Madras Agric. J., 96 (7-12): 339-343, December 2009
https://doi.org/10.29321/MAJ.10.100503

Microbial Inoculants for Enhancing the Biochemical Properties of *Withania somnifera* Variety Jawahar 20

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Use of microbial inoculants to enhance the nutrient uptake of any traditional medicinal plant is prerequisite to keep its medicinal value. In order to optimize the microbial inoculants for *ashwagandha Withania Somnifera (L) Duna* nutrient management, experiments were conducted. The effective strains of *Azospirillum, Azotobacter, Bacillus* and *Pseudomonas* isolated and characterized from different medicinal plants of Tamil Nadu were evaluated for *ashwagandha* under pot-culture condition. The results revealed that combined inoculation of *A. lipoferum* - AAs-11, *Azotobacter* - AAz-3, *Bacillus* - APb-1 and *Pseudomonas fluorescens* - APs-1 enhanced the biochemical constituents of *ashwagandha* such as chlorophyll, protein and total alkaloid contents compared to single and other combinations. Further, this bioinoculant package along with *panchakavya* spray enhanced the rhizosphere microbial activity of *ashwagandha*. It is concluded that these microbial inoculants in combination with *panchakavya* is the best organic nutrient management package for *ashwagandha*.

Key words: *Ashwagandha*, alkaloid, microbial inoculants, medicinal plants, rhizosphere

In India, the use of several medicinal plants to cure specific ailments is in vogue from ancient times. The indigenous systems of medicine namely Siddha, Ayurveda and Unani have been in existence for several centuries. *Withania Somnifera (L) Duna* (sanskrit name: aswagandha) is an important medicinal plant of such traditional medicines, which is used as adaptogen with antistress antioxidant, antitumor, anti-inflammatory, mind boosting and has rejuvenating properties (Singh *et al*., 1990). The pharmacological activity of roots is attributed to the presence of alkaloids like withanolide A and withanolide D (Eastwood *et al*., 1980). The roots also contain monohydric alcohols like withaniol, somnirol and dihydric alcohols like somnitol and withanic acid (Ghossal *et al*., 1988). Among the alkaloids, withanine constitutes 38 per cent of the total alkaloids. (Umadevi *et al*., 1992).

Recently, use of natural medicines or herbal medicines formulated from traditional medicinal plants is gaining progress globally in order to reduce the side effects of pharmaceutical chemicals. The medicinal plants growing without proper management may cause reduction in medicinal values. *ashwagandha* cultivation is gaining important in Tamilnadu and being cultivated in dry and waste lands. Since the principal compounds of these medicinal plants will be influenced by several practices of cultivation, the nutrient management of these crops needs much more attention as any other commercial crops. Adding synthetic fertilizers may affect the medicinal property of these medicinal plants, which should be addressed in future. Alternatively, the natural resources like microbial inoculants could be a potential source of nutrients for these plants. Rhizosphere effect of bacterial inoculants will satisfy the nutrient requirement of these crops. With this hypothesis, evaluation of four different bacterial inoculants to enhance the medicinal property of *ashwagandha* through natural resources was attempted and discussed in this paper.

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Materials and Methods

A pot culture experiment was conducted at the Department of Agricultural Microbiology, TNAU, Coimbatore to study the effect of combined inoculation of rhizobacteria on growth, yield and quality of *ashwagandha* (var. Jawahar 20).

The rhizobacterial isolates *viz*., *Azospirillum lipoferum*-AAs-11, *Azotobacter*-AAz-3, *Bacillus*-APb-1 and *Pseudomonas fluorescens*-APs-1 isolated, characterized from medicinal plants and identified potential inoculants were prepared as carrier based inoculants and used for this study. The pots were filled with potting mixture (soil + sand + FYM at 1:1:1 ratio) and the rhizobacteria treated seeds were sown at 25 seeds per pot and finally 5 seedlings were maintained. The experiment was conducted in completely randomized block design with three replications.

The total chlorophyll content (Talling and Driver, 1961), total protein content (Bradford, 1976) and total alkaloid contents (Srivastava and Iyer, 1960) were

estimated in *ashwagandha* at monthly interval from 3 months to 6 months.

In order to evaluate the performance of these inoculants along with traditional natural nutrient preparation, *panchakavya*, another pot culture experiment was carried out as described earlier. Here, along with biochemical properties of *ashwagandha*, microbial populations in rhizosphere soil were estimated. *Azospirillum* (Dobereiner and Day, 1975 and Baldani and Dobereiner, 1980), *Azotobacter,* phosphate solubilizing bacteria (Sperber, 1958), *Pseudomonas* (King *et al*., 1954) populations were estimated following standard procedures collected from the soil at monthly intervals from 3 to 6 months.

Result and Discussion

Ashwagandha is an important medicinal plant being cultivated extensively in Tamil Nadu under very low nutrient management conditions. The roots of *ashwagandha* are used for extracting withaferin, medicinal property of the plant used for curing stress-related disorders. As agronomical practices including soil, cultivation methods, nutrient management had high influence on the medicinal value of the *ashwagandha* . Keeping this as consideration, several organic nutrient inputs were evaluated for *ashwagandha* like medicinal plants. Composts, traditional nutrient preparations, microbial resources are some of the possible inputs could be effectively explored for *ashwagandha* cultivation. Using these inputs will keep the medicinal value of the *ashwagandha* in higher concentrations and also keep the soil in sustainable manner. The present work is mainly focused to develop and evaluate microbial preparations for supply of nitrogen, phosphorus and growth hormones to improve the biochemical properties of *ashwagandha*, which in turn will improve the medicinal value of crops.

Microbial inoculants

Rhizobacteria present in the roots of several medicinal plants were isolated, characterized and screened for plant growth promotion and identified from potential inoculants *viz., Azospirillum* (AAs-11), *Azotobacter* (AAz-3), *Bacillus* (APb-1) and *Pseudomonas* (APs-1). These microbial inoculants prepared as mixed microbial preparations and used in this study. The inoculants were applied as seed treatment; seedling dip and soil application to *ashwagandha* under pot culture condition and biochemical constituents of the plants were assessed. The results revealed that all the rhizobacteria enhanced the biochemical constituents such as total chlorophyll, protein and alkaloid contents of *ashwagandha* significantly than uninoculated control (Fig.1). Among the different combinations of inoculants, all the four rhizobacterial inoculants treated *ashwagandha* recorded maximum total chlorophyll, protein and alkaloid contents than uninoculated control. These rhizobacterial inoculations enhanced the N and P uptake of crop there by increased the growth (Yan *et al*., 2003). Further, they also produce growth promoting substances such as IAA, which also increased the crop growth (Pal *et al*., 2003). The inoculation usually increased the root and shoot biomass of plant which in turn accelerate the metabolic activities of plant. This was reflected in the present study that inoculation increased the biochemical properties of *ashwagandha* , as chlorophyll and proteins directly influenced by N and P uptake and alkaloid contents by growth hormones influenced by the rhizobacterial inoculation.

***Panchakavya* spray**

After identifying the potential microbial inoculants for enhancing biochemical principles of *ashwagandha*, another experiment was also conducted, with *panchakavya*. *Panchakavya* is a traditional fermented microbial processed growth tonic prepared from products of cow. The final product is enriched with growth hormones, micronutrients and vitamins which enhanced the plant growth. They trigger the stimuli in plant system to uptake the nutrients (Natarajan, 2003). Hence in the present study the selected microbial inoculants in combination with *panchakavya* spray were evaluated for *ashwagandha* in order to recommend as package of practice. For these, along with *panchakavya* spray, individual inoculants and combination of all the four inoculants were tried under pot culture experiment.

The results revealed that biochemical constituents such as chlorophyll and total alkaloid

Table 1. Effect of rhizobacterial inoculation with *panchakavya* on changes in biochemical constituents of *ashwagandha* (var. Jawahar 20) at 180 DAI

Treatments	Biochemical constituents of *ashwagandha*	
	Chloro phyll (mg/g)	Total alkaloid (mg/g)
T_1 - *Panchakavya* 3% spray	2.81	47
T_2 - *Azospirillum* (AAs-11) + *Panchakavya* 3% spray	2.98	61
T_3 - *Azotobacter* (AAz-3) + *Panchakavya* 3% spray	2.90	50
T_4 – *Bacillus* (APb-1) + *Panchakavya* 3% spray	2.84	50
T_5 - *Pseudomonas* (APs-1) + *Panchakavya* 3% spray	2.96	56
T_6 - All the four rhizobacteria + *Panchakavya* 3% spray	3.98	95
T_7 – Control	2.78	44
CD (0.05)	0.55	11.09

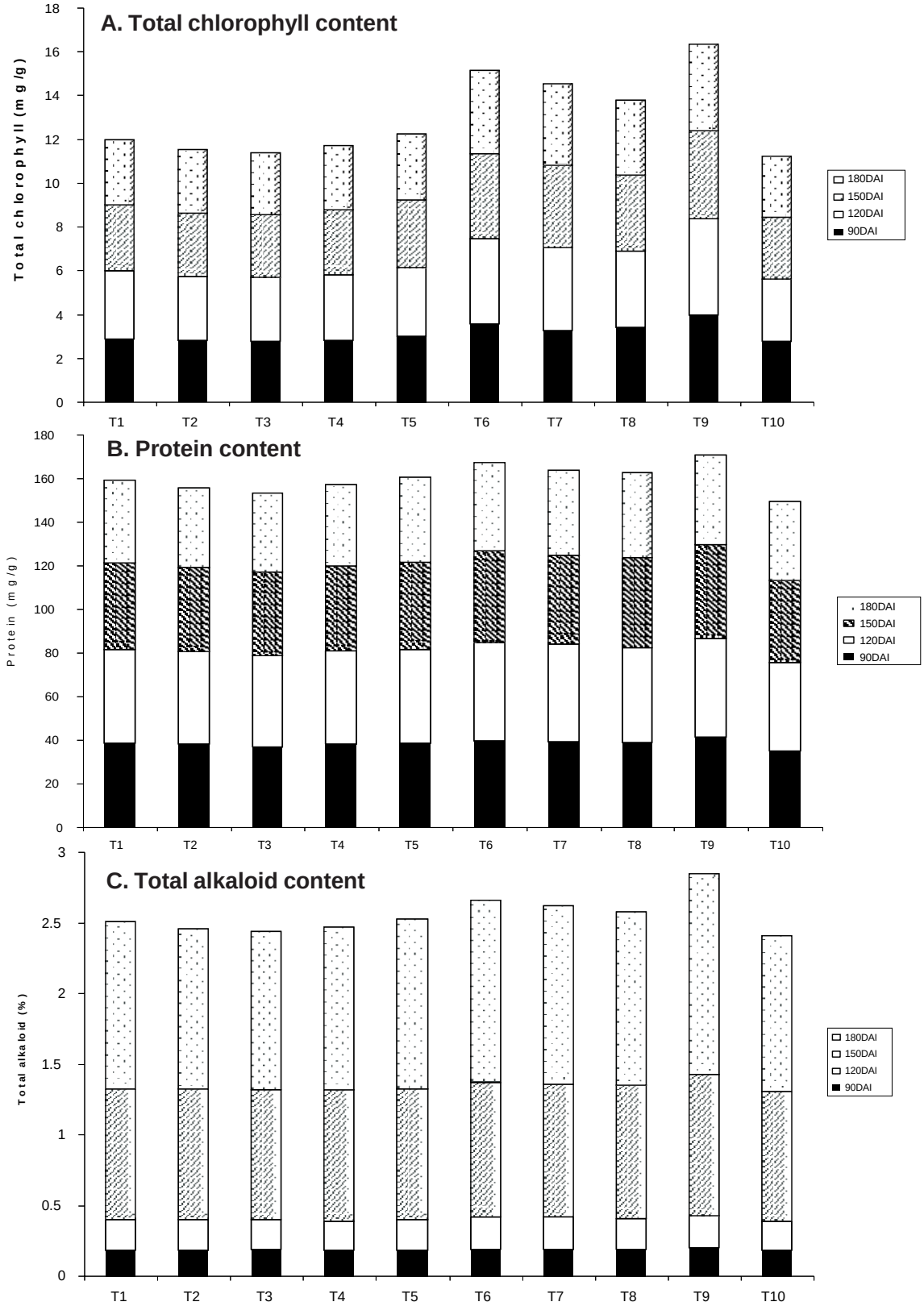

Fig. 1. Influence of microbial inoculants on changes in biochemical constituents(A-Total chlorophyll; B-Protein: C- Total alkaloid) of *ashwagandha* (var. Jawahar 20) under pot culture condition. T_1 – *Azospirillum* (AAs-11) ; T_2 – *Azotobacter* (AAz-3) ; T_3 – *Bacillus* (APb-1) ; T_4 – *Pseudomonas* (APs-1) ; T_5 – T1 + T2 ; T_6 – T1 +T3+T4 ; T_7 – T2 +T3+T4 ; T_8 – T1+T2+T3 ; T_9 – T1+T2+T3+T4 ; T_{10} – Uninoculated control.

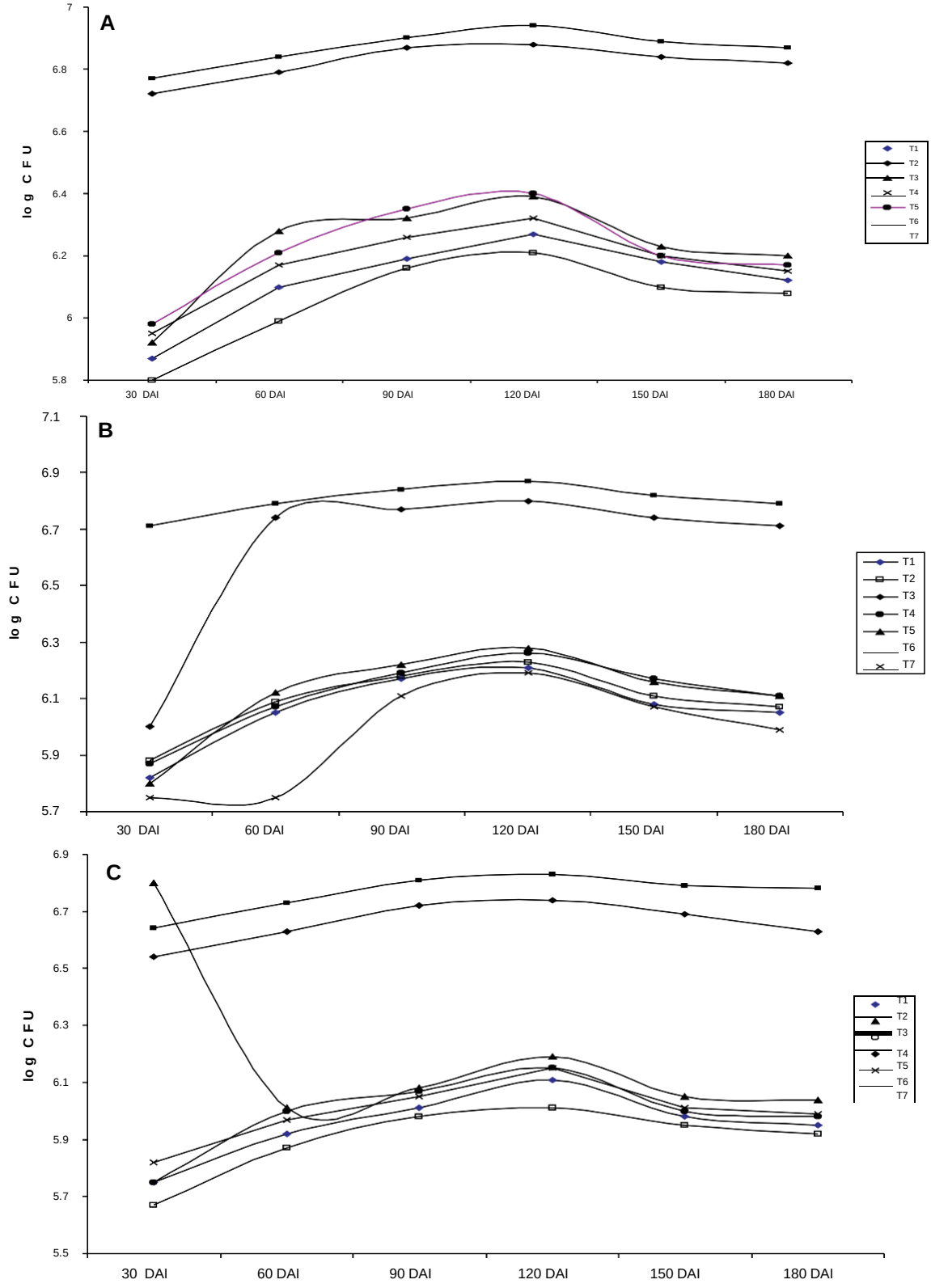

Fig. 2. Influence of microbial inoculants with *panchakavya* on changes in beneficial microflora of *ashwagandha* rhizosphere (var. Jawahar 20) under pot culture condition. T_1 - *Panchakavya* 3% spray; T_2 - *Azospirillum* (AAs-11) + *Panchakavya* 3% spray; T_3 - *Azotobacter* (AAz-3) + *Panchakavya* 3% spray; T_4 – *Bacillus* (APb-1) + *Panchakavya* 3% spray; T_5 - *Pseudomonas* (APs-1) + *Panchakavya* 3% spray; T_6 - All the four hizobacteria + *Panchakavya* 3% spray;T_7 – Control.

contents were significantly increased due to *panchakavya* spray and microbial inoculants (Table 1). The alkaloid content of this combination treatment had two-fold increased alkaloid content than control plants. *Panchakavya* spray alone had little effect on biochemical constituents of plant, when it combined with inoculants drastically increased due to synergestic effects. These two preparations further enhanced the microbial activity in rhizosphere of *ashwagandha*, which is the main cause for this increased biochemical constituents. To reveal this hypothesis, rhizosphere soil samples of these treatments were analysed for individual beneficial microbial population. The results confirmed that microbial inoculations and *panchakavya* spray recorded maximum microbial populations in the rhizosphere (Fig. 2). As the microbial activity enhanced in the rhizosphere soil, the nutrient uptake, growth promotion and triggered metabolic activity are the subsequential activities, which leads to build the biochemical constituents of *ashwagandha* (Chezhiyan *et al*., 2003).

It is concluded from these experiments that microbial inoculant consists of *Azospirillum, Azotobacter*, Phosphobacteria and *Pseudomonas fluorescensc* could be a potential source of nutrients for *ashwagandha*. Further it is also confirmed that synergistic activity of *panchakavya* spray will trigger the activities of these organisms, which in turn enhanced the biochemical constituents of *ashwagandha*.

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Received: November 10, 2009; Revised: December 10, 2009; Accepted: December 14, 2009