

Effect of microbial consortium on plant growth and improvement of alkaloid content in *Withania somnifera* (Ashwagandha)

S. Rajasekar* and R. Elango

Department of Microbiology, Faculty of Science, Annamalai University, Tamilnadu-608 002, India

Abstract

The effect of microbial consortium consisting Plant Growth Promoting Rhizobacteria (PGPR) like *Azospirillum*, *Azotobacter*, *Pseudomonas* and *Bacillus* were tested separately and in combination on *Withania somnifera* for two consecutive years (2009 and 2010). The combinations of above mentioned PGPR strains significantly increased plant height, root length, and alkaloid content in *Withania somnifera* when compared to the uninoculated control. Plant growth promoting rhizobacteria (PGPR) exhibit direct and indirect mechanisms as plant growth promoters and biological control agents. Direct mechanism by PGPR, include the provision of bio-available phosphorus for plant uptake, nitrogen fixation for plant. The results of this study suggest that the PGPR applied in combination have the potential to increase the plant growth, alkaloid content of *Withania somnifera*.

Keywords: *Withania somnifera*, PGPR, Microbial consortium and withaferin- A

INTRODUCTION

Withania somnifera (Ashwagandha) is a plant used in medicine from the time of Ayurveda, the ancient system of Indian medicine. The dried roots of the plant are used in the treatment of nervous and sexual disorders. From chemistry point of view, the drug contains group of biologically active constituents known as Withanolides. The chemical structure of Withanolides have been studied and they widely distributed in family Solanaceae. Withaferin-A is therapeutically active withanolide reported to be present in leaves and root. In animal studies, Withaferin-A has shown significant anti-cancer activity. Majority of the anti-cancer drug like Vinblastine, Vincristine and Taxol have been delivered from green flora. Today there is much interest in natural products with anti-cancer activity. Withanolides are of under research potential as far treatment of cancer is concerned. The article reviews the scope of studies published in favor of anti-cancer potential of Withaferin-A.

An intensive practice that warrants high yield and quality requires the extensive use of chemical fertilizers, which are costly and may create environmental problems. Therefore, more recently there has been a resurgence of interest in environmental friendly, sustainable and organic agricultural practices (Esitken *et al.*, 2005). In this context, the use of biofertilizers containing Plant Growth Promoting Rhizobacteria (PGPR) strains instead of synthetic chemicals may serve as an effective alternative and environmental friendly practice to improve plant growth through the supply of plant nutrients and soil productivity (O'Connell, 1992). Moreover, it has been found that

exploiting these PGPR strains for the growth promotion could reduce that need for chemical fertilizers as well as the cost of cultivation.

Among different group of biofertilizers; nitrogen fixing and phosphorous solubilizing bacteria may be considered to be important since they improve plant nutrition. Plant Growth Promoting Rhizobacteria (PGPR) in the biofertilization of crops (Karlidag *et al.*, 2007) has been a well known fact that these PGPR strains may promote growth either by fixation of atmospheric nitrogen or by solubilization, if minerals such as phosphorous (Karthikeyan *et al.*, 2007; 2008) and they can also promote growth production of plant growth regulators (Klopper and Schroth, 1978; Jaleel *et al.*, 2007).

This PGPR activity was reported in species belonging to *Azospirillum*, *Azotobacter*, *Pseudomonas* and *Phosphate solubilization bacteria* (Rodriguez and Fraga, 1999; Sturz and Nawak, 2000; Sudhakar *et al.*, 2000; Karlidag *et al.*, 2007). The occurrence of *Azospirillum*, *Azotobacter*, *Pseudomonas* and *Bacillus* in the rhizosphere of medicinal plants such as *Withania somnifera* has been documented earlier (Thosar *et al.*, 2005; Attia and Saad, 2001). Furthermore, the strains are also known to stimulate growth and yield in *Catharanthus roseus* and other medicinal plants (Karthikeyan *et al.*, 2008).

However, reports regarding the bioinoculation effect of these PGPR strains in medicinal plants and particularly in *Withania somnifera* have been scarce. Hence the present study was undertaken to investigate the growth promoting effects of root inoculation of *Azospirillum*, *Azotobacter chroococcum*, *Pseudomonas fluorescens* and *Bacillus megaterium* on plant height, root length, Withaferin - A content (root alkaloid).

In addition the effect of bacterial treatment of plant leaves in *Withania somnifera* variety was also evaluated.

MATERIALS AND METHODS

Bacterial strains, Culture conditions, Media and Treatments;

All bacterial strains used in the present study were isolated from the rhizosphere soil of *Withania somnifera*, *Azospirillum* grown in N-free semisolid medium (NFb) *Azotobacter chroococcum*, grown in

*Corresponding Author

S. Rajasekar beroumand
 Department of Microbiology, Faculty of Agriculture Annamalai University,
 Annamalai Nagar – 608 002 Tamilnadu, India

Email: rajasekar.au@rediff.mail.com

Waksman Base Medium (WB) for routine use and maintained in Waksman broth with 15 % glycerol. *Pseudomonas fluorescens* King's B Agar Medium and *Bacillus subtilis* were grown on nutrient agar (NA) for routine use and for long term storage they were maintained in nutrient broth (NB) with 15 % glycerol at -80°C. They isolates were designated based on the location in Tamilnadu, India, where they were collected as Department of Microbiology, Annamalai University. *Azospirillum* (AuAs), *Azotobacter chroococcum* (AuAzc), *Pseudomonas fluorescens* (AuPsf) and *Bacillus megaterium* (AuBm). For each experiment a single colony was transferred to 500 ml flasks containing NfB, Wb, NB and King's B, broth grown aerobically in flasks on a rotating shaker (150 rpm) for 48 hrs. The bacterial suspensions were then diluted in sterile water to a final concentration of 10^9 CFU/ml, and the resulting suspensions were treated with *Withania somnifera* plants.

Field experiments

The seeding of *Withania somnifera* was raised in the pot culture yard, Department of Microbiology, Faculty of Agriculture, Annamalai University in both years 2009 and 2010. Thirty days old seedlings were dipped in the PGPR inoculums and planted in the field. The trial was conducted for two consecutive years with the same treatment. Twelve treatment plots (three plants per pot) were prepared and irrigated immediately for a better accommodation. Three replications were maintained for each treatment subsequent irrigation was done two times in a week to keep the optimum moisture level of the soil. Application of bacterial treatments with *Azospirillum*, *Azotobacter chroococcum*, *Pseudomonas fluorescens* and *Bacillus megaterium* and other combinations were performed by using the dipping methods in which the bacterial suspensions were used to inoculated plants (10^9 CFU/ml in sterile water) and control plants were dipped in sterile water. Growth promoting effects of bacterial treatments were evaluated by determining the plant height, root length (for single

primary root), Withaferin-A content (alkaloid content) on 90, 120, 150 and 180 days after planting (DAP) and the plant nutrient element (DNE) of the plant was analyzed on 180 DAP.

Withaferin-A extraction and quantification:

Withaferin-A extraction from the roots was carried out by following the standard extraction method (Zhao *et al.*, 2000). Identification and quantification of Withaferin-A was done by preparation of the thin layer chromatography using silica gel (Merck) in chloroform; methanol (98:2 v/v) (Renoudin, 1984) by comparing Rf values with authentic Withaferin-A standard (Himedia, Mumbai). Withaferin-A was spotted with dragendrafts reagent (Stahl, 1969).

Statistical analysis

Data was subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) as per procedures described by Gomez and Gomez (1984). Values represent mean \pm SD for three samples in each group. P values are <0.05 were considered as significant.

RESULT AND DISCUSSION

Effect of *Azospirillum*, *Azotobacter*, *Pseudomonas* and *Bacillus* treatments on plant height of *Withania somnifera*.

There was significant variation ($P \leq 0.05$) in plant height of *Withania somnifera* seedlings treated with *Azospirillum*, *Azotobacter*, *Pseudomonas* and *Bacillus*.

When compared with drought stressed and well watered controls (Fig. 1) *Azospirillum*, *Azotobacter*, *Pseudomonas* and *Bacillus* treated plants obtained the maximum height at all sampling periods in *Withania somnifera* (41, 46 and 51 DAP). Individual inoculations of *Azospirillum*, *Azotobacter*, *Pseudomonas* and *Bacillus* increased plant heights of *Withania somnifera*.

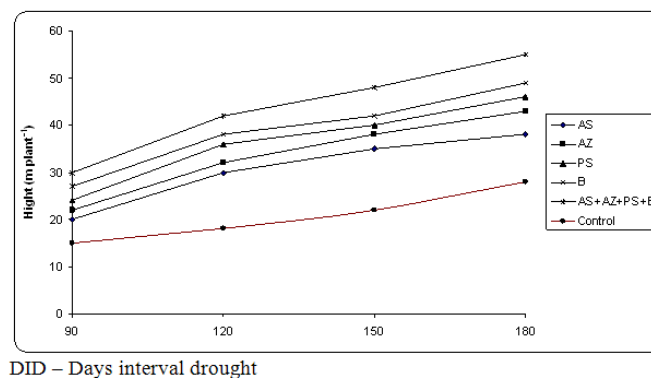
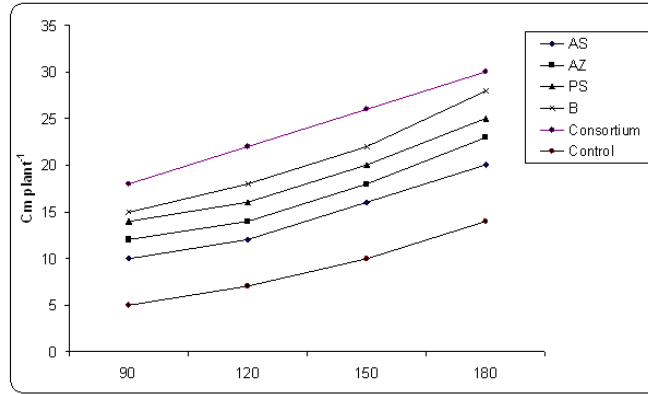


Fig. 1. Effect of *Azospirillum*, *Azotobacter*, *Pseudomonas* and *Bacillus* and their combination on plant height of *Withania somnifera* plants

Effect of *Azospirillum*, *Azotobacter*, *Pseudomonas* and *Bacillus* treatments on root length of *Withania somnifera*.

The root length of *Withania somnifera* seedlings varied in the stress among the isolates of *Azospirillum*, *Azotobacter*,

Pseudomonas and *Bacillus* treatments when compared to well watered controls. The maximum root length was recorded in *Azospirillum*, *Azotobacter*, *Pseudomonas* and *Bacillus* treated plants.



DID – Days interval drought

Fig.2. Effect of drought *Azospirillum*, *Azotobacter*, *Pseudomonas* and *Bacillus* for their combination on root length of *Withania somnifera* plants

Two consecutive years of testing (2009 and 2010) showed that the plant growth and alkaloid content of *Withania somnifera* was

significantly increased by the bacterial treatment in all samples days (Table 1).

Table -1: Effect of plant growth promoting rhizobacterial application on alkaloid content of *Withania somnifera*

S. No	Treatments	Alkaloid content (mg/g of root dry wt.)			
		90	120	150	180
1	AUAS	0.55 ±0.15 ^e	0.58 ±0.20 ^d	0.60 ±0.28 ^f	0.65 ±0.30 ^c
2	AUAT	0.68 ±0.17 ^b	0.70 ±0.23 ^e	0.75 ±0.30 ^d	0.78 ±0.32 ^f
3	AUPf	0.70 ±0.20 ^d	0.72 ±0.25 ^e	0.75 ±0.28 ^f	0.80 ±0.25 ^f
4	AUB	0.72 ±0.25 ^f	0.75 ±0.30 ^d	0.78 ±0.35 ^e	0.83 ±0.42 ^c
5	AUAS+AUAT	0.74 ± 0.45 ^c	0.77 ± 0.38 ^f	0.78 ± 0.41 ^d	0.85 ± 0.60 ^e
6	AUAS+AUPf	0.75 ±0.47 ^e	0.78 ±0.40 ^f	0.80 ±0.44 ^c	0.87 ±0.62 ^e
7	AUAS+ AUB	0.78 ±0.50 ^e	0.80 ±0.44 ^c	0.82 ±0.47 ^c	0.85 ±0.64 ^d
8	AUAT+ AUPf	0.79 ±0.45 ^c	0.80 ±0.40 ^f	0.83 ±0.41 ^d	0.84 ±0.60 ^e
9	AUAT+ AUB	0.80 ±0.75 ^e	0.83 ±0.55 ^c	0.85 ±0.60 ^e	0.87 ±0.75 ^e
10	AUPf+ AUB	0.85 ±0.85 ^d	0.87 ±0.70 ^e	0.88 ±0.55 ^f	0.90 ±0.35 ^c
11	AUAS+AUAT+ AUPf+AUB	0.95 ±0.25 ^a	0.98 ±0.55 ^a	1.55 ±0.35 ^a	2.00 ±0.15 ^a
12	Control	0.35 ±0.15 ^f	0.52 ±0.33 ^f	0.60 ±0.42 ^e	0.70 ±0.16 ^e

There was a significant increase in plant height (45.80%) root length (58.05%) and alkaloid content (189.45%) of *Withania somnifera* obtained with double combined application of PGPR AUAS + AUAT + AUPS + AUBM for 90 days, followed by double combination AUAT + AUBM + AUPS. Testing for consecutive years (2009 and 2010) showed that bacterial treatments increased growth parameter and alkaloid content compared to the control.

Root inoculation with PGPR promoted significant increase in growth and alkaloid content but the growth response varied between different rhizobacterial strains. However in general the growth response was found to be enhanced when the PGPR strains were applied in combination. This growth response was more effective in terms of an increased plant growth and alkaloid compared to control. Earlier reports had shown that combined inoculation of sorghum with *Azospirillum*, *Azotobacter chroococcum*, *Pseudomonas fluorescens* and *Bacillus megaterium* significantly increased grain yield. The stimulatory effects of this PGPR strains on the yield and growth of these crops were attributed to the N₂ fixation ability, plant growth regulator production and phosphate solubilizing capacity (Cakmakci *et al.*, 2007; Kevinvessey, 2003; Karlidag *et al.*, 2007). For *Withania somnifera* microbial consortium is known to enhance alkaloid content Withaferin-A and yield (H. Gopal *et al.*, 2004).

The conclusion the combination of microbial consortium strains were found to have a great potential for use as bioinoculants to increase production in medicinal plants and other crops.

REFERENCES

Alagawadi, A.R.I. Gaur, A.C. 1992: Inoculation of *Azospirillum brasilense* and phosphate solubilizing bacteria on yield of sorghum (*Sorghum bicoloru* (L.) Moench) in dry land. Trop. Agric. 69: 347-350.

Aslantas, R.R. Cakmakci, & F. Sahin, 2007: Effect of plant growth promoting rhizobacteria of young apple tree growth and fruit yield under orchard conditions. Sci. Hort. 111, 371-377.

Atta, F.A. & O.A.O. Saad, 2001: Biotertilizers as potential alternative of chemical fertilizer for *Cathranthus roseus* G. Don. J. Agri. Sci., 26 (11), 7208-7193.

Bremmer, J.M. & C.S. Sulvaner, 1982. Total nitrogen In: A.L. Pageet *et al* (eds) Methods of soil analysis, part Z. agron. Mongr. 9. 2nd ed. ASA and SSA, Madison, WI. P. 595-624.

Cakmakci, R., M.F. Donmez & U. Erdogan, 2007: The effect of plant growth promoting rhizobacteria on barley seedling growth, some soil properties and bacterial counts. Tur J Agric For 31, 189-199.

Esitken, A., S. Ercisli, H. Karlidag & F. Sahin, 2005: Potential use of

- plant growth promoting rhizobacteria (PGPR) in organic apricot production In: proceedings of the International Scientific Conference of environmentally friendly fruit growing, Tartu-Estonia, September 7-9, 2005, pp. 90-97.
- Gomez, K.A. & A.A. GOMEZ, 1984. Statistical procedures for agricultural research John Wiley and Sons. Inc. New York 20-29.
- Jaleel, C.A. P. Manivannan, P. Sankar, B. Krishna Kumar, A., R. Gopi, R. Somasundaram & Pannerselvam, 2007; *Pseudomonas fluorescens* enhances biomass yield and Ajmalicine production in *Catharanthus roseus* under loater deficit stress. Colloid and Surface B: Biointer faces 60 (1), 7-11.
- Jeon, J.S., S.S. Lee, H.Y. Kim, T.S. Ahn & H. G. Song, 2003: Plant growth promotion in soil by some inoculated microorganisms, J. Microbial., 41, 271-276.
- Karlidag, H.A. Esitken, M. Turan & F. Sahin, 2007: Effects of root inoculation of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient element contents of apple. Scientia Horticulture 114, 16-20.
- Karthikeyan, B., C.A. Jaleel, G. Lakshmannan, M. Deiveekasundaram, 2008. Studies on the microbial bio diversity of some commercially important medicinal plants. Colloids and surfaces B. Biointerfaces: 62: 143-145.
- Kevinvessey, J., 2003: Plant growth promoting Rhizobacteria on Radishes, p. 879. Angers (Ed.) Gibert - Clarey, Tours.
- Pandey, P. & D.K. Maheshwari, 2007: Bioformulation of Burkholderia SP M5SP with multi species consortium for growth promoting of *Casuarina cajan* Can. J. Microbial 53, 213-222.
- Patten, C.L., & B.R. Glick, 2002; Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. Apple. Environ. Microbial. 68, 3795-3801.
- Renouhdin, J., 1984; Reversed phase-high performance liquid chromatographic characteristics of Indole alkaloids from cell suspension cultures of *Catharanthus roseus*. J. Chromatogr, 29, 165-174.
- Rodriguez, H. & R. Fraga, 1999; Phosphate solubilizing bacteria and their role in plant growth promotion Bacteriol. Adv., 17, 319-339.
- Stahil, E., 1969: Thin layer chromatography - A Laboratory Hand Book, Springer rerlag Berlin.
- Sturz, A.V. & J. Nowak, 2000: Endophytic communities rhizobacteria and the strategies required to create yield enhancing associates Coith crops, Appl. Soil Ecol., 15, 183-190.
- Sudhakar, P.G.N. Chattopadnyay, S.K. Gangwar & J.K. Ghosh, 2000, Effect of foliar application of *Azotobacter*, *Azospirillum* and *Beijerinckia* on leaf yield and quality of Mulberry (*Morus alba*), J. Agric. Sci. 134, 227-234.
- Sudha, B., V. Natarajam & K. Hari, 2002: Influence of phosphorus solubilizing bacteria on the changes in soil available phosphorus and sugarcane and sugar yields, Filed Crops Res. 77, 43-49.
- Thosar, N.J., V.G. Ingle & J.C. Jadhar, 2005; Effect of FYM and Biofertilizers on dry root and seed yield of Ashwagandha (*Withania somnifera*) crop. Prod., 1 (2) 27-28.
- Watanabe, M., A. Suzuki, S. Komori H. & Bessho, 2004; Comparison of endogenous IAA and cytokinins in shoots of columnar and normal type apple trees, J. Jpn. Soc. Hort. Sci., 73 19-24.
- Zhang, H & K.J. Horgan, P.H.S. Reynolds & P.E. Jameson, 2003: Cytokinins and bud morphology in pinus radiata. Physiol. Plant. 117, 264-269.