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Role of PGPR and heavy metals in Germination and growth of *Andrographis paniculata* (Kalmegh)

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Abstract
Andrographis paniculata is a shrub herbal remedy well-known as echinacea or *Kalmegh*. The present work was carried out to study the role of PGPR and heavy metals in germination and growth of *A. paniculata*. It was found that most of isolates significantly increased growth parameters such as plant height and root length and seed germination was also increased when seeds were pre-treated with PGPR isolates. All these isolates were tested for their tolerance towards different heavy metals. Majority of the bacterial isolates were found tolerant to Cd (100), Ni (200), Zn (100), Co (200), Cu (100), Cr (200), Pb (200) and Hg (100) µg/ml respectively. The isolates, tolerant to cadmium were further tested for PGPR traits such as IAA production, Ammonia Production, catalase, HCN production, PO^{-}_{4} solublization and seed germination test. Fifty potential PGPR were isolated from rhizosphere of Kalmegh. They were found to promote seed germination and plant growth.

Keywords: Kalmegh, PGPR, heavy metals, rhizobacteria

Introduction
Andrographis paniculata (Kalmegh) is a herbaceous plant in the family *Acanthaceae*, native to India and Sri Lanka. It is an annual herb extremely bitter in taste in all parts of the plant body. It is as an ayurveda herb it is known as Kalmegh or Kalamegha. It is also known as Bhui-neem, meaning neem of the ground, since the plant, though being a small annual herb, has a similar strong bitter taste as that of the large Neem tree *(Azadirachta indica).* Plant growth-promoting rhizobacteria (PGPR) was first defined by Kloepper *et al*. (1980) [5]. to describe soil bacteria that colonize the roots of plants following inoculation onto seed and that enhance plant growth. The rhizosphere is populated by a diverse range of microorganisms and the bacteria colonizing this habitat are called rhizobacteria (Schroth and Hancock, 1982) [6]. The major influences that the rhizosphere microorganisms have on plants today become important tool to guard the health of plants in ecofriendly manner (Akhtar *et al*., 2012) [1]. The present work was conducted to study the role of PGPR and heavy metals in Germination and growth of *A. paniculata* (Kalmegh).

Materials and Methods
Experimental Site
The present study was conducted in the Research Laboratory in the Department of Molecular and Cellular Engineering, Sam Higginbottam University of Agriculture Technology and Sciences India.

Heavy metals
For maximum limits and analysis of heavy metals, consult the WHO guidelines on quality control methods for medicinal plants.

Treatment Layout
Effect of rhizobacteria on seed germination

Table 1: Experimental Layout

Treatment (Rhizobacteria)	Inoculation with rhizobacteria
T_0 (Control)	No inoculation
T_1	Inoculation with B-1
T_2	Inoculation with P-3
T_3	Inoculation with P-5
T_4	Inoculation with P-7
T_5	Inoculation with A-4

Effect of heavy metals on seed germination

Table 2: Experimental Layout

Treatment	Concentration of Nickel	Concentration of cadmium
T_0 (Control)	No treatment	No treatment
T_1	12.5 µg/ml	12.5 µg/ml
T_2	25 µg/ml	25 µg/ml
T_3	50 µg/ml	50 µg/ml
T_4	100 µg/ml	100 µg/ml
T_5	200 µg/ml	200 /ml

Combined effect of heavy metals and rhizobacteria

Table 3: Experimental Layout of rhizobactria in Heavy metals

Treatment	Rhizobacteria and concentration of Heavy Metals
T_0 (Control)	Inoculation with rhizobacteria
T_1	Rhizobacteria+12.5 µg/ml
T_2	Rhizobacteria +25 µg/ml
T_3	Rhizobacteria+ 50 µg/ml
T_4	Rhizobacteria+100 µg/ml
T_5	Rhizobacteria+200 µg/ml

Critical difference

If the analysis of variance table showed significant difference between the treatments then compare all positive combination of 2 treatments at time.

C.D. at 1% = SE * t_8 (error degree of freedom at 1%)

SE for Treatment = $\frac{\sqrt{2}\times EMSS}{6}$

SE for Days = $\frac{\sqrt{2}\times EMSS}{12}$

Results and Discussion

Effect of Heavy Metals on Bacterial Isolates

Organisms isolated were also studied for heavy metal tolerance and the results were tabulated in Table 4. Majority of isolates, isolated from rhizosphere were tolerant to Cd, Ni, Zn, Co, Cu, Cr, Pb and Hg at 100, 50, 50, 100, 100, 100, 100 and 25 µg / ml respectively.

There was no significant inhibition in the viable count of Bacterial isolates for many metals at 50 and 100 µg / ml of concentration. On the other hand, treatment of Hg, Cd, Pb, Zn and Cr at 100 and 200µg / ml concentrate significant decline in viable count of complete inhibition of growth. The behavior of indigenous soil bacteria was similar to the previous study where indigenous metal resistant PGPR were directly isolated in significant number on nutrient agar plates supplemented with 200 and 400 µg / ml of metal concentration (Hayat *et al.*, 2002) [4]. Because of the great diversity within this group of bacteria, plate viable count may not be affected at lower concentration of heavy metal of the population is reduced. Selection of all the isolates from different plates indicated that almost 90% of bacteria of Gram positive and are tolerant at higher concentration and remaining 1% is Gram negative. The predominance of Gram negative bacteria at higher concentration of metal is probably due to their higher level of intrinsic metal resistance than Gram positive bacteria. The basis of this difference might be due to the differences in the chemical composition of cell wall of Gram negative bacteria and Gram positive bacteria (Babich and Stotzky, 1977) [2]. There might be another reason for high tolerance power of Gram negative bacteria as majority of them are good extra cellular polysaccharide producers which may further protect cell from the toxic effect of heavy metal (Geesey and Jang, 1990) [3].

Table 4: Heavy Metal Tolerance among rhizobacteria isolated from kalmegh.

S. No.	Organisms	Heavy Metal Tolerance (µG/ML)									
		Co	Zn	Hg	Cu	Cr	Ag	Cd	Ni	As	Pb
1.	KN-1	200	400	100	100	100	200	200	400	200	400
2.	KN -2	200	400	50	50	100	200	100	400	200	200
3.	KN -3	200	100	50	50	200	200	100	200	200	200
4.	KN -4	200	200	25	50	200	100	100	200	100	200
5.	KN -5	200	200	50	200	50	25	50	200	100	400
6.	KN -6	50	100	50	50	100	50	25	50	50	100
7.	KN -7	200	200	100	50	50	100	100	200	50	400
8.	KN -8	100	100	100	100	100	100	25	50	100	400
9.	KN -9	50	400	50	50	100	100	200	400	50	400
10	KN -10	100	200	100	100	100	100	100	200	100	100
11.	KK-1	200	400	100	100	200	200	100	200	100	400
12.	KK-2	200	200	100	100	200	100	100	100	100	400
13.	KK -3	200	100	100	100	100	200	100	200	100	100
14.	KK -4	50	50	50	100	50	100	25	50	50	50
15.	KK -5	50	50	50	100	50	50	25	25	100	50
16.	KK -6	25	50	100	50	50	50	50	100	50	25
17.	KK -7	50	25	50	50	25	50	50	100	25	100
18.	KK -8	200	200	50	200	50	25	50	200	100	400
19.	KK -9	50	100	50	50	100	50	25	50	50	100
20.	KK-10	200	200	100	50	50	100	100	200	50	400
21.	KM-1	100	100	100	100	100	100	25	50	100	400
22.	KM -2	200	100	50	50	200	200	100	200	200	200
23.	KM -3	200	200	25	50	200	100	100	200	100	200
24.	KM -4	200	200	50	200	50	25	50	200	100	400
25.	KM -5	50	100	50	50	100	50	25	50	50	100
26.	KM -6	200	200	100	50	50	100	100	200	50	400
27.	KM -7	100	100	100	100	100	100	25	50	100	400
28.	KM -8	50	400	50	50	100	100	200	400	50	400
29.	KM -9	50	50	50	100	50	50	25	25	100	50
30.	KM -10	25	50	100	50	50	50	50	100	50	25

31.	KA-1	50	25	50	50	25	50	50	100	25	100
32.	KA -2	200	200	50	200	50	25	50	200	100	400
33.	KA -3	50	100	50	50	100	50	25	50	50	100
34.	KA -4	200	200	25	50	200	100	100	200	100	200
35.	KA -5	200	200	50	200	50	25	50	200	100	400
36.	KA -4	50	100	50	50	100	50	25	50	50	100
37.	KA -7	200	200	100	50	50	100	100	200	50	400
38.	KA -8	100	100	100	100	100	100	25	50	100	400
39.	KA -9	50	400	50	50	100	100	200	400	50	400
40.	KA -10	50	50	50	100	50	50	25	25	100	50
41.	KC -1	25	50	100	50	50	50	50	100	50	25
42.	KC -2	50	400	50	50	100	100	200	400	50	400
43.	KC -3	50	50	50	100	50	50	25	25	100	50
44.	KC -4	25	50	100	50	50	50	50	100	50	25
45.	KC -5	50	25	50	50	25	50	50	100	25	100
46.	KC -6	200	200	50	200	50	25	50	200	100	400
47.	KC -7	50	100	50	50	100	50	25	50	50	100
48.	KC -8	200	200	100	50	50	100	100	200	50	400
49.	KC -9	100	100	100	100	100	100	25	50	100	400
50.	KC -10	200	200	25	50	200	100	100	200	100	200

Inoculation of Rhizobacteria on root growth of Kalmegh-
The selection of micro-organisms both metal tolerant and efficient in producing PGP compounds can be useful to speedup the re-colorization of the plant rhizosphere in polluted soil. In the study all the 50 bacterial isolates were undertaken for its role in stimulation of root growth. From the results of obtained from seed germination test it is apparent that the bacterial isolate No. 1, 4, 11, 12, 13, 21, 22, 25, 29, 32, 37, 40, 41, 44, 47 and 50 had significant impact on stimulation of root growth (Table 5). Roots from seeds treated with bacterium were an average 42% longer than the roots from untreated control seeds after 7th days. In this research we showed that the bacterial IAA stimulates the development of the host plant root. Production of phytoharmones such as auxins that stimulate root development and proliferation and that is known to cause a more efficient uptake of water and nutrients by plants. Promotion of root growth is one of the major markers by which the beneficial effect of plant growth promoting bacteria is measured as shown in Table 5.

Table 5: Impact of inoculation of Rhizobacteria on root growth of Kalmegh.

Treatment	Days After Germination (Length In cm)		
	3rd day	5th day	7th day
Control	2.8	3.9	6.0
KN-1	2.7	3.7.	6.0
KN -2	2.9	3.9	6.2
KN -3	2.8	3.8	6.1
KN -4	3.0	4.0	6.4
KN -5	2.8	3.9	6.3
KN -6	3.1	4.2	6.5
KN -7	2.9	4.0	6.1
KN -8	3.0	4.1	6.4
KN -9	3.0	3.9	6.2
KN -10	2.9	4.0	6.3
KK-1	3.0	4.0	6.4
KK-2	2.9	4.0	6.1
KK -3	2.7	3.7	6.0
KK -4	2.9	3.9	6.2
KK -5	3.1	4.2	6.5
KK -6	2.9	4.0	6.1
KK -7	3.0	3.9	6.2
KK -8	2.9	4.0	6.3
KK -9	3.0	4.0	6.4
KK-10	2.8	3.9	6.3
KM-1	3.0	4.1	6.4
KM -2	3.0	3.9	6.2
KM -3	2.9	4.0	6.1
KM -4	3.0	3.9	6.2
KM -5	2.9	4.0	6.3
KM -6	3.0	3.9	6.2
KM -7	2.9	4.0	6.3
KM -8	3.0	4.0	6.4
KM -9	3.1	4.2	6.5

KM -10	2.9	4.0	6.1
KA-1	3.1	4.1	6.3
KA -2	3.1	3.9	6.2
KA -3	2.9	3.9	6.3
KA -4	3.1	4.1	6.4
KA -5	3.2	4.2	6.6
KA -4	2.9	4.0	6.3
KA -7	3.0	4.2	6.4
KA -8	3.1	4.1	6.5
KA -9	2.9	4.0	6.2
KA -10	3.0	3.9	6.1
KC -1	2.9	4.0	6.2
KC -2	2.9	4.1	6.1
KC -3	2.8	3.9	6.2
KC -4	2.9	4.1	6.0
KC -5	2.8	4.1	6.3
KC -6	2.8	3.8	6.1
KC -7	2.7	3.8	6.2
KC -8	2.7	3.7	6.2
KC -9	2.8	3.7	6.1
KC -10	2.7	3.8	6.0

Effect of Rhizobacteria inoculation on root main lateral Kalmegh after germination *in vitro* conditions.

In the above table KN = Kalmegh in Nutrient Agar, KK = Kalmegh in King's B Agar, KM = Kalmegh in MacConkey Agar, KA = Kalmegh in Ashby's Agar and KC = Kalmegh in Cetrimied Agar.

For treatment S.E. (±) = 0.1510, C.D. = 0.3038, F-Tab = 3.80, F-Cal = 39.01.

For days S.E. (±) = 0.1000, C.D. = 0.2055, F-Tab = 5.49, F-Cal = 406.76.

Effect of rhizobacteria on root growth after germination *in vitro* conditions

Root main lateral and adventitious root of Kalmegh was calculated by measuring the length of the roots 7th day after incubation. Root growth was significantly increased by several units as compared to control as lowest root growth was observed at non-treated control. The statistical analysis showed that inoculation of Kalmegh seeds with bacterial strains which increased the root main lateral up to 20% over non-treated control. Results obtained for root main lateral are depicted in Table. 6, Table. 7, Table. 8, Table. 9, Table. 10, Table. 11, Table 12, Table 13, Table 14 and Table 15. Adventitious root length of Kalmegh was also increased up to 45% respectively. Results of adventitious root length are depicted. Thus the result demonstrates that rhizobacteria which shows PGP activities, significantly enhance root growth. Bacterial IAA plays a major role in promotion of root growth.

Table 6: Effect of Co on Root Main Lateral in Kalmegh after germination *in vitro* conditions.

Dose of Co	Days After Germination (Length in cm)		
	3rd day	5th day	7th day
Control	2.8	3.7	5.7
12.5µg/ml	3.1	4.2	6.9
25µg/ml	2.9	4.3	5.3
50µg/ml	2.5	3.4	4.6
100µg/ml	1.2	2.0	3.1
200µg/ml	0.8	0.9	1.7

Table 7: Effect of Zn on Root Main Lateral in Kalmegh after germination *in vitro* conditions.

Dose of Zn	Days After Germination (Length in cm)		
	3rd day	5th day	7th day
Control	2.8	3.7	5.7
12.5µg/ml	3.0	4.3	6.8
25µg/ml	2.7	4.1	5.4
50µg/ml	2.6	3.5	4.5
100µg/ml	1.0	2.1	3.0
200µg/ml	0.7	0.8	1.6

Table 8: Effect of Hg on Root Main Lateral in Kalmegh after germination *in vitro* conditions.

Dose of Hg	Days After Germination (Length in cm)		
	3rd day	5th day	7th day
Control	2.8	3.7	5.7
12.5µg/ml	3.1	4.1	6.9
25µg/ml	2.9	4.3	5.8
50µg/ml	2.5	3.4	4.5
100µg/ml	1.2	2.2	3.0
200µg/ml	0.6	0.9	1.5

Table 9: Effect of Cu on Root Main Lateral in Kalmegh after germination *in vitro* conditions.

Dose of Cu	Days After Germination (Length in cm)		
	3rd day	5th day	7th day
Control	2.8	3.7	5.7
12.5µg/ml	3.1	4.2	6.8
25µg/ml	2.7	4.1	5.4
50µg/ml	2.6	3.3	4.5
100µg/ml	1.2	2.2	3.0
200µg/ml	0.5	0.8	1.5

Table 10: Effect of Cr on Root Main Lateral in Kalmegh after germination *in vitro* conditions.

Dose of Cr	Days After Germination (Length in cm)		
	3rd day	5th day	7th day
Control	2.8	3.7	5.7
12.5µg/ml	2.9	4.3	5.8
25µg/ml	2.7	4.1	5.4
50µg/ml	2.6	3.3	4.5
100µg/ml	1.1	2.1	3.0
200µg/ml	0.5	0.8	1.4

Table 11: Effect of Ag on Root Main Lateral in Kalmegh after germination *in vitro* conditions.

Dose of Ag	Days After Germination (Length in cm)		
	3rd day	5th day	7th day
Control	2.8	3.7	5.7
12.5µg/ml	3.1	4.4	6.9
25µg/ml	2.7	4.1	5.4
50µg/ml	2.7	3.4	4.6
100µg/ml	1.0	2.0	3.0
200µg/ml	0.5	0.9	1.4

Table 12: Effect of Cd on Root Main Lateral in Kalmegh after germination *in vitro* conditions.

Dose of Cd	Days After Germination (Length in cm)		
	3rd day	5th day	7th day
Control	2.8	3.7	5.7
12.5µg/ml	3.0	4.4	6.8
25µg/ml	2.8	4.2	5.5
50µg/ml	2.7	3.3	4.5
100µg/ml	1.1	2.2	3.1
200µg/ml	0.4	0.8	1.7

Table 13: Effect of Ni on Root Main Lateral in Kalmegh after germination *in vitro* conditions.

Dose of Ni	Days After Germination (Length in cm)		
	3rd day	5th day	7th day
Control	2.8	3.7	5.7
12.5µg/ml	3.1	4.5	6.8
25µg/ml	2.7	4.4	5.7
50µg/ml	2.6	3.2	4.4
100µg/ml	1.1	2.3	3.2
200µg/ml	0.5	0.9	1.6

Table 14: Effect of As on Root Main Lateral in Kalmegh after germination *in vitro* conditions.

Dose of As	Days After Germination (Length in cm)		
	3rd day	5th day	7th day
Control	2.8	3.7	5.7
12.5µg/ml	3.0	4.6	6.7
25µg/ml	2.8	4.2	5.6
50µg/ml	2.6	3.3	4.5
100µg/ml	1.3	2.1	3.1
200µg/ml	0.5	0.9	1.5

Table 15: Effect of Pb on Root Main Lateral in Kalmegh after germination *in vitro* conditions.

Dose of Pb	Days After Germination (Length in cm)		
	3rd day	5th day	7th day
Control	2.8	3.7	5.7
12.5µg/ml	3.0	4.4	6.9
25µg/ml	2.8	4.3	5.7
50µg/ml	2.7	3.3	4.6
100µg/ml	1.1	2.2	3.0
200µg/ml	0.5	0.8	1.4

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