bioinoculants), T11 (F2 + AM fungi) and F10 (F2 + *A. chroococcum*) as compared to T1 (control), T2-T4 (bioinoculants) and T5-T6 (F1 and F2 fertilizer levels). Growth parameters were non significant between F1 and F2 dose of chemical fertilizers when inoculated with dual microorganisms. The nitrogen uptake was increased in *Azotobacter* treated plants, while higher P and K uptake were attained in AM fungi inoculated plants. The survival count of inoculated bacteria improved gradually and the highest count was observed on 150 days of inoculation. Economic analysis revealed the net profit from tubers was the highest in T9 using dual inoculation of microorganisms with 80 % of recommended dosage of fertilizer followed by T12 and T11.

Key words: AM fungi, *Azotobacter chroococcum*, *Chlorophytum borivillianum*, cost efficacy, nutrients uptake, yield

Introduction

The tuberous roots of *Chlorophytum borivilianum*, commonly known as safed musli, of family Liliaceae, possess adaptogenic and immunomodulatory properties and are used to treat impotency, sterility and boost male potency. The main active ingredients of roots, saponins are metabolic enhancers and

* Corresponding author: Amar Singh Solanki; e-mail: dr.amarsinghsolanki@yahoo.com

stimulants and also shown to possess anti-tumour activity (Bordia, *et al*., 1995). The extract of dried root tubers of *C. borivilianum* acts as psychostimulant and has a positive effect on the brain and human body by increasing mental ability, intelligence, sexual characters and alertness. Due to its therapeutic activity and diversified uses, demand for *C. borivilianum* is increasing in Indian and the international market. Its seeds have poor germination percentage, low viability and long dormancy period; therefore, it is propagated vegetatively by fleshy tuberous roots bearing shoot buds (Satyabrata and Geetha, 2005).

In India, cultivation of *C. borivillianum* is gaining popularity among farmers due to its profitable commercial value, but the lack of scientific acumen and costly inputs (chemical fertilizers and pesticides) becomes a constraint to produce the high yield to fetch the competitive price and fulfill the market demand nationally and internationally. In the arid and semi arid environments, the crop production is unsustainable and unreliable due to poor soil fertility status, lack of precipitation and uncongenial climatic conditions. Application of appropriate technological approach for overcoming unsuitable cultivation practices may result in increased tuber yield in the arid soil (Singh and Khanuja, 2003).

Microorganisms are important attributes in agriculture to promote circulation of plant nutrients and reduce the need for chemical fertilizers (Kumar *et al*., 2001a,b). Arbuscular mycorrhizal fungi are being frequently used in sustainable agriculture, due to their ability to increase nutrient uptake and water transport. In arid and semi arid areas of India the mycorrhiza are frequently distributed. Mycorrhizal symbiosis plays an important role in nutrient cycling in agricultural and natural ecosystem (Plenchette *et al*. 2005). Mycorrhizal fungi colonize the root cortex of plants, obtain photosynthates and other growth factors from the host and develop an extrametrical hyphal network that can absorb nutrients and water from the soil. The microbiologically solubilized phosphate could, however, be taken up by a mycorrhizal mycelium, thereby, developing a synergistic microbial interaction (Artursson *et al*., 2005). Mycorrhizal plants frequently show resistance to drought and environmental stresses (Dehne, 1986), thus increasing plant growth and yield, improving crop uniformity, and reducing phosphorous and micronutrient requirements.

Azotobacter chroococcum, a free living diazotroph, fixes atmospheric nitrogen, produces growth hormones and solubilizes phosphate (Kumar and Narula, 1999) has been used as a favorable bioinoculant for crops like wheat (Kumar *et al*., 2001b), cotton (Narula *et al*., 2005), sorghum (Kumar *et al*., 1999), sunflower (Kumar *et al*., 2000) and herbal crop, *Withania somnifera* (Kumar *et al*., 2009). Since the literature on the dual inoculation of AM fungi and *A. chroococcum* on *C. borivillianum* is scanty, therefore, the purpose of this study was to understand the co-inoculative effect of AM fungi and *A. chroococcum* on yield, nutrients uptake and cost economics of the *C. borivillianum* in Indian arid region.

Materials and methods

The study was done in October- March 2008-2009 and 2009-2010 on *C. borivillianum* var. MDH Bio-13 in a farmer's field in District Udiapur (24.58°N 73.68°E), Rajasthan (India). The crop was grown in a sandy clay loam soil with good drainage having 45% sand, 25% clay, 30% silt, pH 7.4, organic carbon 0.25 %, available nitrogen 145 kg ha^{-1}, phosphorus 12.31 kg ha^{-1} and potash 149.7 kg ha^{-1}. In the experiment, authors have selected 80% of recommended dose of chemical fertilizer in addition to recommended dose {N-P-K : [30-30-40] kg ha^{-1}} (this chemical fertilizer level was recommended by Agricultural Department, Government of Rajasthan, India). Generally, the use of biofertilizers can save up to 15-25% of chemical fertilizers (Pandey and Kumar, 1998), therefore, a gap of 20% less fertilizer was kept to fulfill by using biofertilizers. Treatments were as follows:-T1 = Control, T2 = *A. chroococcum*, T3 = AM fungi, T4 = *A. chroococcum* + AM fungi, T5 = F1 (N-P-K : 24-24-32) kg ha^{-1} (80% of recommended), T6 = F2 (N-P-K : 30-30-40) kg ha^{-1} (recommended), T7 = F1 + *A. chrococcum*, T8 = F1 + AM fungi, T9 = F1 + *A. chroococcum* + AM fungi, T10 = F2 + *A. chroococcum*, T11 = F2 + AM fungi and T12 = F2 + *A. chroococcum* + AM fungi.

Microbial treatment of tubers and soil

Azotobacter chroococcum nitrogen fixing strain (nitrogenase activity 79.6 n mol C_2H_2/h/mg protein) was obtained from the culture collection of Dr. Shivesh Sharma and AM fungi (*Glomus fasciculatum*) used in the experiment was obtained from the local market, produced and marketed by a government certified company. The fresh separated tubers were sprinkled with 20% sugar solution and then coated with charcoal powder containing *A. chroococcum* cells (10^8 g^{-1}), the tubers were dried for 30 min in shade (ambient temperature 20°C) and sown immediately in experimental plots of 4×3 m^2. AM fungi inoculation was done by layering method (Jackson et al. 1972), the AM fungi granules (at the rate of 4 kg ha^{-1}) were spread in beds before sowing the tubers. Tubers were sown in experimental plots with a spacing of 8″ × 8″. Raised beds of 13″ high and 3.5′ wide for easy tuber growth and proper drainage were prepared with the help of bed raiser. The data was analyzed using ANOVA, where the means of studied treatments were compared using LSD at $P = 0.05$ significant level. MSTAT-C software was used for computing the data.

Survival studies of Azotobacter chroococcum

The survival count of bacteria was carried at regular intervals of 35, 70, 105 and 150 d of inoculation. The selected plants were carefully uprooted and rhizospheric soil adhering intimately to the tubers was separated by gentle tapping and composite samples were prepared. Soil samples were air dried at room temperature (25° C) and *A. chroococcum* counts were determined using dilution technique on *Azotobacter* agar (Hi Media, Mumbai, India) at 30°C.

Growth parameters

Observations on fresh tubers weight (q ha^{-1}), dry yield (q ha^{-1}), number of tubers $bunch^{-1}$, tuber length (cm) and tuber diameter (mm) were recorded at the time of harvest (150 d after sowing). For nutrients analysis in the plants, the plant samples (tubers and leaves) were dried in an oven at 65°C for 48h. The dried samples were powdered and N, P, K was determined using the method of Cottenie *et al.*(1982). The cost efficacy of the experiment was calculated using benefit cost (B:C) ratio as mentioned by Gittinger (1984).

Results

The cultivation data in tables are shown as mean of two years for brevity and clarity. In general, all quantitative plant traits were significantly increased which nearly linear in response to bioinoculants and chemical fertilizer treatments. Inoculation to tubers and soil with bioinoculants in treatments T2 and T3 showed significantly increased in plant growth parameters compared to control (Table 1). Dual inoculation of bioinoculants (treatment T4) performed better than control and individual microbial inoculation. The treatment T4 exhibited a significant increase in fresh tuber yield (39.4%), number of tubers $branch^{-1}$ (40.4%) and tuber length 48.7 %) over control. Inoculation of *A. chroococcum* (treatment T2) produced 24.8% and 30.2 % more fresh tuber yield and number of tubers $branch^{-1}$ more than control, while AM fungi (T3) increased 30.6% and 28.6 % fresh tuber yield and number of tubers $branch^{-1}$ over control. The combined effect of both bioinoculants (*A. chroococcum* and *G. fasciculatum*) and chemical fertilizer as F2 (T12) showed highest fresh tuber yield (52.13 q ha^{-1}), number of tubers $branch^{-1}$ (13.29), tuber length (9.69 cm) and tuber diameter (6.68 mm) followed by treatment T9, but the treatments were non significant with each other. Similarly, inoculation of *Azotobacter* alone and with *G. fasciculatum* in combination with chemical fertilizer increased plant growth parameters. This increment was significantly higher than chemical fertilizer treatments (T5 and T6).

Table 1. Effect of dual inoculation of *Azotobacter chroococcum* and *Glomus fasciculatum* on growth yield parameters of *Chlorophytum borivillianum* under two fertilizer regimes

Treatments	Fresh yield q ha^{-1}	Dry yield q ha^{-1}	Number of tubers bunch^{-1}	Tuber length (cm)	Tuber diameter (mm)
Control	23.06	2.72	6.56	4.51	4.81
A. chroococcum	28.78	3.45	8.54	5.32	5.12
AM fungi	30.13	3.54	8.44	5.45	5.17
A. chroococcum + AM fungi	32.16	3.81	9.21	6.71	5.30
F1	40.48	4.82	9.88	6.97	5.72
F2	42.44	5.02	10.25	7.32	5.95
F1 + *A. chroococcum*	46.32	5.53	11.46	8.06	6.05
F1 + AM fungi	47.15	5.61	11.89	8.19	6.14
F1 + *A. chroococcum* + AM fungi	50.29	6.01	12.27	9.42	6.57
F2 + *A. chroococcum*	47.35	5.60	12.03	8.38	6.20
F2 + AM fungi	48.67	5.79	12.14	8.78	6.41
F2 + *A. chroococcum* + AM fungi	52.13	6.07	13.29	9.59	6.68
LSD (P=0.05)	2.67	0.621	0.367	0.193	0.126

It was observed that N uptake in plant was the highest in treatment T12 followed by T10 and T11. P uptake was the highest in T12 followed by T11 and T10, while the K uptake was also highest in T12 followed by T11 and T10. All these treatments were significantly higher over control. The N, P and K uptake of all the treatments increased with increase in fertility levels which further complimented by *A. chroococcum* and AM fungi inoculation (Fig. 1). Application of *A. chroococcum* resulted in more uptake of N compared to P and K in crop, while the AM fungi inoculation produced more P and K uptake compared to N. The higher N uptake might be an effect of N_2 fixation by *A. chroococcum* which has to be proved in further experiments. The survival count of the inoculated bacteria was found to increase gradually and the highest count was reached at the time of harvest i.e. 150 d (Fig. 2).

The economic analysis of the control treatment was calculated as a total cost in US Dollar (USD) (USD 224.7), gross return (USD 475.3), and net return (USD 250.6). The benefit cost (B:C) ratio was determined to be 2.11 (Figs. 3 and 4). The highest net return was with treatment T9, (USD 2223.1 and a B:C ratio of 2.94), followed by treatment T12 for which the net return and B:C ratio were USD 2213.2 and 2.85, respectively. Treatment T4 (both bioinoculants only) was also good and the net return and B:C ratio were 1022.3 and 2.29, respectively (Figs. 3 and 4).

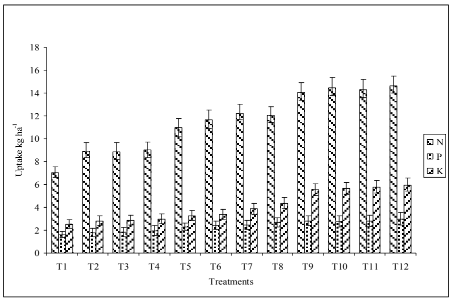

Fig. 1. Effect of dual inoculation of *Azotobacter chroococcum* and AM fungi on nutrients uptake (kg ha-1) by *Chlorophytum borivillianum*.

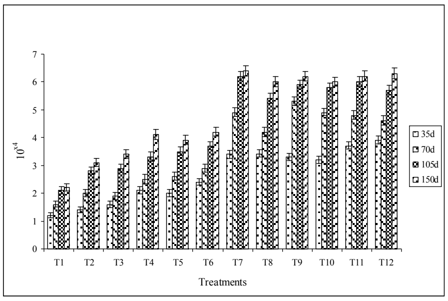

Fig. 2. Survival count (CFU) of *Azotobacter chroococcum* at different intervals of time (d).

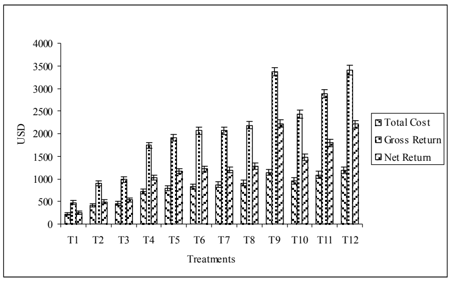

Fig. 3. Economic analysis of different treatments.

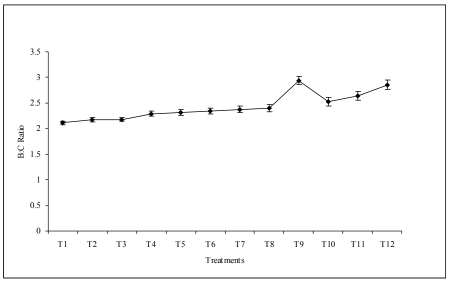

Fig. 4. Benefit cost (B:C) ratio of the treatments.

Discussion

The response of a crop to inorganic fertilizers is well understood; however, a combination of microbial inoculants and fertilizer has shown variable results, partly the promotive effect is attributed to the favorable influences exerted by root exudates which contain organic acids, sugars, phytohormones etc. It is evident from Table 1 that the treatments with single or dual microorganisms, along with fertilizer resulted in higher tuber yield parameters compared to control and fertilizer alone treatments (T5 and T6). In terms of percentage increase the fresh tuber yield in treatments T12 using dual microorganisms resulted in 22.8% higher than T6 and T9 was 24.2% more than T5, similarly the number of tubers branch^{-1} was 29.6% and 23.1% higher than fertilizer alone treatments, also the tuber length was 5.52-5.98% higher than fertilizer alone treatments. This increased in plant growth and yields in T7 to T12 over T5 and T6 which due to the application of beneficial microorganisms, as the same fertility level in T7-T12. Treatments 10 to T12 and T7 to T9 showed higher parameters than T6 and T5, respectively. Although treatment 12

Journal of Agricultural Technology 2011 Vol. 7(4): 983-991
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

was superior to all the treatments but non significant to second best treatment 9, therefore, the application of microorganisms saved the 20% chemical fertilizer.

One of the best described events in *Azospirillum* and *A. chroococcum* root colonization was the impact on the root development in terms of increase in root length, number of lateral roots and root hair (Martin *et al*., 1989; Kumar *et al*., 2001a). Enhanced proliferation of the root system is believed to promote increased minerals uptake (NPK) and consequently to increase the plant growth yield (Narula *et al*., 2000). Inoculation of *A. chroococcum* resulted in more uptake of N compared to P and K. There was 1.25% higher N uptake in treatment 10 over T11, this could be due to nitrogen fixation by bacteria. The AM fungi inoculation also resulted in increased uptake of P (2.54% higher in treatment 11 over T10), similarly, K uptake was 2.48% more in T11 compared to T10. This might be due to increase in surface area of roots by AM fungi to absorb and transport nutrients to the host. Increased in nutrient uptake and productivity of field crops with mycorrhizal fungi inoculation have been reported in basil (Gupta *et al*., 2000), tomato (Gao *et al*., 2001), forage legumes (Sockley *et al*., 2004) and potato (Davies *et al*., 2005).

The stimulatory effect of chemical fertilizer on the survival of *A. chroococcum* may be exerted directly through their effect on the growth and proliferation of bacteria thereby creating a favorable habitat for the growth and survival of inoculated bacteria (Kumar *et al*., 2001a). The *A. chroococcum* count increased till the day of harvesting in all treatments, which may have been attributable to more root exudates, supporting better plant-AM fungi-*A. chroococcum* interaction and nutrient mobilization of fungal (Hetrick *et al*., 1992) and bacterial symbionts. The productiveness of the rhizosphere for AM fungi may be attributed to favorable influence exerted by root exudates (Bais *et al*., 2006), which contain amino acids, carbohydrates, organic acids and growth promoting substances and phytohormones produced by *A. chroococcum*.

Economic analysis revealed higher profitability in the presence of both bacteria with F1 level of fertility. It is evident from Figs. 3 and 4 that treatment 9, followed by treatment 12 was more economically profitable, as net return was higher when compared to the other treatments. Our results are in agreement with Kumar *et al*. (2004), who also calculated the cost economics of three phosphate responsive wheat varieties using phosphate solubilizing *A. chroococcum* mutants and parent strains, they calculated a gain of Rs. 2.87 to 31.28 ha^{-1} by investing one Indian Rupee ha^{-1} (1 USD = 45 Indian Rupee). Our studies suggested that microbial inoculants can be used as an economic input to increase crop productivity and maintaining sustainability of soil and harvesting more nutrients.

Inoculation of *A. chroococcum* and AM fungi under the soil and climatic conditions of arid region, characterized by fluctuations in precipitation, could increase in yield. This study exhibited that dual inoculation of *A. chroococcum* and AM fungi with 80% of recommended fertilizer dose in *C. borivillianum* in arid region could be profitably used to maximize tuber production. Our studies suggested that microbial inoculants can be used as an economic input to increase crop productivity and maintaining sustainability of soil by harvesting more nutrients. The economics of the experiment advocated that application of favorably coinoculated bioinoculants can provide partial substitution to chemical fertilizers, thus saving the fertilizers and exchequer by being cost effective.

References

Artursson, V., Finlay, R.D.and Jansson, J.K. (2005). Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. Environmental Microbiology 8: 1–10.

Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S. and Vivanco, J.M. (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. Annual Review of Plant Biology 57: 233–266.

Bordia, P.C., Joshi, A. and Simlot, M.M. (1995). Safed Musli. In: Advances in Horticultural Medicinal and Aromatic Plants. (Eds. K.L. Chadha and R. Gupta). Malhotra Publishing House, New Delhi, pp. 429–451.

Cottenie, A., Verloo, M., Velghe, M. And Camerlynck, R. (1982). Chemical Analysis of Plant and Soil. In: Manual Laboratory of Analytical and Agrochemistry. Ghent State Univ. Press, Belgium.

Davies, F.T., Calderon, C.H., Huaman, Z. and Gomez, R. (2005). Influence of a flavonoid (formononetin) on mycorrhizal activity and potato crop productivity in the highlands of Peru. Scientific Horticulture 106: 318–329.

Dehne, H.W. (1986). Mycorrhiza and plant stress. Gesunde Pflanzen 38: 117–122.

Gao, L.L., Delp, G. and Smith, S.E. (2001). Colonization patterns in a mycorrhiza-defective mutant tomato vary with different arbuscular-mycorrhizal fungi. New Phytology 51: 477–491.

Gittinger, J.P. (1984). Economic analysis of Agricultural Projects. The Johns Hopkins University Press, World Bank Publications, pp 329-343.

Gupta, M.L., Khaliq, R., Oandey, R., Shukla, R.S., Singh, H.K. and Kumar, S. (2000). Vesicular-arbuscular mycorrhizal fungi associated with *Ocimum* spp. Journal of Herb Spices and Medicinal Plants 7: 343–348.

Hetrick, B.A.D., Wilson, G.W.T. and Leslie, J.F. (1992). Mycorrhizal dependence of modern wheat varieties, land races and ancestors. Canadian Journal of Botany 70: 2032–2040.

Jackson, N.E., Franklin, R.E. and Miller, R.H. (1972). Effects of vesicular-arbuscular mycorrhizae on growth and phosphorus content of three agronomic crops. Soil Science Society of American Proceedings 36: 64-67.

Kumar, V. and Narula, N. (1999). Solubilization of inorganic phosphates by *Azotobacter chroococcum* mutants and their effect on seed emergence of wheat. Biology and Fertility of Soils 28: 301–305.

Kumar, V., Sharma, S., Punia ,S.S., Narula, N., Behl, R.K. and Singh, B.P. (2004). Relative efficacy of *Azotobacter chroococcum* on tall and dwarf wheats (Triticum aestivum L.) in aridisols. Annals of Agri-Bio Research 9: 53–58.

Kumar, V., Solanki, A.S. and Sharma, S. (2009). Yield and economics of *Withania somnifera* influenced by dual inoculation of *Azotobacter chroococcum* and *Pseudomonas putida*. Turkish Journal of Biology 33: 219–223.

Kumar, V., Aggarwal, N.K. and Singh, B.P. (2000). Influence of P- solubilizing analogue resistant mutants of *Azotobacter chroococcum* on yield and quality parameters of *Helianthus annus*. Folia Microbiologica 45: 347–352.

Kumar, V., Behl, R.K. and Narula, N. (2001a). Effect of P-solubilizing *Azotobacter chroococcum* on yield traits and their survival in the rhizosphere of wheat genotypes under field conditions. Acta Agronomica Hungarica. 49: 141–149.

Kumar, V., Behl, R. K. and Narula, N. (2001b). Establishment of P- solubilizing analogue resistant strains of *Azotobacter chroococcum* in rhizosphere and their effect on wheat under green house conditions. Microbiological Research 156: 87-94.

Kumar, V., Punia, S.S., Lakshminarayana, K. and Narula N. (1999). Studies on interaction of phosphate solubilizing *Azotobacter chroococcum* and *Sorghum bicolor*. Indian Journal of Agricultural Sciences 69: 30–32.

Martin, P., Glatzle, A., Kolb, W., Omay, H. and Schmidt, W. (1989). N_2- fixing bacteria in rhizosphere: Quantification and hormonal effects on root development. Z. Pflanzenernahr Bodenk., 152: 237–245.

Narula, N., Kumar, V., Behl, R.K., Deubal, A., Gransee, A. and Merbach, W. (2000). Effect of P-solubilizing *Azotobacter chroococcum* on N, P, K uptake in P-responsive wheat genotypes grown under green house conditions. Journal of Plant Nutrition and Soil Science 163: 393–398.

Narula, N., Saharan, B.S., Kumar, V., Bhatia, R., Bishnoi, L.K., Lather, B.P.S. and Lakshminarayana, K. (2005). Impact of use of biofertilizers on cotton (*Gossypium hirusutum*) crop under irrigated agro-ecosystem. Archives of Agronomy and Soil Science 51: 69–77.

Pandey, A. and Kumar, S. (1998). Potential of *Azotobacter* and *Asozprillum* for upland agriculture: A review. Journal of Scientific and Industrial Research 48:134-144.

Plenchette, C., Clermont-Dauphin, C., Meynard, J.M and Fortin, J.A. (2005). Managing arbuscular mycorrhizal fungi in cropping systems. Canadian Journal of Plant Sciences 85: 31-40.

Satyabrata, M. and Geetha, K.A. (2005). Characterization, genetic improvement and cultivation of *Chlorophytum borivilianum*-an important medicinal plant of India. Plant Genetic Resources 3: 264–272.

Singh, A. and Khanuja, S.P.S. (2003). Cultivation and processing of certain high value medicinal herbs for income and employment generation among smaller communities in rural areas. Journal of Rural Technology 1: 42–47.

Sockley, F.W., McGraw, R.L. and Garett, H.E. (2004). Growth and nutrient concentration of two native forage legumes inoculate with *Rhizobium* and mycorrhiza in Missouri, USA. Agro Forestry System 60: 137–142.

(Received 16 February 2011; accepted 30 May 2011)