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Effect Of Plant Growth Promoting Rhizobacteria On Growth And Antimicrobial Activity Of Andrographis Paniculata.

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ABSTRACT

Andrographispaniculata is one of the medicinal plant in Tamilnadu. By using the chemical fertilizers it may create an environmental problems. Hence, Plant Growth Promoting Rhizobacteria (PGPR) have been known to applied in various medicinal plants for their growth enhancements. PGPR shows the direct as well as indirect mechanisms in plants. In the present study, the Plant Growth Promoting Rhizobacteria such as *Azospirillumbrasilense*, *Bacillus subtilis*, *Pseudomonas fluorescenes* were inoculated to observe the effect of growth characters and also an antimicrobial activity of *Andrographispaniculata* were studied. PGPR organisms were inoculated in various treatments. Thus, the results revealed that, among the various treatments the consortium T_7 (*Azospirillumbrasilense+Bacillus subtilis+Pseudomonasfluorescenes*) shows the maximum growth characters of shoot length (39.75 and 64.21cm), root length (21.32 and 24.48 cm), plant height (79.02 and 83.17 cm), Number of leaves (118.00 and 130.00 plants^{-1}) and Number of branches (13.00 and 20.57 plants^{-1}) were observed on 45 and 90 DAS. And also the antimicrobial activity of test organisms *Staphylococcus aureus,Escherichia coli* and *Salmonella typhi* shows maximum inhibition zone in leaf extract.

INTRODUCTION

Medicinal plant sector has traditionally occupied an important position in the socio-cultural, spiritual and medicinal area of rural and tribal lives of India (Gupta and Chadha, 1995). *Andrographispaniculata* is one of the important medicinal plant in Tamilnadu. It has a main role in Siddha, Ayurveda and Unani system. It is used as an Asian medicine for centuries. It is an annual herb (shrubs), which grows up to 3 feet height in moist and shady places. Plant growth promoting rhizobacteria (PGPR) have been known to be applied to a wide range of medicinal crops for the purpose of growth enhancement of yields. Numerous varieties of microorganisms inhabitat in soil, especially in the rhizosphere region and play a major role in plant growth and improve the quality of soil. They mainly involve in biological nitrogen fixation, phytohormones production, siderophore production, solubilize phosphorus to as a biocontrol agents and induce development of the plant yield (Kanchana *et al.,* 2013). The potential effect of plant growth promoting rhizobacteria on the association with medicinal plants will enhance the standardizing microbial technique to develop the crop yield and quality of *A.paniculata.* At present use of biofertilizer technology for sustainable production for medicinal plants are lacking. Efficient strains of *Azotobacter sp. Azospirillum sp. Phosphobacteria* and Rhizobacter can provide a significant amount of nitrogen to Helianthus annus and to increase the plant height, number of leaves, stem diameter, percentage of seed filling and seed dry weight. Similarly, in rice, the addition of *Azotobacter sp. Azospirillumsp.,* and Rhizobium promotes the physiology and improves the root morphology (Choudhury and Kennedy, 2004).

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MATERIALS AND METHODS

Effect of plant growth promoting rhizobacterial (PGPR) inoculantson growth characters of *A. paniculata*

The plants were removed from each treatment without damaging the root and washed with tap water. The plants were first dried under sunlight for 24 hrs to removing the water content and cut the root and shoot parts separately; the shoot length and root length of the plant were determined by measuring the length by using the measuring tape and recorded the values in cm on 45 and 90 DAS.

The average number of lateral branches per plant was counted manually and recorded the values on 45^{th} and 90^{th} DAS.

The dry matter content of root and shoot were estimated by drying the samples in hot air oven at 60°C until a constant weight obtained and calculated the values in g plant^{-1} on 45 and 90 DAS.

ANTIMICROBIAL ACTIVITY OF *A. paniculata*

1. Test organisms and culture media

The clinical pathogenic bacterial strains were aseptically collected from Rajah Muthiah Medical College and Hospital, Annamalai University. The bacterial cultures *viz*., *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi* were maintained in nutrient broth. The bacterial broth cultures were stored in 4 ºC for their future studies.

2. Extraction of plant materials

The different plant parts *viz*., root stem and leaf of *A. paniculate* were separately collected from all the treatments. Then they were shade dried for two weeks and then ground into powder. The powder materials were packed in a separate plastic zip covers and stored under refrigeration until use. About 5.0 g of each powder sample was extracted with 25 ml of methanol and kept in shaker for overnight. The extracts were concentrated by using rotary evaporator at 45°C and stored at 4°C in air tight container until use. Different solvents are used such as ethanol, acetone, methanol and hot water extracts of *A. paniculata* were screened for antimicrobial activity using disc diffusion assay method according to (Bauer *et al.,* 1996).

RESULTS

Effect of plant growth promoting rhizobacterial (PGPR) inoculants on growth characters of *A. paniculata*.

The growth characters of *A. paniculata* such as shoot length, root length, plant height, number of leaves and branches were induced by consortium, dual and single treatment of growth promoting bacteria. The 90 DAS results were evaluated in the (**Table 1).**

The treatment T_7 recorded the highest shoot length, root length, plant height, number of leaves and branches on 90 DAS of *A. paniculata* and the results were 64.21 cm, 24.48 cm, 83.17 cm, 130 plant $^{-1}$ and 20.57 plant^{-1} respectively and it was followed by the T_5. Un inoculated (T_8) recorded very low value than all other treatments.

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TABLE – 1

Effect of plant growth promoting rhizobacterial (PGPR) inoculants on growth characters of *A. paniculata*

S.N	Treatments	Shoot length (cm)		Root length (cm)		Plant height (cm)		Number of leaves (Plant^{-1})		Number of branches (Plant^{-1})	
		45 DAS	90 DAS	45DAS	90 DAS	45 DAS	90 DAS	45 DAS	90 DAS	45 DAS	90 DAS
1.	T_1– (*A. brasilense*)	20.54	33.02	9.82	13.11	71.12	78.45	48.00	90.00	5.62	9.08
2.	T_2 – (*B. subtilis*)	33.21	55.70	11.03	17.00	74.42	81.97	67.00	72.00	9.36	13.05
3.	T_3 – (*P.fluorescens*)	27.87	42.11	10.00	16.01	72.53	79.13	51.00	73.00	8.07	11.54
4.	T_4 - (*A. brasilense* + *B. subtilis*)	37.65	59.62	12.93	18.54	78.61	82.99	90.00	119.00	11.64	18.72
5.	T_5– (*A. brasilense* +*P. fluorescens*)	38.92	61.65	14.12	22.04	79.00	83.00	96.00	117.00	12.90	19.11
6.	T_6– (*B. subtilis* + *P. fluorescens*)	36.06	59.54	11.73	17.27	76.79	82.00	71.00	110.00	11.27	18.08
7.	T_7 – Consortium (*A. brasilense* + *B. subtilis* + *P. fluorescens*)	39.75	64.21	21.32	24.48	79.02	83.17	118.00	130.00	13.00	20.57
8.	T_8 - Control (Un inoculated)	18.09	27.01	6.59	9.42	70.08	73.00	29.00	70.00	14.42	8.42
	SED	**0.317**	**0.536**	**0.125**	**0.168**	**0.721**	**0.777**	**0.687**	**0.951**	**0.097**	**0.140**
	CD=(p=0.05)	**0.682**	**1.152**	**0.260**	**0.361**	**1.551**	**1.670**	**1.478**	**2.046**	**0.210**	**0.302**

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Antimicrobial activity of selective human pathogen with different solvents of *A. paniculata*

The antibacterial activities of *A. paniculata* plant parts such as root, stem and leavesin different solvents (ethanol, acetone, methanol and hot water) were studied by disc diffusion assay against certain important human pathogens *viz*., *S. aureus*, *E.coli* and *S. typhi.* These antimicrobial activities were compared with standard antibacterial (streptomycin) (**Table – 2 to 4).**

The present study clearly revealed that the methanolic leaf extract of 100 µl showed highest inhibitory action on the pathogens tested than that of root and stem extract of *A. paniculata*in all the treatments. It was observed that, the antibacterial agent (streptomycin) showed higher zone of inhibition against all three bacterial pathogens tested *viz.*, 18.02 mm (*S. aureus*), 32.04 mm (*E. coli*) and 25.05 mm (*S. typhi*). Among the treatments, the maximum inhibition zone of leaf extract of *A. paniculata* was recorded as 32.04 mm against *E. coli* and followed by 25.05 mm (*S. typhi*) these results were equal to commercial bacterial agent of streptomycin.

The antimicrobial activity of leaf and stem extract of *A.paniculata*was higher than root extract. It may be due to the presence of active principle compounds like diterpene lactone (andrographolide, neoandrographolide,deoxyandrographolide, 14- deoxy-11, 12-didehydroandrographolide and andrograpanin), alkaloids, flavonoids, tannins, glycosides, saponins, steroids and phenols.

TABLE – 2

Antibacterial activity of *A. paniculata* extract against *Staphylococcus aureus*

Plant	Extract	Solvents used	Area of inhibition zone (mm)			D.W
			50 µl	100 µl	150 µl	*R
A. paniculate	Root	Ethanol	10.06	11.01	10.03	12.00
		Acetone	11.09	12.02	10.04	12.03
		Methanol	11.03	13.00	10.04	13.91
		Hot Water	8.00	10.01	9.00	10.06
	Stem	Ethanol	7.01	8.00	6.05	13.00
		Acetone	12.02	13.02	10.05	13.02
		Methanol	14.06	16.03	14.06	18.00
		Hot Water	7.00	10.00	10.04	10.00
	Leaf	Ethanol	7.93	13.01	10.03	12.62
		Acetone	13.01	14.06	13.00	14.99
		Methanol	18.00	18.02	15.02	18.06
		Hot Water	7.65	8.03	9.00	10.00

*R – positive control (streptomycin), DW- Distilled water

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TABLE – 3

Antibacterial activity of *A. paniculata* extract against *Escherichia coli*

Plant	Extract	Solvents used	Area of inhibition zone (mm)			D.W
			50 µl	100 µl	150 µl	*R
A. paniculate	Root	Ethanol	16.00	16.02	13.04	18.00
		Acetone	11.04	12.00	11.00	14.00
		Methanol	12.03	15.01	12.04	15.00
		Hot Water	13.00	14.02	11.05	17.00
	Stem	Ethanol	9.01	11.00	10.00	13.00
		Acetone	15.03	17.01	13.03	18.00
		Methanol	18.02	18.93	11.00	19.04
		Hot Water	9.06	10.00	10.01	10.03
	Leaf	Ethanol	18.02	19.01	17.03	19.06
		Acetone	24.01	25.00	20.00	27.05
		Methanol	30.02	32.04	27.04	33.00
		Hot Water	10.01	11.03	10.06	13.01

*R – positive control (streptomycin), DW- Distilled water

TABLE – 4

Antibacterial activity of *A. paniculata* extract against *Salmonella typhi*

Plant	Extract	Solvents used	Area of inhibition zone (mm)			D.W
			50 µl	100 µl	150 µl	*R
A. paniculate	Root	Ethanol	13.07	15.00	11.00	16.00
		Acetone	16.09	18.04	15.06	19.0
		Methanol	17.08	18.03	17.05	19.00
		Hot Water	10.01	12.02	10.01	12.00
	Stem	Ethanol	20.06	20.07	20.0	22.00
		Acetone	17.00	19.01	13.02	13.08
		Methanol	17.03	19.05	16.6	19.00
		Hot Water	11.04	13.2	11.2	15.00
	Leaf	Ethanol	24.05	25.00	24.01	27.00
		Acetone	27.05	28.2	20.4	23.0
		Methanol	20.04	25.05	25.03	26.00
		Hot Water	10.03	11.05	10.07	13.00

*R – positive control (streptomycin), DW- Distilled water

DISCUSSION

A pot culture study was conducted to compare the application effect of PGPR and its related products on the growth and development of the medicinal plant *A. paniculata*. The

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treatments include control, recommended dose of inorganic fertilizers, and supplementation of Consortium T_7 as individual and combination.

The present investigation clearly revealed that, PGPR application in *A. paniculata* significantly supported over recommended dose of inorganics in terms of growth (shoot length, root length and number of branches per plant, yield parameters (root dry weight and shoot dry weight). Further the results of the present study clearly revealed that supplementation of PGPR products were produced significant growth and yield parameters in *A. paniculata*. The beneficial effects of plant growth and development were already reported by many researchers (Kloepper and Ryu 2006; Egamberdiyeva D. 2007; Shaharoona*et al.*, 2006; Aparna. 2000; Choudhury and Kennedy. 2004; Bloemberg and Lugtenberg. 2001;Van Loon. 2007; Yan li *et al.*,2011).

The antimicrobial (antibacterial and antifungal) activities of methanolic extract of different parts like leaf, stem and root of *A. paniculata*were studied by disc diffusion assay against certain important human pathogens *viz., S. aureus, S. typhi,* and *E. coli* (bacteria).The results clearly revealed that the methanolic extract of leaves showed higher inhibitory action on the pathogens tested than that of stem and root extract of *A. paniculata*in all the treatments.

The antimicrobial activity of stem and leaf and extracts of *A. paniculata* were higher than root extract.

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