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Effect of bacterial isolates on organic and sustainable farming of medicinal plant: ashwagandha (*Withania angustifolia*)

Dinesh Kumar, Jagdish Parshad, Raj Pal Dalal and KS Yadav

Abstract
A total of 104 isolates of rhizobacteria were obtained from rhizosphere and rhizoplane of Ashwagandha plants collected from three locations of Haryana. Of these 36 were from rhizosphere and 68 were from rhizoplane. All the isolates were screened for their growth promoting activities in terms of biomass production in sandy soil without addition of any organic or inorganic fertilizers. Inoculation with these isolates produced more plant biomass than control to a varying level. Only four isolates (HRP-7, RRP-8, and RRP-26 & YRP-11) produced plant biomass more than 10g/plant. Highest plant biomass was produced by inoculation of isolate YRP-11 (16.49 g/plant) followed by RRP-26 (15.86 g/plant). Isolate HRP-7 produced least plant biomass. Isolates selected on the basis of higher plant biomass production showed useful traits like nitrogen fixation, and production of growth promoting substances like indole acetic acid. Inoculation with selected isolates increased plant biomass more in presence of farmyard manure than without farmyard manure. Mixture of these isolates produced biomass at par with mixed biofertilizer formulation containing *Azotobacter* and phosphorus solubilizing bacteria.

Keywords: Bacterial isolates, farming, medicinal plant

Introduction
Intensive agriculture practiced with the motive of immediate profit has posed great danger to environment, since the green revolution. Intensive cultivation practices lead to loss of soil fertility in terms of physical, chemical and biological properties. The decreased response to applied chemical fertilizers due to loss of soil organic matter, less profit from conventional crops, loss of soil fertility and high cost of fertilizers have forced farmers and policy makers to think of alternative crops like medicinal and aromatic plants which give more profits (Gulati, 2004) [5].

Cultivation of Medicinal Plants is presumed to have immense potential for diversification of land use pattern, more remuneration per unit area, increased employment opportunities, optimum utilization of waste lands, ensuring health security, attraction of entrepreneurs and upliftment of rural/ farming communities. The fact that the modern medicines contain about 25% drugs derived from such medicinal plants and its parts, make their production more beneficial and economic both in developing and developed countries (Ahlawat, 2004) [1]. Haryana has wide diversity of wild medicinal plants among the hilly tracts of Shivalik and also has many agro climatic zones with various micro-climatic conditions. Among the various medicinal plants, Ashwagandha is an important medicinal plant whose roots have been employed in Indian traditional systems of medicine, Ayurveda and Unani medicines. Its natural habitat is in Rajasthan, M.P., Haryana, Punjab, H.P, and western U.P and its root production is approximately 2000 tones, but its annual requirement is about 7000 tones. Roots of Ashwagandha contain several alkaloids and are used for curing chronic joint diseases, mental disorders and gynecological problems like leucorrhea. Its roots can be dried and used as tonic for hiccup, cold, cough and female disorders. It is also an ingredient of medical ailments prescribed for curing disability and sexual weakness in males (Sangwan *et al*., 2004). Farmers are getting attracted toward this crop as it requires less water, animal do not eat it and grows well in poor soils. But little information is available on its nutrient management and even use of biofertilizers has not been studied in a scientific manner. Only few sporadic reports are available in few crops like Isabgol, *Artemisia annura*, *Solanum nigrum*, *curcuma* (turmeric), *Asparagus*, *Oscimum*, *Andrographis* and *Aloe vera* etc. in which biofertilizers like

Azotobacter, *Azospirillum*, vesicular arbuscular mycorrizae (VAM), *Pseudomonas* and *Bacillus polmyxa* were used.

The earlier reports suggested that the bacteria like *Pseudomonas* present in rhizosphere of medicinal plants like A*chyranthus*, *Eleacgnus* and *Hereclum* in addition to production of phytohormones showed antimicrobial activity (Kaur *et al.*, 2005) [7] and improved bioavailability of nutrients (Ratti *et al*., 2001 and Nuthan *et al*., 2005) [9, 11]. The rhizosphere microorganisms exert the beneficial effect on plant growth as a result several activities individually or as a combined effect. These activities include bioavailability of nutrients, production of phytohormones and antimicrobial substances. Keeping in view the above effects of rhizosphere microorganisms, present investigation was undertaken to Isolate and characterize rhizobacteria from rhizosphere and rhizoplane of Ashwagandha (*Withania angustifolia*) and to evaluate selected efficient isolates under pot conditions with farmyard manure.

Materials and Methods

Soil samples from rhizosphere and rhizoplane of Ashwagandha (W*ithania angustifolia*) were collected from CCS, HAU, Hisar; Krishi Vigyan Kendra-Rampura, Rewari and Krishi Vigyan Kendra-Damla, Yamuna Nagar. The representative soil of the field was collected from 5 different spots randomly upto 15cm depth, mixed well and a composite sample was used for determining pH, total nitrogen, total organic carbon and available phosphorus.Following steps were followed further to conduct the experiment.

A. Isolation of bacterial inoculants

For the isolation of rhizobacteria from rhizosphere and rhizoplane soil, serial dilution spread plate method using Luria Bertani (LB) medium was used (Sambrook *et al*., 1989) [10]. The plates were incubated at 28±1 ºC till visible colonies appeared. Individual colonies of different bacterial isolates showing different morphological features were picked up, purified by streaking on solidified LB agar plates. The further experiment was carried out on Jensen's nitrogen free media, Luria Bertani (LB) medium (Sambrook *et al*., 1989) [10] and on Pikovskaya's media with tri-calcium phosphate.

B. Screening of rhizobacteria for plant growth promoting activities under pot house conditions

To evaluate the plant growth promoting activity of rhizobacteria from rhizosphere and rhizoplane in terms of biomass production, a pot house experiment was conducted using sandy soil without addition of FYM or chemical fertilizers. Seedlings of Ashwagandha were dipped in 2 days old culture broth for 15 minutes and transplanted in earthen pots. Plants were harvested after growth period of 60 days and root and shoot dry weight was determined.

C. Characterization of selected isolates

The isolates which showed higher biomass production were used for characterization in terms of possession of useful traits like nitrogen fixing ability in terms of nitrogenase activity, phosphate solubilizing activity by growing them on solidified Pikovskaya's agar medium plates containing tricalcium phosphate and production of plant growth promoting substances such as IAA in presence and absence of tryptophan. The efficient isolates were then subjected to morphological and biochemical tests.

D. Selection of best isolates on the bases of growth and biomass production of Ashwagandha under pot house conditions with and without FYM

To study the effect of efficient isolates on growth and biomass production of Ashwagandha plant, second experiment under pot house conditions was carried out with and without FYM. Seedlings of Ashwagandha were dipped in 2 days old culture broth for 15 minutes and transplanted in earthen pots and with control. Plants were harvested along with the roots after growth period of 60 days and root and shoot dry weight was determined. The experiment was carried out with the following treatments:

1. Control-NO FYM + No isolate
2. NO FYM + Isolate no. HRP-7
3. NO FYM + Isolate no. RRP-8
4. NO FYM + Isolate no. RRP-26
5. NO FYM + Isolate no. YRP-11
6. NO FYM + Isolates [HRP-7+RRP-8+RRP-26+YRP-11]
7. NO FYM + Biomix (*Azotobacter* + PSB)
8. FYM @5t/ha + No isolate
9. FYM @5t/ha + Isolate no. HRP-7
10. FYM @5t/ha + Isolate no. RRP-8
11. FYM @5t/ha + Isolate no. RRP-26
12. FYM @5t/ha + Isolate no. YRP-11
13. FYM @5t/ha + Isolates [HRP-7+RRP-8+RRP-26+YRP-11]
14. FYM@5 t/ha+ Biomix (*Azotobacter* + PSB)

Results and Discussion

Nine rhizosphere soil samples along with roots collected from three different places i.e. Hisar (MAUUP, Deptt. of Plant Breeding CCS HAU, Hisar), Rewari (KVK, Rampura) and (KVK, Damla) Yamuna Nagar of Haryana were analyzed for pH, percent organic carbon, total nitrogen and available phosphorus (Table 1)

Table 1: Chemical properties of soil samples collected from Ashwagandha growing areas of Haryana

Location	Soil properties				
	Soil Texture	pH	Organic Carbon (%)	Total Nitrogen (%)	Available P (kg/ha)
Hisar-1	Sandy loam	7.6	0.30	0.044	22
Hisar-2	Sandy loam	7.4	0.39	0.028	24
Hisar-3	Sandy loam	7.5	0.30	0.034	20
Mean		7.5	0.33	0.035	22
Rewari-1	Loamy sand	8.1	0.25	0.031	20
Rewari-2	Loamy sand	8.0	0.28	0.033	16
Rewari-3	Loamy sand	7.9	0.26	0.031	18
Mean		8.0	0.26	0.032	18
Yamunanagar-1	Silt loam	7.3	0.39	0.036	18
Yamunanagar-2	Silt loam	7.2	0.34	0.029	22
Yamunanagar-3	Silt loam	7.1	0.41	0.027	14
Mean		7.2	0.38	0.031	18

The soil types were sandy loam, loamy sand and silt loam for Hisar, Rewari and Yamuna Nagar soils respectively. The pH of the soil ranged between 7.1 to 8.1. The organic carbon varied from 0.25-0.41%, highest being in Yamuna Nagar soils (average 0.38%) while lowest organic carbon (0.26%) was found in Rewari soils. The total nitrogen in soil samples varied from 0.027 to 0.044%, highest mean nitrogen being in Hisar (0.035%) soils. There was no difference in mean nitrogen of Rewari and Yamuna Nagar soils. The available P in these soils ranged between 16-24 kg/ha of soil. The phosphorus status of these soils was in the medium range.

I. Isolation of rhizobacteria from Ashwagandha rhizosphere and rhizoplane

Bacteria were isolated using dilution plating technique from rhizosphere and rhizoplane samples collected from Ashwagandha plant (*Withania angustifolia*) from Hisar, Rewari and Yamuna Nagar areas of Haryana using half strength LB agar medium. Total of 104 (36 from rhizosphere and 68 from rhizoplane) isolates were picked up from the samples and were numbered as RRS for isolates from Rewari Rhizosphere soil, RRP Rewari rhizoplane, YRS for Rhizosphere from Yamuna Nagar, YRP for Yamuna Nagar Rhizoplane, HRP, and HRS for isolates from Rhizoplane and Rhizosphere of Hisar soil, respectively**,** as tabulated below in Table 2.

Table 2: Bacterial Isolates obtained from rhizosphere and rhizoplane of Ashwagandha from Hisar, Rewari and Yamuna Nagar

S. No.	Isolates	Sample from	Location	Number of Isolates
1.	HRS-1 HRS-2 HRS-3 HRS-4 HRS-5 HRS-6 HRS-7 HRS-8 HRS-9 HRS-10 HRS-11 HRS-12	Rhizosphere	Hisar	12
2.	HRP-1 HRP-2 HRP-3 HRP-4 HRP-5 HRP-6 HRP-7 HRP-8 HRP-9 HRP-10 HRP-11 HRP-12 HRP-13 HRP-14 HRP-15 HRP-16 HRP-17 HRP-18 HRP-19 HRP-20 HRP-21 HRP-22	Rhizoplane	Hisar	22
3.	RRS-1 RRS-2 RRS-3 RRS-4 RRS-5 RRS-6 RRS-7 RRS-8 RRS-9 RRS-10 RRS-11 RRS-12 RRS-13 RRS-14 RRS-15	Rhizosphere	Rewari	15
4.	RRP-1 RRP-2 RRP-3 RRP-4 RRP-5 RRP-6 RRP-7 RRP-8 RRP-7 RRP-8 RRP-9 RRP-10 RRP-11 RRP-12 RRP-13 RRP-14 RRP-15 RRP-16 RRP-17 RRP-18 RRP-19 RRP-20 RRP-21 RRP-22 RRP-23 RRP-24 RRP-25 RRP-26 RRP-27 RRP-28 RRP-29 RRP-30 RRP-31 RRP-32 RRP-33	Rhizoplane	Rewari	33
5.	YRS-1 YRS-2 YRS-3 YRS-4 YRS-5 YRS-6 YRS-7 YRS-8 YRS-9	Rhizosphere	Yamuna Nagar	9
6.	YRP-1 YRP-2 YRP-3 YRP-4 YRP-5 YRP-6 YRP-7 YRP-8 YRP-9 YRP-10 YRP-11 YRP-12 YRP-13	Rhizoplane	Yamuna Nagar	13
Total number of isolates				104

It was seen that more number of rhizobacteria were present in rhizoplane at all the three locations. More number of rhizobacteria in rhizoplane area than rhizosphere area may be due to the presence of various carbohydrates and organic acids, amino acids vitamins etc. present in root exudates. (*Arun et al*, 2012 and *Eman et al*., 2014) [2, 4].

II. Screening of bacterial isolates for plant growth promoting activities under pot house conditions

A. Screening of isolates from Hisar soils

The biomass (root + shoot) production of Ashwagandha plant with and without inoculation of rhizosphere and rhizoplane bacterial isolates from Hisar soils are given in Table 2 and 3. The root biomass production varied between 0.76 g/plant (HRS-1) and 2.80 g/plant (HRS-6) while the shoot dry weight varied between 2.14 g/plant to 6.17 g/plant by the rhizosphere isolates HRS-1 and HRS-6 respectively (Table 3).

Table 3: Screening of isolates from Hisar-rhizosphere soil of Ashwagandha for plant growth promoting activities

Isolate No.	Dry weight (g/plant)			Isolate No.	Dry weight (g/plant)		
	Root	Shoot	Total		Root	Shoot	Total
Control	0.59	1.99	2.58	HRS-7	2.36	4.63	6.99
HRS-1	0.76	2.14	2.90	HRS-8	0.85	2.70	3.55
HRS-2	2.15	4.25	6.40	HRS-9	1.37	3.35	4.72
HRS-3	2.32	4.64	6.96	HRS-10	1.03	2.90	3.93
HRS-4	2.00	5.75	7.75	HRS-11	1.14	2.84	3.98
HRS-5	2.20	5.20	7.40	HRS-12	1.42	2.66	4.08
HRS-6	2.80	6.17	8.97				

The minimum root and shoot biomass produced by rhizoplane bacterial isolates varied between 0.80 g/plant and 2.63 g/plant. Maximum root biomass was achieved due toinoculation of isolate HRP-7 which was 3.51 g/plant (root) and 8.64 g/plant (shoot). The bacterial isolate no. HRP-7 produced maximum plant biomass (root + shoot) of 12.15 g/plant as compared to 2.58 g/plant in control (Table 4).

Table 4: Screening of Isolates from Hisar-rhizoplane of Ashwagandha for Plant growth promoting activities

Isolate No.	Dry weight (g/plant)			Isolate No.	Dry weight (g/plant)		
	Root	Shoot	Total		Root	Shoot	Total
Control	0.59	1.99	2.58	HRP-12	0.98	2.93	3.91
HRP-1	1.78	3.42	5.20	HRP-13	0.80	2.63	3.43
HRP-2	2.65	4.20	6.85	HRP-14	0.90	2.78	3.68
HRP-3	2.25	4.73	6.98	HRP-15	0.88	2.83	3.71
HRP-4	2.43	6.40	8.83	HRP-16	1.95	5.15	7.10
HRP-5	2.84	4.22	7.06	HRP-17	0.98	2.95	3.93
HRP-6	2.51	4.34	6.85	HRP-18	0.81	2.83	3.64

HRP-7	3.51	8.64	12.15	HRP-19	0.82	2.96	3.82
HRP-8	2.17	4.66	7.13	HRP-20	2.03	4.80	6.83
HRP-9	0.90	2.75	3.65	HRP-21	0.97	2.32	3.29
HRP-10	1.06	3.11	4.17	HRP-22	1.76	3.40	5.16
HRP-11	0.93	2.88	3.81				

The total biomass (root + shoot) due to inoculation of rhizobacteria from rhizosphere of Ashwagandha ranged between 2.90 g/plant (isolate HRS-1) and 8.97 g/plant with isolate HRS-6 (Table 4) while in control it was only 2.50 g /plant. The dry weight of root and shoot biomass varied between 0.80 and 3.51 gram/plant produced by isolate no. HRP-13 and HRP-7 respectively. Likewise the highest and lowest shoot biomass was produced by isolate HRP-7 and HRS-13 respectively.

B. Screening of isolates from Rewari soils

The effect of inoculation of various isolates obtained from Ashwagandha rhizosphere soil and rhizoplane soil of Rewari on biomass production is given Table 5 and 6. A total of 48 isolates (15 rhizosphere + 33 rhizoplane) were screened. In control the root biomass and shoot biomass was 0.59 and 1.99 gram respectively. Maximum root biomass (2.35 g/plant) and shoot biomass (4.25 g/plant) was obtained when isolate no. RRS-10 was inoculated from rhizosphere, and minimum root biomass, (0.80g), and shoot biomass (2.11g/plant) was obtained when isolate RRS-1 was inoculated (Table 5).

Table 5: Screening of Isolates from Rewari-rhizosphere soil of Ashwagandha for plant growth promoting activities

Isolate No.	Dry weight (g/plant)			Isolate No.	Dry weight(g/plant)		
	Root	Shoot	Total		Root	Shoot	Total
Control	0.59	1.99	2.58	RRS-8	1.30	3.52	4.82
RRS-1	0.80	2.11	2.91	RRS-9	0.98	2.41	3.39
RRS-2	1.07	3.40	4.47	RRS-10	2.35	4.25	7.60
RRS-3	1.51	3.81	5.32	RRS-11	1.44	3.89	5.33
RRS-4	2.02	4.11	6.13	RRS-12	0.86	2.44	3.30
RRS-5	1.72	3.11	4.73	RRS-13	0.98	2.56	3.54
RRS-6	1.77	3.12	4.89	RRS-14	0.92	2.67	3.59
RRS-7	1.60	3.86	5.46	RRS-15	1.15	3.33	4.48

Among the isolates from rhizoplane of Ashwagandha from Rewari area the isolate RRP-26 produced highest root biomass (3.86g/plant) and shoot biomass (12.00g/plant). Followed by isolate RRP-8 which produced 3.16g/plant root biomass and 10.23 g/plant shoot biomass (Table 6).

Table 6: Screening of Isolates from Rewari rhizoplane of Ashwagandha for Plant growth promoting activities

Isolate No.	Dry weight (g/plant)			Isolate No.	Dry weight (g/plant)		
	Root	Shoot	Total		Root	Shoot	Total
Control	0.59	1.99	2.58	RRP-17	1.68	3.64	5.32
RRP-1	1.12	2.50	3.62	RRP-18	1.24	3.31	4.55
RRP-2	1.11	2.58	3.69	RRP-19	1.26	2.41	3.67
RRP-3	1.21	2.59	3.80	RRP-20	1.15	2.80	3.95
RRP-4	1.12	2.68	3.80	RRP-21	1.20	2.60	3.80
RRP-5	2.10	4.05	6.25	RRP-22	1.14	2.83	3.97
RRP-6	2.35	6.73	8.08	RRP-23	1.12	2.64	3.76
RRP-7	1.80	2.85	4.65	RRP-24	1.28	3.32	4.60
RRP-8	3.16	10.23	13.79	RRP-25	1.18	2.45	3.63
RRP-9	1.03	2.40	3.43	RRP-26	3.86	12.00	15.86
RRP-10	1.24	3.28	4.52	RRP-27	1.28	3.44	4.72
RRP-11	2.73	5.66	8.39	RRP-28	1.20	2.85	4.05
RRP-12	1.00	2.30	3.30	RRP-29	1.20	3.20	4.40
RRP-13	1.12	2.88	4.00	RRP-30	1.37	2.43	3.80
RRP-14	1.47	3.43	4.90	RRP-31	1.25	3.11	4.16
RRP-15	1.18	2.88	3.96	RRP-32	1.19	3.44	5.63
RRP-16	1.34	2.43	3.77	RRP-33	1.28	3.67	4.95

The total plant biomass of Ashwagandha as a result of inoculation of rhizoplane isolates from Rewari varied between 2.40g with isolates RRP-9 and 12.00 g with isolate RRP-26. The total plant biomass also showed same trend. Therefore taking into consideration the total plant biomass the isolates RRP-26 and RRP-8 were selected for further investigation from the isolates from Rewari location.

Screening of isolates from Yamuna Nagar soils

A total number of 22 isolates, 9 from rhizosphere and 13 from rhizoplane of Ashwagandha from Yamuna Nagar were screened for production of plant biomass (Table 7 and 8). The root biomass production varied between 0.80 to 2.83 g/plant in case of rhizosphere soil isolates while in rhizoplane isolates the root biomass production varied from 2.20 g/plant to 5.35 g/plant. The total plant biomass ranged from 3.00g/plant to 8.18 g/plant among rhizosphere isolates. Among the rhizosphere isolates highest root biomass (2.83g/plant) was obtained with isolate no YRS-1 while the lowest root biomass (0.80g/plant) was obtained with isolate YRS-7 (Table 7).

Table 7: Screening of Isolates from Yamuna Nagar rhizosphere soil of Ashwagandha for plant growth promoting activities

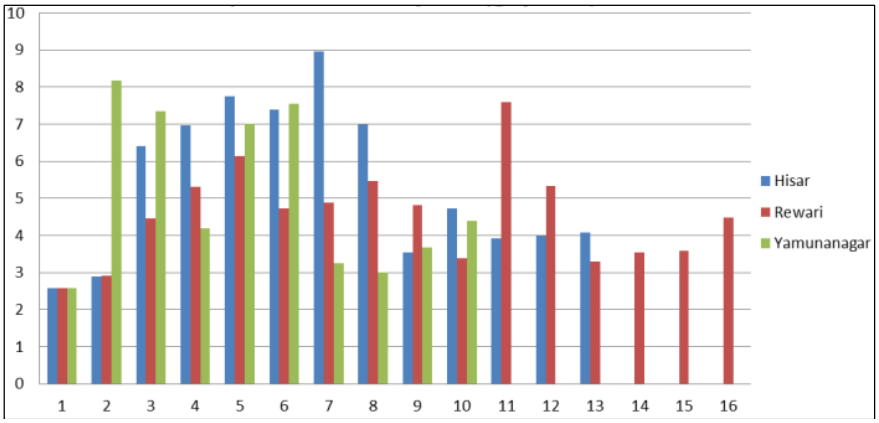
Isolate No.	Dry weight (g/plant)			Isolate No.	Dry weight (g/plant)		
	Root	Shoot	Total		Root	Shoot	Total
Control	0.59	1.99	2.58	YRS-5	2.58	4.95	7.54
YRS-1	2.83	5.35	8.18	YRS-6	0.90	2.36	3.26
YRS-2	2.68	4.68	7.36	YRS-7	0.80	2.20	3.00
YRS-3	1.30	2.90	4.20	YRS-8	1.00	2.67	3.67
YRS-4	2.13	4.88	7.01	YRS-9	1.15	3.25	4.40

Highest root biomass (4.73g/plant) was obtained when isolate YRP-11 was inoculated while the minimum (1.04g/plant) was obtained with isolate YRP-13. Among rhizoplane bacterial isolates, the isolate no YRP-11 produced maximum dry weight (10.66g/plant), while minimum was produced by bacterial isolate no. YRP-13 (Table 8).

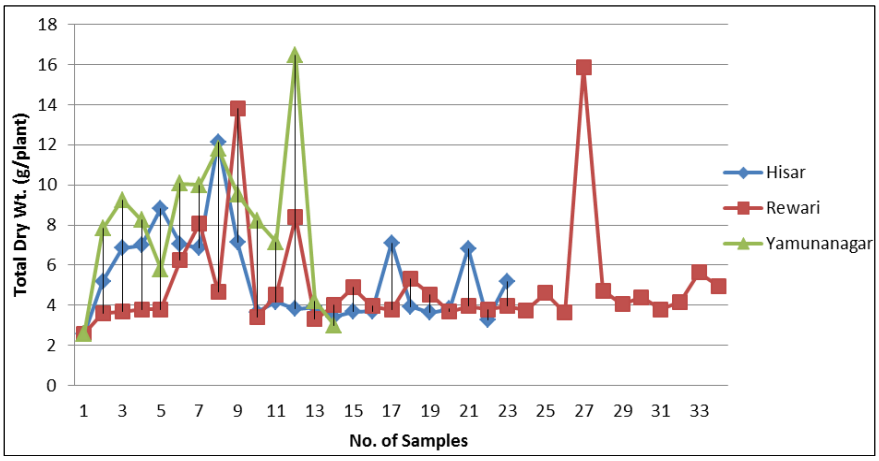
Table 8: Screening of Isolates from Yamuna Nagar rhizoplane of Ashwagandha for plant growth promoting activities

Isolate No.	Dry weight (g/plant)			Isolate No.	Dry weight (g/plant)		
	Root	Shoot	Total		Root	Shoot	Total
Control	0.59	1.99	2.58	YRP-7	4.29	7.52	11.81
YRP-1	2.96	4.86	7.82	YRP-8	3.74	5.80	9.54
YRP-2	3.31	5.95	9.26	YRP-9	3.03	5.20	8.23
YRP-3	3.03	5.23	8.26	YRP-10	2.99	4.16	7.15
YRP-4	2.91	2.89	5.80	YRP-11	5.73	10.66	16.49
YRP-5	3.89	6.17	10.06	YRP-12	1.04	3.17	4.21
YRP-6	3.81	6.08	9.99	YRP-13	1.81	2.19	3.00

Based on plant biomass production by rhizosphere and rhizoplane isolates isolate no. YRP-11 produced maximum plant biomass (15.49 g/plant), hence this isolate was selected for further studies. This pattern of more increase in biomass due to inoculation of rhizoplane isolates may be due to reason that carbohydrate, organic acids, amino acids and hormones may be excreted from the roots, as well as synthesis of growth hormones by the bacterial isolates.



Rhizosphere Total Dry Wt.(g/plant) Production



Rhizoplane Total Dry Wt.(g/plant) Production

III. Characterization of selected isolates for useful traits

All the 104 bacterial isolates obtained from Ashwagandha rhizosphere as well as rhizoplane were screened for their plant growth promoting activity in terms of biomass production. Based on higher plant biomass (root + shoot) at least one isolate each from one location i.e. Hisar, Rewari and Yamuna

Nagar were selected to examine the presence of various useful traits like nitrogen fixing ability (acetylene reduction activity), phosphate solubilization and production of plant growth promoting substances like in dole acetic acid. The four efficient isolates selected for this study were (1) HRP-7, (2) RRP-8, (3) RRP-26 and (4) YRP-11 (Table 9). After examination of above traits, these isolates were examined for their effect on plant biomass production with and without addition of organic matter (Farmyard manure @5tons/ha.). The efficient bacterial isolates which produced higher biomass atleast one bacterial isolate from each location i.e. Hisar, Rewari and Yamuna Nagar was selected for determining effect on plant growth in terms of biomass production in the absence and presence of farmyard manure @ 5 tons/ha under pot house conditions (Table 9).

Table 9: Effect of efficient isolates on biomass production of Ashwagandha under pot house conditions with and without FYM

Treatments	Dry weight (g/plant)		% increase due to FYM
	No FYM	FYM (5 t/ha)	
Control	7.12	7.88	10.7
Isolate-HRP-7	8.03 (12.8)	9.30 (18.0)	15.8
Isolate-RRP-8	8.33 (17.0)	9.54 (21.1)	14.5
Isolate-RRP-26	8.56 (20.2)	9.80 (24.4)	14.5
Isolate-YRP-11	8.23 (15.6)	9.29 (17.9)	12.9
Mixture of Isolates (HRP-7+ RRP-8+ RRP-26+ YRP-11)	8.82 (23.9)	10.20 (29.4)	15.6
Biomix (*Azotobacter* Mac-27+MSX-9+ PSB)	8.95 (25.7)	10.30 (30.7)	15.1
CD at 5% FYM	0.304		
CD at 5% Inoculation (I)	0.568		
CD at 5% Interaction (FYM x I)	NS		
Figures in parenthesis indicate % increase due to inoculation over respective uninoculated control			

It was observed that the biomass production was 7.12 g/plant without addition of FYM while with addition of FYM it was 7.88 g/plant which is about 10.7% higher than without FYM. Among four bacterial isolates, isolate no. RRP-26 produced biomass of 8.56 g/plant which was higher by 20.2% than control, followed by bacterial isolate no.RRP-8 which produced 8.33 g/plant, 17.0% higher than the control. The bacterial isolate no. YRP-11 though produced higher biomass (8.03 g) but the increase was least among all isolates (12.8%) over uninoculated control. It was interesting to note that the combination of all the four bacterial isolates HRP-7+ RRP-8+ RRP-26+ YRP-11, produced highest biomass 8.82 g/plant which was 23.8% higher than the uninoculated control. The effect of biomix (*Azotobacter* strain Mac 27 + MSX-9 and phosphate solubilizing bacteria) produced a biomass of 8.95 g/plant (25.7%) higher than the uninoculated

The selected bacterial isolates were evaluated for growth and biomass production with farm yard manure at 5 tonnes/ha. The mixture of all the four bacterial isolates was also used along with Biomix (mixture of standard *Azotobacter chroococcum* a phosphate solubilizing bacteria) as check for comparison. Increased plant biomass was seen when the bacterial isolates were inoculated singly or as mixture with and without farm yard manure. The increase ranged between 12.8-23.9% without farm yard manure and 18.0 to 29.4% with FYM. The increase in biomass production with mixture of selected bacterial isolates was at par with biomix. The treatments of inoculation of bacterial isolates and the use of farm yard manure gave significantly higher biomass yields but the interaction of both was non-significant.

Conclusion

Total of 104 bacterial isolates obtained from rhizosphere and rhizoplane of Ashwagandha (*Withania angustifolia*) increased plant biomass (root + shoot) on inoculation in sandy soil without FYM. More number of isolates were obtained from rhizoplane (68) than rhizosphere (36) of Ashwagandha. All the efficient isolates which increased biomass belonged to rhizoplane. Characterization of the efficient isolates indicated that bacteria possessed useful traits like nitrogen fixation, production of growth promoting substances like Indole Acetic Acid. Mixture of efficient isolates increased more plant biomass when inoculated with farmyard manure than without farmyard manure and individual isolates. By inoculating rhizobacteria the plant biomass of Ashwagandha (*Withania angustifolia*) can be increased and similar studies may be conducted in other medicinal plants as well as other crops in Horticulture and Agronomy for sustainable growth and development of plants and organic approach of crop production. It was also seen in several investigations that use of rhizobacteria or rhizosphere microorganisms like *Azotobacter*, *Pseudomonas* etc. along with organic manures, increased the germination, plant growth and plant biomass. The growth attributing characters also improved. When these microorganisms were used the efficiency of inorganic fertilizer also increased as was seen by Balakumbahan *et al*., (2005) [3]; Kedia and Kasera (2006) [8] in case of *Phyllanthus*; Puttanna *et al*., (2006) [12] in *Centella*; Kavitha and Vadivel (2006) [6] in *Mucuna pruriens*.

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