

Phyto-Remediation Studies on Bacterial-Fungal Interaction in Stimulation, Growth and Amelioration of Physiological Condition of Citrus Crop

Rajesh Kumar Dubey¹

Director, Prakriti Educational & Research Institute, Lucknow, UP, India¹

ABSTRACT: The availability of quality planting material effect of AMF and MHB on physical and physiological attributes of citrus rootstocks (Troyer citrange and Cleopatra mandarin) was studied. The objective of this study was to evaluate the effects of bacterial inoculation like phosphorus solubilising bacteria (PSB) (*Bacillus subtilis* + *B. megatherium*), *Azospirillum* sp. and *Providencia* cultures on the growth and physiology of the symbiosis between Troyer citrange and Cleopatra mandarin and AM fungi *Glomus intraradices*. Troyer citrange co-inoculated with *G. intraradices* and PSB significantly gave the higher root colonization at 30, 60 and 120 days after inoculation (DAI). In the case of Cleopatra mandarin, the highest root colonization was found in seedlings co-inoculated with *G. intraradices* and *Azospirillum* at all the growth stages. Troyer citrange co-inoculated with *G. intraradices* and PSB gave significantly highest plant height at 210 DAI. In case of Cleopatra mandarin, significantly highest plant height was observed for seedlings co-inoculated with *G. intraradices* and *Azospirillum* at 210 DAI. Co-inoculation of *G. intraradices* and PSB recorded significantly highest stem diameter for Troyer citrange and Cleopatra mandarin at 210 DAI. The number of leaves was recorded significantly more for Troyer citrange and Cleopatra mandarin co-inoculated with *G. intraradices* and PSB at 210 DAI. The significantly maximum leaf area was exhibited by Troyer citrange co-inoculated with *G. intraradices* + helper bacteria PSB or *Azospirillum* or *Providencia* (4.13, 6.97 and 10.33 cm²) at 60, 120 and 210 DAI, respectively. In case of Cleopatra mandarin, *G. intraradices* + PSB gave highest leaf area (13.66, 14.73 and 15.61 cm²) at all the growth stages. At 60 DAI, Troyer citrange inoculated with mixed strains and PSB showed significantly highest relative water content (79.04%), while at 120 and 210 DAI, seedlings treated with *G. intraradices* and PSB showed significantly highest relative water content (86.09 and 88.19%, respectively). In case of Cleopatra mandarin significantly highest relative water content was found in seedlings co-inoculated with *G. intraradices* + PSB at all the growth stages (82.81, 87.56 and 89.18%, respectively). Chlorophyll a and total chlorophyll content was recorded more in *G. intraradices* + PSB treatment for Troyer citrange at 120 and 210 DAI. Total carotenoids were more in Troyer citrange seedlings inoculated with *G. intraradices* + PSB at 120 and 210 DAI. For Cleopatra mandarin, similarly co-inoculation of *G. intraradices* + PSB produced highest chlorophyll a and total chlorophyll at 120 and 210 DAI. Total carotenoids content was more in *G. intraradices* + PSB at 120 and 210 DAI, respectively. Total phenols were recorded significantly highest in *G. intraradices* + *Azospirillum*-inoculated Troyer citrange and Cleopatra mandarin.

KEYWORDS: Phosphorus Solubilising Bacteria (PSB), Rhizosphere, Mycorrhizal Symbiosis.

I. INTRODUCTION

An emerging area of study in the field of fruit crop improvement involves getting more quality returns per unit area by use of improved varieties and adopting good agronomic practices, considering the health of soil and environment for sustainable crop production. Increased concern about the hazardous impact of agrochemicals on the environment has stimulated continued interest in the use of bio-fertilizers. There are abundant microorganisms thriving in soil, especially in the rhizosphere of plants. It is well known that a considerable number of bacterial and fungal species possess a

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 1, January 2015

functional relationship and constitute a holistic system with plants. They are able to exert beneficial effects on plant growth (Vessey, 2003).

Mycorrhizal symbiosis is the most important mutualistic association between specific group of fungi from soils and the roots of terrestrial plants (Gadkar *et al.*, 2001). Agricultural and horticultural crops have been shown to benefit from AM fungi on a world-wide basis (Mosse, 1973). Ubiquitous AM fungi and plant-growth-promoting rhizosphere bacteria are components of natural systems that benefit the host plant (Barea and Jeffries, 1995). As a result of microbial-plant associations, crop production can be increased and mixed inocula have been recommended for re-vegetation purposes (Bowen and Rovira, 1999). The cooperation of AM fungi and bacteria in nutrient uptake by plants may be due to specific attributes of microorganisms, such as the ability of certain bacteria to stimulate mycorrhizal formation and development (Garbaye, 1994). This group of bacteria are referred as mycorrhizal helper bacteria or MHB (Meyer and Linderman, 1986).

Citrus has few and short root hairs and is highly dependent on arbuscular mycorrhizae, because the most common mutualistic symbiosis replaces part of the root-hair functions (Graham and Syvertsen, 1985). The study on co-inoculation of AM fungi and MHB is a promising approach either for low-input agricultural technologies (Gianinazzi and Schuepp, 1994), or for the reestablishment of natural vegetation in a degraded area (Jeffries and Barea, 2001).

Thus, it is necessary to determine the most promising combination for maximum growth response for a particular crop. Keeping these points in view, a study was undertaken on two citrus rootstocks to evaluate the effect of AM fungi and mycorrhizal helper bacteria on growth and physiological attributes.

II. MATERIALS AND METHODS

The study was conducted on two citrus rootstocks, viz. Troyer citrange (*Citrus sinensis* × *Poncirus trifoliata*) and Cleopatra mandarin (*C. reshni*) collected from the Citrus Germplasm Block of the Division of Fruits and Horticultural Technology, Indian Agricultural Research Institute (IARI), New Delhi during 2009-2011. Seeds of the two citrus rootstocks were surface sterilized by immersing in 70% alcohol for five minutes, followed by rinsing with sterile distilled water and then germinated on wet filter paper in Petri dishes at 28°C. After 7 days these seedlings were transplanted in plastic containers (12 cm × 20 cm) containing mixture of soil: sand: FYM (2:2:1) under glasshouse conditions. The potting mixture had EC_(1:2) 6.35 mS m⁻¹, pH_(1:2) 7.92, HCO₃⁻ 1.14 g kg⁻¹ and Cl⁻ 5910.75 ppm. The AMF strain, *Glomus intraradices* and three mycorrhizal helper bacterial strains, viz., phosphate solubilising bacteria (*Bacillus subtilis* + *B. megatherium*), *Azospirillum* and *Providencia* were used in the experiment. *Glomus intraradices* and all the bacterial strains were purchased from Division of Microbiology, IARI, New Delhi.

Table 1. Root colonization of arbuscular mycorrhizal citrus seedlings as influenced by mycorrhizal helper bacteria.

Treatment	Root colonization (%)		
	30 DAI	60 DAI	120 DAI
T ₁	0.00 (0.72)*	0.00 (0.72)	6.25 (14.48)
T ₂	0.00 (0.72)	0.00 (0.72)	9.37 (17.59)
T ₃	0.00 (0.72)	0.00 (0.72)	9.37 (17.59)
T ₄	0.00 (0.72)	0.00 (0.72)	6.25 (14.48)
T ₅	21.87 (27.83)	34.37 (35.87)	46.87 (43.21)
T ₆	34.37 (35.87)	59.37 (50.42)	71.87 (58.01)
T ₇	31.25 (33.99)	46.87 (43.21)	68.75 (56.01)
T ₈	25.00 (29.82)	40.63 (39.58)	62.50 (52.30)

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 1, January 2015

T ₉	0.00 (0.72)	0.00 (0.72)	6.25 (14.48)
T ₁₀	0.00 (0.72)	0.00 (0.72)	12.50 (20.71)
T ₁₁	0.00 (0.72)	0.00 (0.72)	9.37 (17.59)
T ₁₂	0.00 (0.72)	0.00 (0.72)	6.25 (14.48)
T ₁₃	18.75 (25.35)	28.13 (31.99)	37.50 (37.70)
T ₁₄	18.75 (25.66)	37.50 (37.76)	56.25 (48.59)
T ₁₅	25.00 (29.82)	43.75 (41.41)	56.25 (48.62)
T ₁₆	21.87 (27.83)	34.37 (35.87)	50.00 (45.00)
SE±	3.32	2.30	3.189
CD _{0.05}	9.73	6.75	9.365

* Figures in parenthesis are Arc Sin $\sqrt{\%}$ transformation data. DAI= Days after inoculation

AM fungi (approx. 20 g inocula per pot) either alone or in combination with helper bacteria were put at 5 cm below the seedlings at transplanting in plastic containers. The seedlings were maintained in glasshouse with day night temperatures ranging from 27±1°C. Day lengths were being extended up to 16 h with cool white fluorescent lights at 630 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Seedlings were watered on alternate days with sterile tap water. After two months, the seedlings along with plastic containers were transferred to open conditions at High-Density Orchard of Division of Fruits and Horticultural Technology, IARI, New Delhi. The seedlings were irrigated at two day intervals. Water used for irrigation had EC_(1:2) 288 $\mu\text{S m}^{-1}$, pH_(1:2) 7.48, HCO_3^- 1.0 meq l^{-1} and Cl^- 110.76 ppm.

Table 2 Physical parameters of citrus seedlings as influenced by microbial inoculants.

Treatm ent	Plant height (cm)			Stem diameter (mm)			No. of leaves/seedling			Leaf area (cm ²)		
	60 DAI	120 DAI	210 DAI	60 DAI	120 DAI	210 DAI	60 DAI	120 DAI	210 DAI	60 DAI	120 DAI	210 DAI
T ₁	9.25	20.95	37.20	2.33	3.02	6.01	14.50	40.50	126.50	1.09	1.82	2.96
T ₂	11.40	43.45	93.80	2.49	3.17	7.61	19.00	59.00	142.50	1.63	2.60	3.30
T ₃	10.00	40.05	86.95	2.74	3.55	7.27	15.50	42.50	102.00	1.77	2.66	3.21
T ₄	10.40	49.95	93.45	3.01	5.41	12.24	23.50	67.00	141.00	1.93	3.06	3.94
T ₅	13.35	48.60	58.55	3.08	5.18	15.38	12.50	35.00	108.50	2.13	3.44	3.93
T ₆	12.60	61.50	111.75	3.65	7.05	16.47	20.50	55.00	180.50	4.13	5.50	7.46
T ₇	10.75	55.60	107.55	3.18	6.76	12.76	18.50	45.00	160.00	3.86	6.97	9.44
T ₈	9.65	54.25	106.90	2.67	5.79	10.57	13.50	33.00	123.00	3.09	6.86	10.33
T ₉	7.35	19.40	26.05	2.31	3.59	3.59	6.50	16.50	42.00	3.78	4.60	5.76
T ₁₀	7.70	27.15	36.55	2.84	4.90	6.08	8.50	18.50	54.50	6.03	6.97	7.93

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 1, January 2015

T ₁₁	8.85	26.80	42.80	2.91	4.39	7.05	12.50	30.00	88.00	7.15	8.27	9.39
T ₁₂	9.10	27.50	40.85	3.04	5.73	6.92	11.50	28.50	77.50	6.12	6.75	7.98
T ₁₃	7.25	31.30	32.85	2.68	4.74	4.57	11.00	17.00	47.00	5.79	6.38	8.46
T ₁₄	7.55	48.50	61.20	2.89	5.22	8.17	18.50	43.50	136.50	13.66	14.73	15.61
T ₁₅	7.30	46.80	64.10	2.59	3.93	5.86	8.50	15.50	73.50	7.06	7.96	9.23
T ₁₆	8.90	32.50	48.85	2.45	4.69	5.31	11.00	24.00	90.00	4.79	5.79	7.05
SE±	0.72	2.43	1.93	0.12	0.33	0.60	2.32	3.69	4.46	0.46	0.46	0.48
CD _{0.05}	2.10	7.13	5.66	0.34	0.98	1.76	6.81	10.83	13.13	1.34	1.36	1.41

The experimental design adopted was completely randomised design with 16 treatments and two replications per treatment. The treatment included T₁ = control Troyer citrange, T₂ = Troyer citrange inoculated with phosphate solubilising bacteria (PSB), T₃ = Troyer citrange inoculated with *A. brasilense*, T₄ = Troyer citrange inoculated with *Providencia* sp., T₅ = Troyer citrange inoculated with *G. intraradices*, T₆ = Troyer citrange co-inoculated with *G. intraradices* and PSB, T₇ = Troyer citrange co-inoculated with *G. intraradices* and *A. brasilense*, T₈ = Troyer citrange co-inoculated with *G. intraradices* and *Providencia* sp., T₉ = control Cleopatra mandarin, T₁₀ = Cleopatra mandarin inoculated with PSB, T₁₁ = Cleopatra mandarin inoculated with *A. brasilense*, T₁₂ = Cleopatra mandarin inoculated with *Providencia* sp., T₁₃ = Cleopatra mandarin inoculated with *G. intraradices*, T₁₄ = Cleopatra mandarin co-inoculated with *G. intraradices* and PSB, T₁₅ = Cleopatra mandarin co-inoculated with *G. intraradices* and *A. brasilense*, T₁₆ = Cleopatra mandarin co-inoculated with *G. intraradices* and *Providencia* sp. The two years experimental data were analyzed with SPSS package (SPSS 11.0) and significance of variance was estimated by applying F test at 5% level of significance.

Table 3: Leaf relative water content of citrus seedlings as influenced by microbial inoculants (Mean of 2009-10 and 2010-11).

Treatment	Leaf relative water content (%)		
	60 DAI	120 DAI	210 DAI
T ₁	58.18	68.61	69.39
T ₂	66.66	70.76	71.83
T ₃	64.56	78.59	79.06
T ₄	58.92	74.51	75.96
T ₅	65.66	74.41	77.53
T ₆	72.37	86.09	88.19
T ₇	67.78	82.13	82.84
T ₈	61.74	76.52	80.47
T ₉	59.12	69.02	71.11
T ₁₀	63.43	82.27	82.48

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 1, January 2015

T ₁₁	60.85	80.74	81.54
T ₁₂	67.99	75.38	76.69
T ₁₃	77.52	82.13	82.57
T ₁₄	82.81	87.56	89.18
T ₁₅	80.12	83.25	84.61
T ₁₆	75.54	76.78	81.17
SE $\pm$	0.39	0.68	0.59
CD _{0.05}	0.121	1.16	2.01

The percentage of AM colonization in roots was analyzed at 30, 60 and 120 days after inoculation (DAI) by clearing and staining of roots by the method (Phillips and Haymans, 1970). Seedlings were analyzed for morphological parameters like plant height, stem diameter, number of leaves and leaf area at 60, 120 and 210 DAI. Relative water content (RWC) of the fifth fully expanded leaf from the shoot apex was evaluated at 60, 120 and 210 DAI by the method of Barrs and Weatherley (1962). Leaf pigments (chlorophyll a, chlorophyll b, total chlorophyll and total carotenoids) were assayed at 120 and 210 DAI according to Hiscox and Israelstam (1979). Total phenols content in leaf was estimated according to Malik and Singh (1980). Absorbance was read at 750 nm. The concentration of phenols in the test sample was calculated by comparing with a standard curve drawn with known concentrations of catechol and then values were expressed as mg catechol equivalent of phenol g⁻¹ leaf sample.

III. RESULTS AND DISCUSSION

Root Colonisation

Arbuscular mycorrhizal inoculation increased root colonization (Table 1). Mycorrhizal helper bacteria synergistically increased root colonization of AM fungi at different growth stages in both the citrus rootstocks used in the experiment. Troyer citrange co-inoculated with *G. intraradices* and PSB significantly showed the higher root colonization at 30, 60 and 120 DAI (34.37, 59.37 and 71.87%, respectively), which was statistically at par with Troyer citrange co-inoculated with *G. intraradices* and *Azospirillum* (31.25%) and *G. intraradices* and *Providencia* (25.00%) at 30 DAI and Troyer citrange co-inoculated with *G. intraradices* and *Azospirillum* (68.75%) at 60 DAI, respectively. In the case of Cleopatra mandarin, the highest root colonization was found in seedlings inoculated with *G. intraradices* and *Azospirillum* at all the growth stages (25.00, 43.75 and 56.25% at 30, 60 and 120 DAI, respectively), which was statistically at par with seedlings inoculated with *G. intraradices* and PSB (37.50%) at 60 DAI and *G. intraradices* and PSB (56.25%) at 120 DAI. Non-mycorrhizal control citrus seedlings or seedlings inoculated with either of the bacteria alone did not exhibit any AM fungal inoculation at 30 and 60 DAI, but at 120 DAI these seedlings were also infected by native AM fungal strains, while their root colonization was less than 13%. Many of the descriptions of bacteria-induced promotion of fungal growth or development involve interactions between fungi and mycorrhiza helper bacteria (MHB), which aid mycorrhizal development between the fungus and the plant host (Kobayashi and Crouch, 2009). The present finding is in agreement with those of Marulanda *et al.* (2003) and Pivato *et al.* (2009).

Table 4. Pigment content (mg g⁻¹ f. w. of leaf) of citrus seedlings as influenced by microbial inoculants.

Treatment	Chlorophyll a		Chlorophyll b		Total chlorophyll		Carotenoids	
	120 DAI	210 DAI	120 DAI	210 DAI	120 DAI	210 DAI	120 DAI	210 DAI
T ₁	0.42	1.37	0.31	0.26	0.73	1.64	0.14	0.32
T ₂	0.65	1.53	0.45	0.40	1.10	1.93	0.23	0.47
T ₃	0.55	1.40	0.46	0.27	1.01	1.66	0.21	0.35

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 1, January 2015

T ₄	0.74	1.55	0.48	0.30	1.22	1.85	0.26	0.40
T ₅	0.62	1.51	0.46	0.33	1.07	1.83	0.19	0.37
T ₆	0.89	1.76	0.48	0.28	1.37	2.22	0.32	0.43
T ₇	0.84	1.60	0.46	0.31	1.30	1.99	0.28	0.42
T ₈	0.85	1.53	0.52	0.34	1.36	1.85	0.28	0.40
T ₉	0.16	0.88	0.05	0.06	0.20	0.94	0.08	0.30
T ₁₀	0.45	1.03	0.08	0.07	0.54	1.10	0.18	0.33
T ₁₁	0.35	1.51	0.09	0.18	0.44	1.85	0.16	0.43
T ₁₂	0.54	1.29	0.12	0.14	0.66	1.43	0.21	0.40
T ₁₃	0.42	1.04	0.10	0.11	0.52	1.15	0.14	0.35
T ₁₄	0.69	1.67	0.11	0.14	0.80	1.89	0.27	0.43
T ₁₅	0.64	1.56	0.10	0.16	0.74	1.71	0.23	0.41
T ₁₆	0.65	1.32	0.15	0.08	0.80	1.40	0.23	0.34
SE _±	0.01	0.01	0.02	0.02	0.047	0.03	0.01	0.01
CD _{0.05}	0.04	0.04	0.07	0.06	0.11	0.09	0.02	0.01

Physical parameters

Troyer citrange exhibited significant response in plant height to single inoculation of *G. intraradices* alone (13.35 cm) at 60 DAI, which was at par with seedlings inoculated with *G. intraradices* and PSB (12.60 cm) and seedlings inoculated with PSB alone (11.40 cm). At 120 and 210 DAI, co-inoculation of *G. intraradices* and PSB highest plant height (61.50 and 111.75 cm, respectively), which was at par with *G. intraradices* and *Azospirillum* (55.60 and 107.55 cm) and *G. intraradices* and *Providencia* (106.90 cm) at 120 and 210 DAI, respectively. In case of Cleopatra mandarin, highest plant height was obtained in seedlings inoculated with *Providencia* alone (9.10 cm) at 60 DAI, while at 120 and 210 DAI, it was more in seedlings co-inoculated with *G. intraradices* and PSB (48.50 and 61.20 cm) and seedlings inoculated with *G. intraradices* and *Providencia* (46.80 and 64.10 cm) (Table 2). Jaizme-Vega *et al.* (2004) also found that combined inoculation (*G. manihotis* + *Bacillus* spp.) of 'Grande Naine' banana plants resulted in an increase in plant development compared with single inoculation of either AM fungi or plant growth promoting rhizobacteria.

Stem diameter was found to be better in microbial treatments. The maximum stem diameter was recorded in seedlings co-inoculated with *G. intraradices* and PSB at 60, 120 and 210 DAI in case of Troyer citrange (3.65, 7.05 and 16.47 mm, respectively) and seedlings singly inoculated with *Providencia* culture at 60 and 120 DAI (3.04 and 5.73 mm, respectively) and co-inoculated with *G. intraradices* and PSB (8.17 mm) at 210 DAI in case of Cleopatra mandarin (Table 2). It is well known that presence of MHB caused better AM fungal colonization on plant roots. Furthermore, AM fungal inoculation significantly stimulated growth parameters like stem diameter, as observed by Wu *et al.* (2007). Significantly maximum number of leaves was produced in Troyer citrange by single inoculated treatment of *Providencia* culture (23.50 and 67.00 at 60 and 120 DAI, respectively). At 210 DAI, co-inoculation of *G. intraradices* and PSB significantly recorded maximum number of leaves (180.50). In case of Cleopatra mandarin, co-inoculation of *G. intraradices* and PSB significantly produced maximum number of leaves (18.50, 43.50 and 136.50 at 60, 120 and 210 DAI, respectively) (Table 2).

For Troyer citrange, significantly higher leaf area was recorded by the treatment of *G. intraradices* and PSB (4.13 cm²), *G. intraradices* and *Azospirillum* (6.97 cm²) and *G. intraradices* and *Providencia* (10.33 cm²) at 60, 120 and 210 DAI, respectively. For Cleopatra mandarin, co-inoculated treatment of *G. intraradices* and PSB significantly produced maximum leaf area at all the growth stages (13.66, 14.73 and 15.61 cm²) (Table 2).

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 1, January 2015

The present result is in harmony with the findings of Mamatha *et al.* (2002) in mulberry and Bashan and Levanony (1990) in papaya. It is largely known that rhizobacteria and AM fungi increase plant growth (Basham, 1999; Barea *et al.*, 2002).

Physiological Parameters

The present study showed that co- inoculation of mixed strains and PSB recorded highest leaf relative water content (79.04 %) at 60 DAI and *G. intraradices* and PSB at 120 and 210 DAI (86.09 and 88.19%) in Troyer citrange and *G. intraradices* and PSB in Cleopatra mandarin (82.81, 87.56 and 89.18 per cent) at different growth stages, respectively (Table 3). Higher relative water content of citrus seedlings inoculated with AM fungi and PSB implied PSB improved the mutualistic interaction of AM fungi with citrus seedlings as stimulation of bacteria can promote the establishment and development of mycorrhizae (Dai *et al.*, 2008).

The co-inoculation of *G. intraradices* and PSB significantly increased chlorophyll a content in Troyer citrange (0.89 and 1.76 mg g⁻¹ f. w.) and Cleopatra mandarin (0.69 and 1.67 mg g⁻¹ f. w.) at 120 and 210 DAI, respectively (Table 3). The Troyer citrange seedlings inoculated with PSB alone significantly had higher chlorophyll b content at 210 DAI (0.40 mg g⁻¹ fresh weight). In case of Cleopatra mandarin, seedlings inoculated with *Azospirillum* alone had highest chlorophyll b content (0.18 mg g⁻¹ fresh weight) at 210 DAI. The AM fungus *G. intraradices* in association with the mycorrhizal helper bacteria PSB significantly increased total leaf chlorophyll content (a+b) at 120 and 210 DAI in both Troyer citrange (1.37 and 2.22 and mg g⁻¹ fresh weight, respectively) and Cleopatra mandarin (0.80 and 1.89 mg g⁻¹ fresh weight, respectively) (Table 4). An increase in chlorophyll content due to inoculation with AM fungi has been reported previously (Boby *et al.*, 2008). Thus observed increase in chlorophyll content in the present study might be due to changes in the plant metabolism, as affected by AM fungi and the helper bacteria (Kim *et al.*, 2010).

Table 5: Leaf osmolyte contents of citrus seedlings as influenced by microbial inoculants

Treatment	Phenol (mg/100 g f.w)		Proline (µg/ g f.w)		Total soluble sugars (%)	
	120 DAI	210 DAI	120 DAI	210 DAI	120 DAI	210 DAI
T ₁	37.36	23.05	97.92	62.40	46.40	29.56
T ₂	41.23	24.76	117.72	78.30	47.61	29.39
T ₃	42.82	24.44	114.92	73.52	53.62	38.48
T ₄	41.32	23.99	125.26	85.71	59.39	37.39
T ₅	43.32	36.98	175.36	103.10	78.26	47.42
T ₆	49.12	42.83	255.20	197.38	80.40	51.40
T ₇	50.54	47.68	238.30	118.00	82.40	55.47
T ₈	47.23	43.06	207.10	107.66	80.44	50.19
T ₉	30.83	21.10	109.58	75.18	56.42	31.46
T ₁₀	31.78	21.84	122.36	82.22	57.40	39.49
T ₁₁	32.84	22.78	119.10	78.30	63.35	45.77
T ₁₂	32.16	22.16	128.40	90.60	69.50	49.27
T ₁₃	41.08	36.48	176.22	107.30	82.47	58.70
T ₁₄	48.27	44.41	262.00	189.24	87.44	65.44
T ₁₅	49.05	47.46	250.04	129.96	87.02	63.21
T ₁₆	44.80	39.70	213.36	113.18	86.29	59.40

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 1, January 2015

SE±	0.33	0.24	0.39	0.34	0.13	0.09
CD _{0.05}	0.97	0.69	1.15	1.01	0.39	0.26

The present study also revealed that *G. intraradices* + PSB significantly increased total carotenoids content in both Troyer citrange and Cleopatra mandarin seedlings at 120 DAI (0.32 and 0.27 mg g⁻¹ f. w., respectively). At 210 DAI, highest production of carotenoids was recorded in Troyer citrange seedlings inoculated with PSB alone (0.47 mg g⁻¹ f. w.) and Cleopatra mandarin seedlings co-inoculated with *G. intraradices* and PSB (0.43 mg g⁻¹ f. w.) and *Azospirillum* alone (0.43 mg g⁻¹ f. w.) (Table 4). Similar result was found by Manoharan *et al.* (2010) in *Erythrina variegata*. The enhancement of the photosynthetic rate in co-inoculated plants affects the translocation of soluble sugars to host roots, thus increasing fungal growth and activity in the roots and thereby increasing leaf chlorophyll and carotenoid contents. Plant phenolics are the most widespread classes of secondary metabolites known to be involved in the plant-microbe interactions (Morandi, 1996). Phenols are important components of plant defence mechanism against diseases. Phenolic compounds occur naturally in plant system and owing to their anti-microbial properties inhibit fungal spore germination and toxin production by pathogens (Vidhyasekaran, 1973). AM fungi can increase the total phenols content, which might be one of the factors responsible for increased disease resistance found in mycorrhizal plants (Krishna and Bagyaraj, 1984). The present study revealed that *Glomus intraradices* in combination with *Azospirillum* had significant effect on level of phenol in both Troyer citrange (50.54 and 47.68 mg 100 g⁻¹ f. w.) and Cleopatra mandarin (49.05 and 47.46 mg 100 g⁻¹ f. w.) at 120 and 210 DAI, respectively (Table 5).

Proline, an important osmolyte in leaf cytoplasm which protects and stabilise macro-molecules from oxidative damage, tend to decrease with passage of time in both Troyer citrange and Cleopatra mandarin seedlings, irrespective of treatments. However, the control seedlings recorded lowest proline content in leaf cytoplasm in Troyer citrange (97.92 and 62.40 µg g⁻¹ f.w.) and Cleopatra mandarin (109.58 and 75.18 µg g⁻¹ f.w.) at 120 and 210 DAI, respectively. The highest proline content was recorded in *G. intraradices* + PSB inoculated Troyer seedlings (255.20 and 197.38 µg g⁻¹ f.w.) and Cleopatra seedlings (262.00 and 189.24 µg g⁻¹ f.w.) at 120 and 210 DAI, respectively (Table 5). Vivas *et al.* (2003) also reported highest proline content in lettuce seedlings co-inoculated with *G. intraradices* and phosphorus solubilizing bacteria *Bacillus* sp. The greater concentration of amino acids in AM shoots and roots may indicate a greater capability for osmotic adjustment through amino acid accumulation in these plants (Auge, 2001).

The total soluble sugars in the leaves recorded declining trend with the passage of time in both the citrus plants (Table 5). It was found highest in Troyer citrange seedlings inoculated with *G. intraradices* + *A. brasilense* at 120 and 210 DAI (82.40 and 55.47%, respectively) followed by *G. intraradices* + PSB and *G. intraradices* + *Providencia* at 120 DAI (80.40 and 80.44%) and at 210 DAI (51.40 and 50.19%), as compared to control (46.40 and 29.56%) at 120 and 210 DAI, respectively. In case of Cleopatra mandarin, the highest total soluble sugars was observed in seedlings co-inoculated with *G. intraradices* and PSB (87.44 and 65.44%) at 120 and 210 DAI, respectively, followed by seedlings inoculated with *G. intraradices* + *A. brasilense* (87.02 and 63.21%) and *G. intraradices* + *Providencia* (86.29 and 59.40%) at 120 and 210 DAI, respectively, while the uninoculated control seedlings significantly recorded lowest leaf total soluble sugars at both the growth stages (56.42 and 31.46%, respectively). The result is in harmony with Selvaraj *et al.* (2009). Phosphorus plays the most important role during the breakdown of carbohydrates and synthesis of polysaccharides. In particular, phosphorus is very effective in the synthesis of starch from glucose (Demir, 2004). Since, AM fungi increase the uptake of phosphorus; they may also increase the synthesis of carbon compounds (Graham, 2000).

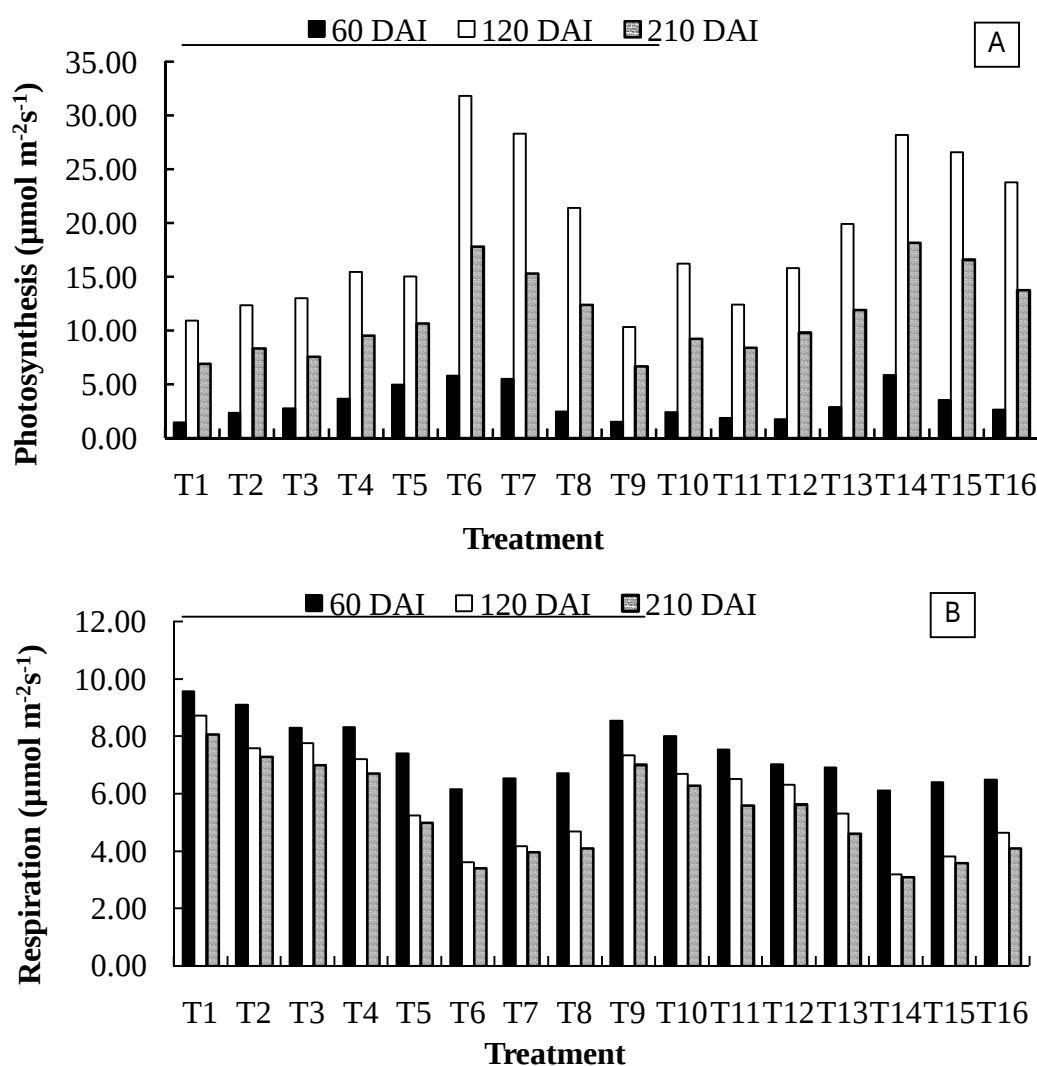
Troyer citrange and Cleopatra mandarin seedlings exhibited significant response to co-inoculation of *G. intraradices* and PSB for photosynthetic rate at all the growth stages, as compared to other treatments, while the control seedlings exhibited the lowest photosynthetic rate. For Troyer citrange, *G. intraradices* and PSB had significantly enhanced the photosynthetic rate at all the growth stages (5.76, 31.82 and 17.82 µmol m⁻²s⁻¹, respectively), which was non-significant with co-inoculation of *G. intraradices* and *A. brasilense* (5.48 µmol m⁻²s⁻¹) at 60 DAI. Cleopatra mandarin also had positive response to *G. intraradices* and PSB for photosynthetic rate at all the growth stages (5.89, 28.19 and 18.19

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 1, January 2015

$\mu\text{mol m}^{-2}\text{s}^{-1}$, respectively). Irrespective of treatment, photosynthetic rate showed first increasing trend from 60 to 120 DAI and then showed decline at 210 DAI in both Troyer citrange and Cleopatra mandarin (Fig. 1).



International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 1, January 2015

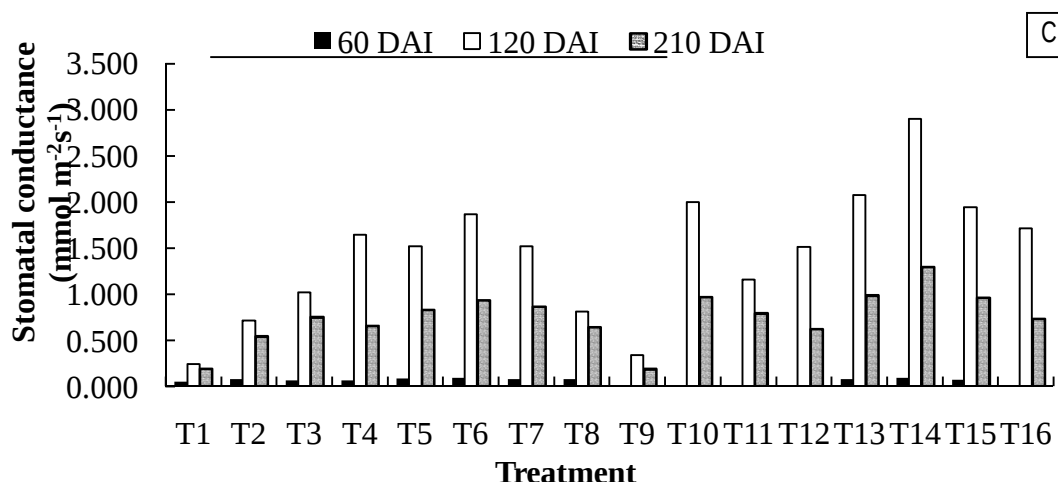


Fig. 1 Response of Troyer citrange and Cleopatra mandarin to AM fungi and MHB on (A). leaf photosynthetic rate, (B). Leaf respiration rate and (C). Leaf stomatal conductance.

The observation recorded for leaf respiration rate of Troyer citrange and Cleopatra mandarin seedlings, as influenced by AM fungi and MHB, revealed that Cleopatra seedlings had comparatively lesser leaf respiration rate than Troyer citrange at different growth stages and respiration rate declined with passage of time, irrespective of any treatment for both the citrus plants (Fig. 2). At 60 DAI, Troyer citrange and Cleopatra mandarin seedlings co-inoculated with *G. intraradices* and PSB exhibited the lowest leaf respiration rate (6.17 and $6.11 \mu\text{mol m}^{-2}\text{s}^{-1}$, respectively), which was non-significant with seedlings co-inoculated with *G. intraradices* and *A. brasilense* and *G. intraradices* and *Providencia* (6.53 and $6.71 \mu\text{mol m}^{-2}\text{s}^{-1}$, respectively) in case of Troyer citrange and *G. intraradices* and *A. brasilense* and *G. intraradices* and *Providencia* (6.41 and $6.48 \mu\text{mol m}^{-2}\text{s}^{-1}$, respectively) in case of Cleopatra mandarin. At 120 DAI, *G. intraradices* + PSB and *G. intraradices* + *A. brasilense* recorded lowest respiration rate for Troyer citrange (3.62 and $4.17 \mu\text{mol m}^{-2}\text{s}^{-1}$, respectively) and Cleopatra mandarin (3.19 and $3.81 \mu\text{mol m}^{-2}\text{s}^{-1}$, respectively). At 210 DAI, co-inoculation of *G. intraradices* and PSB resulted in minimum leaf respiration rate in Troyer citrange and Cleopatra mandarin (3.40 and $3.10 \mu\text{mol m}^{-2}\text{s}^{-1}$, respectively), which was non-significant with dual inoculated treatment of *G. intraradices* + *A. brasilense* (3.96 and $3.59 \mu\text{mol m}^{-2}\text{s}^{-1}$) and *G. intraradices* + *Providencia* (4.10 and $4.11 \mu\text{mol m}^{-2}\text{s}^{-1}$) in case of Troyer citrange and Cleopatra mandarin, respectively.

Glomus intraradices and PSB had a synergistic effect for increased stomatal conductance at 60, 120 and 210 DAI in both Troyer citrange (0.098 , 1.866 and $0.938 \text{ mmol m}^{-2}\text{s}^{-1}$, respectively) and Cleopatra mandarin (0.095 , 2.905 and $1.296 \text{ mmol m}^{-2}\text{s}^{-1}$, respectively), which was non-significant with co-inoculation of AMF mixed AMF strains and PSB ($0.091 \text{ mmol m}^{-2}\text{s}^{-1}$) at 60 DAI and co-inoculation of *G. intraradices* and *A. brasilense* ($0.870 \text{ mmol m}^{-2}\text{s}^{-1}$) and single inoculation of *G. intraradices* ($0.833 \text{ mmol m}^{-2}\text{s}^{-1}$) at 210 DAI in case of Troyer citrange (Fig. 3).

The increase in photosynthesis and stomatal conductance due to inoculation of AM fungi was also reported in chilli (Aguilera-Gomez *et al.* (1999) and maize (Boomsma and Vyn, 2008). Mycorrhiza can increase lateral root formation, which can indirectly increase cytokinin production and also enhances net photosynthetic rate (Davies *et al.*, 1996). These authors attributed the increase in the rate of photosynthesis to increased sink strength resulting from the presence of the mycobiont. One of the most common explanations for reduced leaf respiration rate in microbial inoculated seedlings is the strongly increased absorbing surface caused by soil-growing hyphae combined with the fungal ability to take up water from soils with low water potential (Lehto and Zwiazek 2011).

In the present investigation, the application of bio-fertilizers containing beneficial microorganisms gave a promoting effect on the growth of citrus rootstocks and also improved the physiological status. The co- inoculation of AM fungi and helper bacteria resulted in significant improvement in growth performance, chlorophyll content, carotenoids

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 1, January 2015

production and accumulation of osmolytes like total phenols. In particular, *Glomus intraradices* and phosphate solubilising bacteria had synergistic effect on higher growth of Troyer citrange and Cleopatra mandarin seedlings, which can be used as bio-fertilizers in the propagation media.

REFERENCES

1. Vessey, K.V. (2003). Plant growth promoting rhizobacteria as biofertilizers, *Plant soil*, **255**: 571-586.
2. Gadkar, V., David-Schwartz, R., Kunik, T. and Kapulnik, Y. (2001). Arbuscular mycorrhizal fungal colonization and factors involved in host recognition. *Plant Physiology*, **127**: 1493-1499.
3. Mosse, B. (1973). Advances in the study of vesicular-arbuscular mycorrhiza. *Ann. Rev. Phytopathol.*, **11**: 171-196.
4. Barea, J. M. and Jeffries, P. (1995). Arbuscular mycorrhizas in sustainable soil-plant systems. In: Varma A., and Hock, B. (Eds), *Mycorrhiza: structure, function, molecular biology and biotechnology*. Springer, Berlin Heidelberg New York, pp 521-561.
5. Bowen, G. D. and Rovira, A. D. (1999). The rhizosphere and its management to improve plant growth. *Adv Agron*, **66**: 1-102.
6. Garbaye, J. (1994). Tansley Review No. 76. Helper bacteria: a new dimension to the mycorrhizal symbiosis. *New Phytol.*, **128**: 197-210.
7. Meyer, J.R. and Linderman, R.G. (1986). Response of Subterranean clover to co- inoculation with vesicular arbuscular mycorrhizal fungi and a plant growth promoting bacterium, *Pseudomonas putida*. *Soil Biology and Biochemistry*, **18**: 185-190.
8. Graham, J. H. and Syvertsen, J. P. (1985). Host determinants of mycorrhizal dependency of citrus rootstock seedlings. *New Phytology*, **101**: 667-676.
9. Jeffries, P. and Barea, J. M. (2001). Arbuscular mycorrhiza: a key component of sustainable plant-soil ecosystems. In: Hock, B. (Ed.), *The Mycota*, Vol IX: fungal associations, Springer, Berlin Heidelberg New York, pp. 95-113.
10. Philips, J. M. and Hayman, D. S. (1970). Improved procedure for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.*, **55**: 158- 161.
11. Barrs, H. D. and Weatherley, P. E. (1962). A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Australian Journal of Biological Sciences*, **15**: 413-428.
12. Hiscox, J. D. and Israelstam, G. F. (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany*, **57**: 1332-1334.
13. Malik, C.P. and Singh, M.B. (1980). In: *Plant Enzymology and Histochemistry*. Kalyani Publishers, New Delhi, pp. 53.
14. Kobayashi, D. Y. and Crouch, J. A. (2009). Bacterial/Fungal Interactions: From Pathogens to Mutualistic Endosymbionts. *Annual Review of Phytopathology*, **47**: 63-82.
15. Marulanda, A., Azcon, R. and Ruiz-Lozano, J. M. (2003). Contribution of six arbuscular mycorrhizal fungal isolates to water uptake by *Lactuca sativa* L. plants under drought stress. *Physiologia Plantarum*, **119**: 526-533.
16. Pivato, B., Offre, P., Marchelli, S., Barbonaglia, B., Mougé, C., Lemanceau, P. and Berta, G. (2009). Bacterial effects on arbuscular mycorrhizal fungi and mycorrhiza development as influenced by the bacteria, fungi, and host plant. *Mycorrhiza*, **19**: 81-90.
17. Jaizme-Vega, M. C., Rodriguez-Romero, A. S. and Pinero-Guerra, M. S. (2004). Potential use of rhizobacteria from the *Bacillus* genus to stimulate the plant growth of micropropagated banana. *Fruits*, **59**: 83-90.
18. Wu, Q. S., Xia, R. X., Zou, Y. N. and Wang, G.Y. (2007). Osmotic solute responses of mycorrhizal citrus (*Poncirus trifoliata*) seedlings to drought stress. *Acta Physiol Plant*, **29**: 543-549.
19. Mamatha, G., Bagyaraj, D. J. and Jaganath, S. (2002). Inoculation of field-established mulberry and papaya with arbuscular mycorrhizal fungi and a mycorrhiza helper bacterium. *Mycorrhiza*, **12**: 313-316.
20. Bashan, Y. and Levanony, H. (1990). Current status of Azospirillum inoculation technology: *Azospirillum* as a challenge for agriculture. *Can J Bot*, **36**: 591-608.
21. Bashan, Y. (1999). Interactions of *Azospirillum* spp. in soils: a review. *Biol. Fert. Soils*, **29**: 246-256.
22. Barea, J.M., Azcon, R. and Azcon, A. (2002). Mycorrhizosphere interactions to improve plant fitness and soil quality. *Antonie van Leeuwenhoek*, **81**: 343-351.
23. Dai, M., Wang, H. X., Yin, Y. Y., Wu, X., Wang, M. Y. and Liu, R. J. (2008). Effects and mechanisms of interactions between arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria. *Acta Ecol. Sin.*, **28**: 2854-2860.
24. Bobby, V.U., Balakrishna, A. N., Bagyaraj, D. J. (2008). Interaction between *Glomus mosseae* and soil yeasts on growth and nutrition of cowpea. *Microbiol Res*, **163**: 693-700.
25. Kim, K., Yim, W., Trivedi, P., Madhaiyan, M., Hari, P., Boruah, D., Islam, R., Lee, G. and Sa, T. (2010). Synergistic effects of inoculating arbuscular mycorrhizal fungi and *Methylobacterium oryzae* strains on growth and nutrient uptake of red pepper (*Capsicum annum* L.). *Plant Soil*, **327**: 429-440.
26. Manoharan, P.T., Shanmugiah, V., Balasubramanian, N., Gomathinayagam, S., Sharma, P. M. and Muthuchelian, K. (2010). Influence of AM fungi on the growth and physiological status of *Erythrina variegata* Linn. grown under different water stress conditions. *European Journal of Soil Biology*, **46** (2): 151-156.
27. Morandi, D. (1996). Occurrence of phytoalexins and phenolic compounds in endo-mycorrhizal interactions, and their potential role in biological control. *Plant and Soil*, **185**: 241-251.
28. Krishna, K. R. and Bagyaraj, D.J. (1984). Phenols in mycorrhizal roots of *Arachis hypogea*. *Experientia*, **40**: 85-86.
29. Vidhyasekaran, P. (1973). Possible role of ortho-dihydroxy phenolics in grape vine anthracnose disease resistance. *Indian J. Exp. Biol.*, **11** (5): 473-475.
30. Lehto T., Zwiazek J.J. (2011). Ectomycorrhizas and water relations of trees: a review, *Mycorrhiza*, **21**: 71-90.
31. Boomsma, C.R. and Vyn, T.J. (2008). Maize drought tolerance: Potential improvements through arbuscular mycorrhizal symbiosis? *Field Crops Research*, **108**: 14-31.

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 1, January 2015

32. Davies, F.T., Svenson, S.E., Cole, J.C., Phavaphutanon, L., Duray, S.A., Olalde-Portugal, V., Meier, C.E. and Bo, S.H. (1996). Non-nutritional stress acclimation of mycorrhizal woody plants exposed to drought. *Tree Physiology*, **16**: 985-993.
33. Vivas A, Marulanda A, Manuel J, Barea J, Azcon R. (2003). Influence of a *Bacillus* sp. on physiological activities of two arbuscular mycorrhizal fungi and on plant responses to PEG-induced drought stress, *Mycorrhiza*, **13**: 249-256.
34. Auge RM. (2001). Water relations, drought and vesicular arbuscular mycorrhizal symbiosis. *Mycorrhiza*, **11**: 3-42.
35. Demir, S. (2004). Influence of arbuscular mycorrhiza on some physiological growth parameters of pepper. *Turkish Journal of Biology*, **28**: 85-90.
36. Selvaraj, T., Nisha, M.C. and Rajeshkumar, S. (2009). Effect of indigenous arbuscular mycorrhizal fungi on some growth parameters and phytochemical constituents of *Pogostemon patchouli* Pellet. *Maejo International Journal of Science and Technology*, **3**(1): 222-234.
37. Aguilera-Gomez, L., Davies, F.T.J., Olalde-Portugal, V., Duray, S.A. and Phavaphutanon, L. (1999). Influence of phosphorus and endomycorrhiza (*Glomus intraradices*) on gas exchange and plant growth of chile ancho pepper (*Capsicum annum* L. cv. San Luis). *Photosynthetica*, **36** (3): 441-449.

BIOGRAPHY



Rajesh Kumar Dubey is M. Tech & Ph.D. (Biotechnology), a nationally reputed biotechnologist & sustainable development facilitator, academician, policy maker & administrator. He worked as Visiting Professor & Director, Centre for Technology Development and Research, (CTDR), JRRS University, Jaipur, Rajasthan India. He has rich & versatile experience of more than 2 decades in the field of science & technology and biotechnology for poverty alleviation and sustainability. Associated with the World Bank supported projects on

diversification support & micro-credit action research and demonstrated technology based societal transformations through knowledge connectivity. His major contributions are with innovations in rural technology for green growth, eco-livelihood and sustainability. Carrying forward with Council of Science & Technology, Organic Missions, Nature based livelihood development programmes, National Agriculture Technology Project (NATP) & Agriculture Technology Management (ATMA) development, clean energy & climate mitigation programmes etc for different national & international development institutions across the globe, Dr. Dubey is winner of more than a dozen national & international citations and awards.

--