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Effect of rhizobacterial inoculation on rhizosphere microflora of ashwagandha cv. JAWAHAR 20

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ABSTRACT
Rhizobacteria from different medicinal plants *viz*., *Withania somnifera, Coleus forskohlii* and *Vinca rosea* grown in different parts of Tamil Nadu were isolated and characterized. Under pot culture conditions the rhizosphere population of *Azospirillum, Azotobacter*, phosphate solubilizing bacteria and *Pseudomonas* increased up to 120 days after inoculation and then declined. Witnessed increase in the population of respective inoculants due to biofertilizer inoculation offers more scope for plant growth promotion.

Key words: Rhizosphere population, Ashwagandha

INTRODUCTION

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. The WHO has estimated that over 80 per cent of the world population meets their primary health care needs through traditional medicine (Lambert, 1997). Ashwagandha is used as a tonic in geriatrics, being efficacious in relieving hand and limb tremors of people at old age (Atal *et al*., 1975). It has been equated to ginseng (*Panax ginseng*) of China and is popularly known as the "Indian Ginseng". The most important pharmacological use of ashwagandha is as adaptogen with antistress antioxidant, antitumor, anti-inflammatory, mind boosting and has rejuvenating properties (Singh *et al*., 1990). Studies on the rhizobacterial population in the rhizosphere region and testing the suitability of the isolated rhizobacteria as seed and soil inoculant will be highly useful in improving the productivity and quality of this commercially important medicinal plant.

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 were prepared as carrier based inoculants as described earlier and used for this study. The pots were filled with potting mixture (soil + sand + FYM) 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 populations of *Azospirillum, Azotobacter*, phosphate solubilizing bacteria and *Pseudomonas* in ashwagandha rhizosphere were estimated following standard procedures at 30 days interval upto 180 DAI by using nitrogen free semisolid malate medium for *Azospirillum* (Dobereiner and Day, 1975), Waksmann No. 77 medium for *Azotobacter*(Allen 1953), Pikovskaya's medium for phosphate solubilizing bacteria (Pikovskaya, 1948) and King's B medium for *Pseudomonas* (King *et al*.,1954). The population was expressed as cfu g^{-1} of oven dry soil.

RESULTS AND DISCUSSION

Population of Azospirillum in ashwagandha rhizosphere

In various treatments, *Azospirillum* population increased upto 120 DAI and then declined. The results revealed that population of *Azospirillum* was maximum (78.33 x10^5 cfu g^{-1}) on 120 DAI in the rhizosphere of ashwaganda receiving all the inoculants (Table 1).

Population of Azotobacter in ashwagandha rhizosphere

On 120th day, the *Azotobacter* population was maximum (67.33 x 10^5 cfu g^{-1}) in the combined inoculation of all the organisms (AAs-11, AAz-3, APb-1 and APs-1) followed by the treatment receiving the *Azotobacter*-AAz-3, *Bacillus*-APb-1 and *Pseudomonas fluorescens*-APs-1 (63.66 x 10^5 cfu g^{-1}). Among the individual inoculants, *Azotobacter* inoculation resulted in higher rhizosphere population than others when compared to individual inoculants and uninoculated control (Table 2).

Population of phosphate solubilizing bacteria in ashwagandha rhizosphere

Inoculation of all the four rhizobacterial isolates recorded higher population of phosphate solubilizing bacteria in the rhizosphere soil on 120 DAI (59.33 x 10^5 cfu g^{-1}) when compared to uninoculated control (10.66 x 10^5 cfu g^{-1}) as well as other treatments (Table 3).

Population of Pseudomonas in ashwagandha rhizosphere

Inoculation of rhizobacterial isolates either alone or in different combinations increased the rhizosphere population of *Pseudomonas* upto 120 days. Among the various treatments, combined inoculation of all the organisms recorded higher population than other treatments at all the stages of sampling. Maximum population (88 x 10^5 cfu g^{-1}) was recorded in this treatment on 120 DAI. The uninoculated control recorded the minimum rhizosphere population of *Pseudomonas* at all the growth stages (Table 4).

The establishment of introduced rhizobacteria in rhizosphere is essential to obtain desired effect on the growth and yield of crop plants. Migration of beneficial rhizosphere bacteria towards their respective host plants provides an important ecological advantage. Also, species of rhizobacteria with poor survival in soil should reach the root environment for their survival (Rovira, 1969). Root colonization by beneficial bacteria (active/passive attachment to the root surface of inoculated bacteria) is fundamental for increased plant productivity (Burdman *et al*., 2000 and Mehnaz *et al.*, 2001).

In the present study rhizosphere of inoculated plants recorded higher rhizobacterial population. The favourable condition in that area might have supported the proliferation of the introduced rhizobacterial isolates. The population of microorganisms is normally more in the rhizosphere than in soil away from plant roots due to the greater availability of carbon and other nutrients. Plant root exudates serve as a major nutrient for microbial colonization (Lynch and Whipps, 1990). Since rhizobacteria are able to utilize the root exudates effectively, they are able to colonize the rhizosphere region in large numbers than in the near by soil. In the present study the survival of rhizobacteria was found to be more in the presence of root exudates of ashwagandha plants as evidenced by survival studies under controlled conditions in Jensen's nutrient solution. This explains the

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Table 1 : Effect of rhizobacterial inoculation on the population of *Azospirillum* in ashwagandha (var. Jawahar 20) rhizosphere

Treatments	*Azospirillum* population (cfu x 10^5 g^{-1} of soil)					
	30 DAI	60 DAI	90 DAI	120DAI	150 DAI	180 DAI
T_1 – *Azospirillum* (AAs-11)	48.00 (6.68)	57.66 (6.76)	65.33 (6.81)	67.66 (6.83)	62.66 (6.80)	58.66 (6.77)
T_2 – *Azotobacter* (AAz-3)	7.66 (5.88)	13.33 (6.12)	18.33 (6.26)	20.33 (6.30)	17.33 (6.24)	15.00 (6.17)
T_3 – *Bacillus* (APb-1)	7.33 (5.86)	13.00 (6.11)	17.00 (6.23)	19.00 (6.28)	15.66 (6.19)	13.66 (6.13)
T_4 – *Pseudomonas* (APs-1)	8.33 (5.92)	14.66 (6.16)	19.66 (6.29)	20.33 (6.31)	18.66 (6.27)	16.33 (6.21)
T_5 – T1 + T2	50.33 (6.70)	59.33 (6.77)	66.33 (6.82)	69.00 (6.84)	63.66 (6.80)	60.00 (6.78)
T_6 – T1 +T3+T4	52.33 (6.72)	62.66 (6.80)	71.33 (6.85)	74.33 (6.87)	68.66 (6.83)	65.33 (6.81)
T_7 – T2 +T3+T4	9.66 (5.98)	15.33 (6.18)	22.66 (6.35)	25.00 (6.40)	20.33 (6.30)	17.66 (6.24)
T_8 – T1+T2+T3	51.00 (6.70)	60.33 (6.78)	68.66 (6.83)	71.33 (6.85)	65.33 (6.81)	62.66 (6.79)
T_9 – T1+T2+T3+T4	54.66 (6.73)	65.33 (6.81)	76.66 (6.88)	78.33 (6.89)	72.33 (6.86)	68.00 (6.83)
T_{10} – Uninoculated control	6.66 (5.82)	9.00 (5.95)	14.66 (6.17)	16.33 (6.21)	13.00 (6.11)	11.66 (6.07)

Values in parentheses are $\log_{10}$ transformed

	SEd	CD (P=0.05)
T	0.012	0.024
D	0.009	0.019
T X D	0.023	0.059

Table 2 : Effect of rhizobacterial inoculation on the population of *Azotobacter* in ashwagandha (var. Jawahar 20) rhizosphere

Treatments	*Azotobacter* population (cfu x 10^5 g^{-1} of soil)					
	30 DAI	60 DAI	90 DAI	120 DAI	150 DAI	180 DAI
T_1 – *Azospirillum* (AAs-11)	6.33 (5.80)	10.00 (5.99)	13.33 (6.12)	15.33 (6.18)	12.66 (6.10)	10.33 (6.01)
T_2 – *Azotobacter* (AAz-3)	36.66 (6.56)	47.00 (6.67)	52.66 (6.72)	55.00 (6.74)	50.66 (6.70)	46.33 (6.66)
T_3 – *Bacillus* (APb-1)	6.00 (5.78)	9.33 (5.97)	13.00 (6.11)	15.66 (6.19)	12.00 (6.08)	11.00 (6.04)
T_4 – *Pseudomonas* (APs-1)	6.66 (5.82)	11.33 (6.05)	14.00 (6.14)	16.33 (6.21)	13.00 (6.11)	11.33 (6.05)
T_5 – T1 + T2	39.00 (6.59)	48.66 (6.69)	55.66 (6.74)	58.33 (6.77)	54.33 (6.73)	50.66 (6.70)
T_6 – T1 +T3+T4	7.66 (5.88)	11.66 (6.07)	16.00 (6.20)	17.33 (6.24)	14.66 (6.17)	12.00 (6.08)
T_7 – T2 +T3+T4	43.00 (6.63)	53.33 (6.72)	61.00 (6.78)	63.66 (6.80)	59.33 (6.77)	55.33 (6.74)
T_8 – T1+T2+T3	40.66 (6.61)	51.00 (6.70)	58.33 (6.76)	60.00 (6.77)	56.66 (6.75)	52.66 (6.72)
T_9 – T1+T2+T3+T4	45.66 (6.66)	56.33 (6.75)	64.66 (6.81)	67.33 (6.83)	63.00 (6.80)	60.00 (6.78)
T_{10} – Uninoculated control	5.33 (5.73)	9.33 (5.97)	12.66 (6.10)	14.00 (6.14)	11.33 (6.05)	10.00 (5.99)

Values in parentheses are $\log_{10}$ transformed

	SEd	CD (P=0.05)
T	0.056	0.146
D	0.043	0.113
T X D	0.136	0.357

Table 3 : Effect of rhizobacterial inoculation on the population of phosphate solubilizing bacteria in ashwagandha (var. Jawahar 20) rhizosphere

Treatments	Phosphate solubilizing bacteria (cfu x 10^5 g^{-1} of soil)					
	30 DAI	60 DAI	90 DAI	120DAI	150 DAI	180 DAI
T_1 – *Azospirillum* (AAs-11)	5.33 (5.72)	9.33 (5.97)	11.00 (6.04)	12.33 (6.09)	10.33 (6.01)	9.00 (5.95)
T_2 – *Azotobacter* (AAz-3)	5.00 (5.70)	9.00 (5.95)	10.66 (6.02)	12.00 (6.08)	10.00 (5.99)	8.66 (5.93)
T_3 – *Bacillus* (APb-1)	28.66 (6.46)	37.33 (6.57)	43.00 (6.63)	46.00 (6.66)	40.33 (6.60)	35.66 (6.55)
T_4 – *Pseudomonas* (APs-1)	5.00 (5.70)	9.33 (5.97)	9.66 (5.98)	11.33 (6.05)	10.66 (6.03)	9.00 (5.95)
T_5 – T1 + T2	5.33 (5.72)	9.00 (5.95)	10.00 (5.99)	12.00 (6.08)	10.66 (6.03)	9.00 (5.95)
T_6 – T1 +T3+T4	32.00 (6.50)	42.33 (6.63)	53.33 (6.73)	56.66 (6.75)	50.00 (6.70)	45.33 (6.66)
T_7 – T2 +T3+T4	30.00 (6.47)	39.00 (6.59)	46.00 (6.66)	48.33 (6.68)	43.00 (6.63)	39.00 (6.59)
T_8 – T1+T2+T3	30.66 (6.48)	40.00 (6.60)	48.66 (6.68)	51.00 (6.70)	46.33 (6.66)	41.66 (6.62)
T_9 – T1+T2+T3+T4	36.33 (6.56)	46.00 (6.66)	57.00 (6.75)	59.33 (6.77)	55.33 (6.74)	51.00 (6.71)
T_{10} – Uninoculated control	4.66 (5.67)	8.66 (5.94)	9.33 (5.97)	10.66 (6.03)	9.00 (5.95)	8.00 (5.90)

Values in parentheses are $\log_{10}$ transformed

	SEd	CD (P=0.05)
T	0.018	0.037
D	0.014	0.038
T X D	0.045	0.119

Table 4. Effect of rhizobacterial inoculation on the population of *Pseudomonas* in ashwagandha (var. Jawahar 20) rhizosphere

Treatments	*Pseudomonas* population (cfu x 10^5 g^{-1} of soil)					
	30 DAI	60 DAI	90 DAI	120 DAI	50 DAI	180 DAI
T_1 – *Azospirillum* (AAs-11)	11.00 (6.04)	14.33 (6.15)	20.00 (6.30)	23.33 (6.37)	17.66 (6.25)	16.00 (6.20)
T_2 – *Azotobacter* (AAz-3)	10.66 (6.02)	13.66 (6.13)	19.66 (6.29)	23.00 (6.36)	17.00 (6.24)	15.66 (6.19)
T_3 –*Bacillus* (APb-1)	10.00 (5.99)	13.00 (6.11)	18.00 (6.25)	21.33 (6.33)	16.00 (6.20)	15.00 (6.17)
T_4 – *Pseudomonas* (APs-1)	55.33 (6.74)	64.66 (6.81)	71.00 (6.82)	74.00 (6.87)	68.66 (6.83)	66.66 (6.82)
T_5 – T1 + T2	10.33 (6.01)	13.33 (6.12)	19.00 (6.28)	21.33 (6.33)	16.00 (6.20)	15.66 (6.20)
T_6 – T1 +T3+T4	59.33 (6.77)	68.00 (6.83)	80.33 (6.90)	83.00 (6.92)	78.00 (6.89)	74.66 (6.87)
T_7 – T2 +T3+T4	57.66 (6.76)	66.33 (6.82)	76.6 (6.88)	78.33 (6.89)	73.00 (6.86)	71.00 (6.85)
T_8 – T1+T2+T3	11.66 (6.06)	15.00 (6.17)	23.33 (6.36)	25.66 (6.41)	20.00 (6.30)	17.66 (6.24)
T_9 – T1+T2+T3+T4	62.66 (6.56)	70.33 (6.85)	85.33 (6.93)	88.00 (6.94)	83.66 (6.92)	78.00 (6.89)
T_{10} – Uninoculated control	9.33 (5.97)	12.66 (6.10)	17.00 (6.23)	20.00 (6.30)	15.00 (6.17)	13.00 (6.11)

Values in parentheses are $\log_{10}$ transformed

	SEd	CD (P=0.05)
T	0.011	0.022
D	0.008	0.017
T X D	0.027	0.053

better survival of rhizobacteria in the rhizosphere of inoculated plants when compared to uninoculated control.

The population of all the rhizobacteria was higher in combined inoculation than in individual inoculation and synergism among the organisms might have played a role in this higher activity. Better survival of *Azospirillum* and phosphate solubilizing bacteria in the rhizosphere of rice and maize was observed in combined inoculation than the individual inoculation (Lakshmipriya, 1997 and Phalalochanan, 2001).Though phosphate solubilizing microorganisms has been found in the rhizosphere of plants, it constitutes only a small percent of the total microbial population. It is widely accepted that additional quantity of efficient phosphate solubilizing microorganisms has to be introduced into soils in order to increase the availability of phosphorus from insoluble sources (Vassileva *et al*., 1997).

Rao *et al*. (1999) stated that the population of fluorescent pseudomonads on lentil roots varied with the strain and ranged from 10^4 to 10^6 cfu g^{-1} of root fresh weight. de Freitas and Germida (1992) also found an increase in *P. fluorescens* population as plants grow old which is similar to the findings of the present investigation. Rhizobacterial population increased upto 120 DAI only and with further ageing of the crop the rhizosphere population started to decline. The decline in the population might be due to the reduction in the quantum of available root exudates during the later stages of crop growth or in the quality of the root exudates. Proliferation of *Azospirillum* in the root environment of plants is controlled by a number of factors like age of the host, root depth and soil conditions (Okon, 1985). Gayathri (2002) observed that several biotic and abiotic factors influenced the survival of *Azotobacter* in rice rhizosphere. Better establishment of *Azotobacter* was observed in young rice seedlings with green manuring and limited irrigation. Similar trend of the results were obtained in our studies. Inoculation of more than one type of bio-inoculant like the inoculation of nitrogen fixing diazotroph (Azospirillum / *Azotobacter*) along with phosphate solubilizing bacterium (*Bacillus* sp.) and plant growth promoting bacterium (*Pseudomonas fluorescens*) pave the better way for higher rhizosphere bioinoculants population and ultimate plant growth promotion.

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