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

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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 Vinblastime, Vincvistine 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 Prompting 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 Prompting 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 chrooccum, 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 chrococcum,* grown in

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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 -80ºC. They isolates were designated based on the location in Tamilnadu, India, where they were collected as Department of Microbiology, Annamalai University. *Azospirillum* (AuAs), *Azotobacter chrococcum* (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 than 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 chrococcum, 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 dragendraffs 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 ± 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≤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.*

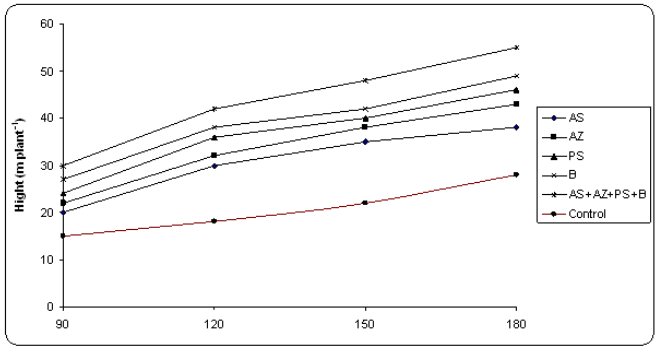


Fig. 1. Effect of *Azospirillum, Azotobacter, Pseudomonas* and *Bacillus* and their combination on plant height of *Withania somnifera* plants

### 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.

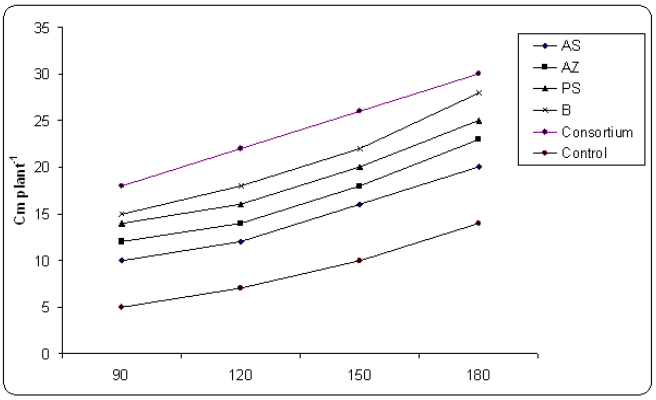


Fig.2. Effect of drought *Azospirillum, Azotobacter, Pseudomonas* and *Bacillus* for their combination on root length of *Withania somnifera* plants

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 *rhizobactorial* application on alkaloid content of *Withania somnifera*

| S. No | Treatments | Alkaloid content (mg/g of root dry wt,) | | | |
|---|---|---|---|---|---|
| | DAP * | 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 + AUAZ + AUPS + AUBM for 90 days, followed by double combination AUAZ + 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 chrococcum, 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 $N_2$ 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.

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