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Enhancement of Yield and Quality Parameters of *Withania somnifera* by Indigenous Endophytic Bacterial Isolates

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A B S T R A C T

Ashwagandha (*Withania somnifera* L. Dunal) is an important medicinal plant containing active constituent withanolides providing anti-stress ability, energy, and immunity. An experiment in Randomized Block Design (factorial) was laid out at medicinal and aromatic plants farm, AAU, Anand to study the effect of individual five endophytic bacterial isolates on growth and yield of *W. somnifera* along with FYM and with/without recommended dose of fertilizer (RDF 30:15:0, N:P:K kg/ha). Amongst two levels of fertilizer doses, F1 receiving RDF was superior to F0 control. *Pseudomonas aeruginosa* showed significantly higher yield and yield attributing parameters *viz.* plant height (104.45 cm), no. of branches (6.50), root length (29.85 cm), root girth (6.40 cm), green leaves yield (22698 kg/ha), dry root yield (480 kg/ha) and dry foliage yield (8574 kg/ha) when compared with un-inoculated control (72.30 cm, 4.00, 19.42 cm, 4.35 cm, 7907 kg/ha, 280 kg/ha, 5704 kg/ha), respectively. The results indicated that soil application of endophytic bacteria @ 1L/ha as well as RDF (30:15:0 NPK kg/ha) improved dry root quality parameters starch and withanolides. Endophytic *P. aeruginosa* strain AAU K5 proved to be the best plant growth promoting endophytic bacteria for Ashwagandha.

Introduction

Withania somnifera (L.) Dunal commonly known as Ashwagandha / Indian ginseng or winter cherry, is one of the most valued medicinal plants used in Indian Ayurveda for ≃ 3000 years. The Sanskrit name "*ashva*" meaning horse and "*gandha*" meaning smelling was given to this plant due to the smell of the roots resembling a sweating horse. It belongs to the family *Solanaceae* and attains a height of 0.5-2 m. The whole plant or its different parts are widely used in Ayurvedic and Unani *i.e.* indigenous systems of in India for its medicinal properties and has been used since ancient times (Dar *et al.*, 2015).

It is used as herbal medicine in various forms (decoctions, infusions, ointments, powder and syrup) in different parts of the world, for all age groups of patients (Chatterjee *et al.*, 2010). It possesses a wide array of therapeutic properties including anti-arthritic, anti-aging, anti-cancer, anti-inflammatory, immuno-regulatory, chemoprotective, cardioprotective

and recovery from neurodegenerative disorders (Singh *et al.,* 2015). The traditional use of 'Ashwagandha' was to increase energy, youthful vigour, endurance, strength, health, increase vital fluids, muscle fat, blood, lymph, semen and cell production. It helps counteract chronic fatigue, weakness, dehydration, bone weakness, loose teeth, thirst, impotency, premature aging emaciation, debility, convalescence and muscle tension. It helps invigorate the body by rejuvenating the reproductive organs, just as a tree is invigorated by feeding the roots (Ishnava *et al.,* 2012).

The medicinal properties of *W. somnifera* are due to its chemical constituents (alkaloids and withanolides) present primarily in roots and also in leaves. The species is also known as 'Indian ginseng' as its roots possess restorative properties similar to *Panax ginseng* (Datta *et al.,* 2011). The molecules such as withaferin A, withanolide A and withanolide D isolated from this plant are potential bioactive molecules. Due to the remarkable biological activity of *W. somnifera* and its constituents, it will be appropriate to develop them as a medicine and make them more potent by chemical modifications and biotransformations (Mir *et al.,* 2014).

Many chemical fertilizers are used to increase the yield of plants and improve the nutritional composition of the plants. But continuous use of chemical fertilizers is reported to have deleterious effects on soil health due to their ill effects on physical, chemical and biological properties of soil. Organic manure contains plant growth regulating materials/ regulators, such as humic acids and auxins like gibberellins and cytokinins which are responsible for improved plant growth and yield of many crops (Kumari *et al.,* 2016). The growing interest in environmental sustainability has led to considerable efforts to minimize the use of chemical fertilizers and pesticides, replacing/integrating these conventional approaches with more eco-friendly methods, such as the application of beneficial microorganisms (Abbamondi *et al.,* 2016).

Endophytes 'hidden' within the host plants are a poorly investigated reserve of microorganisms. Endophytes are relatively unstudied as potential sources of novel natural products for medical and commercial exploitation. Since, there are so many of them occupying literally millions of unique biological niches (higher plants) growing in so many unusual environments, exciting potential exist in the endeavor into the wild and unexplored territories of the world to engage in the discovery of endophytes, their biology and potential usefulness (Mehanni and Safwat, 2010). Endophytic microbes have been under increased investigation due to their intimate interaction with the host; it is believed that the phytochemical constitutes of plants are related either directly or indirectly to endophytes and their interactions with host plants (Egamberdieva *et al.,* 2017).

In this effort, endophytic bacteria were isolated from medicinal plants, established their PGP traits and further confirmed their efficacy on *W. somnifera*.

Materials and Methods

Total thirty bacterial endophytes were isolated from various plants like *Azadirachta indica, Zingibe rofficinale* Rosc., *Chlorophytum borivilianum, Curcuma longa, Tinospora cordifolia, Withania somnifera* and *Ocimum sanctum* Lin. from various plant parts like leaf, fruit, rhizome and stem using different media *viz.* Nutrient agar (NA), Starch casein agar (SCA), Glycerol asparagine agar (GAA), Kuster's agar (KUS), King's B (KB) and Inorganic salt starch (ISP4). Further they were screened for *in vitro* PGP traits *viz.* phosphate

solubilization, potash mobilization, production of IAA and siderophore, production of various enzymes like ACC-deaminase, cellulose, protease, lipase, chitinase; antifungal activity and biochemical tests. Five promising endophytic bacterial isolates out of thirty were selected on the basis of PGP traits as stated above. Selected five isolates were then identified on the basis of biochemical and molecular characterization. Isolate Z-1 was identified as *Bacillus tequilensis* strain AAU K1 accn. MF034733, CH-1 as *Bacillus endophyticus* strain AAU K2 acc.no. MF034734, C-1 as *Beijerinckia fluminensis* strain AAU K3 accn. MF034735, T-1 as *Bacillus safensis* strain AAU K4 accn. MF034736 and W-1 as *Pseudomonas aeruginosa* strain AAU K5 accn. MF034737. Nitrogen fixing ability was also confirmed with *nif* H gene which was found in three isolates namely isolate C-1, T-1 and W-1. Further their efficacy was tested on Ashwagandha (*Withania somnifera* L. Dunal) along with FYM and with/without recommended dose of fertilizer in pot as well as field.

The research trial was carried out in field at medicinal and aromatic plants research farm of the Anand Agricultural University during October-March 2016. Various physiological and biochemical studies were undertaken both in field as well in laboratory. The soil of the experimental plots was sandy loam, locally known as "Goradu". The soil was well drained and retentive of moisture. It responded well to irrigation and manuring and was reasonably suitable for Ashwagandha cultivation. The field experiment was laid during October 2015 to March 2016 in Randomized Block Design (factorial) with three replications, Gross plot size: 2.7 x 4.0 m^2, Net Plot size: 1.8 x 3.0 m^2, seed rate: 7 to 10 kg/ha, irrigations: 5 to 6, bacterial treatments: soil drenching at 1L/ha having 10^9 cfu/ml as per treatment, crop and variety: Ashwagandha *cv.*GAA-1. Treatments comprised as, B0: control, B1: *Bacillus tequilensis*, B2: *Bacillus endophyticus*, B3: *Beijerinckia flumensis*, B4: *Bacillus safensis*, B5: *Pseudomonas aeruginosa* with two levels of fertilizer doses F0: control and F1: RDF (30:15:0 NPK kg/ha). FYM 10 ton/ha was applied common in all plots as basal (Table 1).

Plant observations recorded were; days to flower initiation (no.), plant height (cm), branches/plant (no.), root length (cm), root girth (cm), green leaves yield (kg/ha), dry root yield (kg/ha), dry foliage yield/plant (kg/ha), chlorophyll content of leaves (µg /cm^2) measured by chlorophyll meter (Made: at LEAF Ver. 1.0, WILMINGTON, US) at harvest.

Withanolide content (%)

The roots was measured by LC-MS (ekspert™ ultraLC Systems) technique. For extraction of withanolides, one g dried root sample powder was taken. To convert it into fine powder with even particles it was crushed in liquid nitrogen. 100 mg of finely powdered root sample was taken in eppendorf vial (2 ml) for further extraction of withanolides. 1.5 ml 50% v/v Methanol in 1.2 M HCl was taken and added to the sample. It was sonicated and kept in water bath at 80°C. Sonication was continued till 1 h with a break after every 15 min for 1 min (to avoid bumping of solvent).

Centrifugation was carried out at 5,500 rpm for 5 min. Supernatant was transferred to falcon tube (10 ml). To the residue, the solvent was added and extraction was continued as described previously for 5 times. Pooled supernatant was kept at room temperature for evaporation of solvent. Next day, tubes were kept in water bath at 70°C till 2 ml was left in tube. It was further filtered using 0.2 µm syringe filter (Axiva). This was directly used as sample.

Estimation of the withanolide content of roots of Ashwagandha was carried out using liquid chromatography (LC) connected to a mass spectrometer (MS). For liquid chromatography, a QTRAP 4500 ion trap mass spectrometer (ABSCIEX) was connected to the Ekspert ultra LC 100 (Eksigent) instrument via an ESI interface. For liquid chromatography ultra LC 100 from Eksigent was used in conjunction with ABSCIEX QTRAP 4500 mass analyser. The LC-100 was equipped with degasser, quaternary pump, column oven and auto-sampler. Gradient elution was optimized for getting good resolution and noise control during acquisition. For Liquid Chromato-graphy ACQUITY UPLC-BEH C18 (2.1 X 50 mm, 1.7µm) column (WATER Company make) was used.

The column temperature and sample temperature were maintained at 65ºC and 4ºC respectively. The mobile phase was (A: 10%) water and (B: 90%) Methanol (MeOH) with 0.1 % formic acid each. The gradient column elution was as follows: (i) 0 min, 95% A and 5%B; (ii) 0–2 min, 90% A and 10% B; (iii) 2–15 min, 50% A and 50% B; (iv) 15–22 min, 50% A and 50% B; (v) 22–24 min, 10% A and 90% B; (vi) 24–25 min, 10% A and 90% B; (vii) 25–35min, 10% A and 90% B and (viii) 35–60min, 95% A and 5% B. The flow rate of the mobile phase was set to 0.4 µl/ml with an injection volume fixed at 5 µl.

Starch content

Starch content was estimated by Anthrone method described by MacCready (1950). Dry root powder 200 mg, 5 ml of distilled water and 25 ml of 80% ethyl alcohol were taken in 50 ml centrifuge tube and centrifuged at 8000 rpm for 6 min., supernatant was discarded, to the pellet 30 ml of 80% ethyl alcohol was added and centrifuged again, supernatant was discarded, to the residue 20 ml of distilled water plus 6.5 ml of perchloric acid was added and centrifuged. Repeated twice and then transferred the aqueous phase to 100 ml volumetric flask and final volume made up to 100 ml. One ml of filtrate was taken and diluted to 100 ml with distilled water. Again 5 ml from this was taken and 10 ml of freshly prepared 10 % anthrone reagent was added and boiled for 7.5 min in boiling water bath.

The tubes were allowed to cool down at room temperature and read at 630 nm in spectrophotometer. Starch content was calculated as per the following formula:

Starch % = O.D. × Graph factor × 0.9

Soil microbial population (cfu/g)

Soil samples were collected before sowing and at the time of harvest from different treatments separately and stored in polythene bags and kept in refrigerator till processed. Bacterial counts were done by taking 10 g soil sample in sterile 90 ml D/W and shaken it for 1 h and 0.5 ml sample was taken aseptically from it and transferred in 4.5 ml D/W containing dilution tube to make up to 10^{-6} dilutions by serial dilution method from which 0.1 ml aliquot was spreaded on NA plates, incubated for 24-48 h and colony counts were taken for calculating cfu/g.

$$\text{Soil bacterial population cfu/g} = \frac{\text{No. of colonies x dilution factor}}{\text{Aliquot taken}}$$

Results and Discussion

A field trail was laid out during October 2015 to March 2016 at Medicinal and Aromatic farm AAU, Anand. Data regarding efficacy of endophytic bacterial PGPB isolates on flower initiation, plant height, branches per plant, root length, root girth, green leaves weight per

plant, dry root weight, dry biomass weight and leaf chlorophyll content of *Withania somnifera* (L.) Dunal are described below.

Days to flower initiation (No.)

The results indicated that the fertilizer dose and bacterial treatment showed significant influence on days to flower initiation. The data (Table 2) indicated that days to flowering was significantly the least in the fertilizer dose F1 (59.61) as compared to F0 (62.22). Treatment B5 receiving soil application of *P. aeruginosa* showed early days to flowering (53.67) as compared to other bacterial treatments. Effect of FxB interaction was non-significant.

Plant height (cm)

In an experiment to study effect of bacterial treatment and fertilizers in ashwagandha under field conditions, data (Table 3) indicated that plant height was found significant. Dose F1 gave significantly the highest plant height (92.17 cm) as compared to F0 (87.42 cm). Among various bacterial treatments, B5 showed significantly the highest plant height (104.45 cm) when compared with all the other treatments and it was found at par with treatment B2 (102.30 cm). Effect of FxB interaction was non-significant.

Branches/plant (no.)

The results presented in Table 4 indicated that the fertilizer dose and bacterial treatment showed their significant influence on number of branches per plant. The mean data indicated that number of branches per plant was significantly the highest in F1 (5.78) as compared to F0 (5.17).

Among different bacterial treatments, B5 gave the highest number of branches (6.50) which was at par with B2. Effect of FxB interaction was non-significant.

Root length (cm)

Soil application of all the 5 bacterial treatments and fertilizer doses had significant effect on growth and development of Ashwagandha roots which is represented in Table 5. Root length was found significantly the highest in F1 (25.31 cm) as compared with F0 (24.08 cm). Bacterial treatment B5 gave significantly the highest root length (29.85 cm) when compared with all the other treatments.

The effect of FxB interaction was found significant. Treatment F1B5 was found at par with treatment F0B5 suggesting that for organic farming of ashwagandha, treatment F0B5 receiving soil application of *P. aeruginosa* with fertilizer comprising basal FYM can be applied and for conventional farming treatment F1B5 comprising soil application of *P. aeruginosa* with fertilizer dose RDF can be beneficial. Also treatment F1B2 was found at par with treatment F0B2.

Root girth (cm)

Soil application of all the 5 bacterial treatment had significant effect on growth and development of Ashwagandha roots which is represented in Table 6. Effect of fertilizer dose was found non-significant. Bacterial treatment B5 gave significantly the highest root girth (6.40 cm). Effect of FxB interaction was found non-significant.

Green leaves yield (kg/ha)

Data (Table 7) indicated that green leaves yield was found significant. Dose F1 gave significantly the highest green leaves yield (15776 kg/ha) as compared to F0 (15370 kg/ha). Among various bacterial treatments, B5 showed significantly the highest yield (22698 kg/ha). Effect of FxB interaction was found non-significant.

Table.1 Treatment details

Factor B	Bacterial isolate	Factor F	Fertilizer
B0	Control	F0	Control
B1	*Bacillus tequilensis* strain AAU K1	F1	RDF : N:P:K (30:15:0) kg/ha
B2	*Bacillus endophyticus*strain AAU K2		
B3	*Beijerinckia fluminensis* strain AAU K3		
B4	*Bacillus safensis* strain AAU K4		
B5	*Pseudomonas aeruginosa* strain AAU K5		

Table.2 Days to flower initiation (No.)

Fertilizer dose (F)	Bacterial treatment (B)						
	B0	**B1**	**B2**	**B3**	**B4**	**B5**	**Mean F**
F0	71.33	66.33	56.33	60.67	63.67	55.00	62.22
F1	68.67	61.67	56.00	57.67	61.33	52.33	59.61
Mean B	70.00	64.00	56.17	59.17	62.50	53.67	
Effect	**B**	**F**	**BxF**				
S.Em.±	0.80	0.46	1.13				
lsd	2.35	1.36	NS				
CV%	3.22						

Table.3 Plant height (cm)

Fertilizer dose (F)	Bacterial treatment (B)						
	B0	**B1**	**B2**	**B3**	**B4**	**B5**	**Mean F**
F0	66.13	84.60	101.57	91.37	78.54	102.33	87.42
F1	78.47	90.40	103.03	94.20	80.37	106.57	92.17
Mean B	72.30	87.50	102.30	92.78	79.45	104.45	
Effect	**B**	**F**	**BxF**				
S.Em.±	1.67	0.97	2.37				
lsd	4.91	2.84	NS				
CV%	4.57						

Table.4 Branches/plant (no.)

Fertilizer dose (F)	Bacterial treatment (B)						
	B0	**B1**	**B2**	**B3**	**B4**	**B5**	**Mean F**
F0	3.33	5.33	6.00	4.67	5.33	6.33	5.17
F1	4.67	5.67	6.00	6.00	5.67	6.67	5.78
Mean B	4.00	5.50	6.00	5.33	5.50	6.50	
Effect	**B**	**F**	**BxF**				
S.Em.±	0.29	0.17	0.41				
lsd	0.85	0.49	NS				
CV%	12.89						

Table.5 Root length (cm)

Fertilizer dose (F)	Bacterial treatment (B)						
	B0	B1	B2	B3	B4	B5	Mean F
F0	17.30	22.73	27.13	23.87	22.53	29.03	24.08
F1	21.53	24.03	27.23	25.73	24.50	30.67	25.31
Mean B	19.42	23.38	27.18	24.80	23.52	29.85	
Effect	B	F	BxF				
S.Em.±	0.52	0.30	0.74				
lsd	1.53	0.89	2.16				
CV%	5.16						

Table.6 Root girth (cm)

Fertilizer dose (F)	Bacterial treatment (B)						
	B0	B1	B2	B3	B4	B5	Mean F
F0	3.90	4.83	5.87	5.57	5.50	6.23	5.32
F1	4.80	4.93	5.93	6.00	5.77	6.57	5.67
Mean B	4.35	4.88	5.90	5.78	5.63	6.40	
Effect	B	F	BxF				
S.Em.±	0.22	0.13	0.31				
lsd	0.64	NS	NS				
CV%	9.78						

Table.7 Green leaves yield (kg/ha)

Fertilizer dose (F)	Bacterial treatment (B)						
	B0	B1	B2	B3	B4	B5	Mean F
F0	7,337	14,233	18,692	15,944	13,378	22,633	15,370
F1	8,478	14,570	18,978	16,126	13,741	22,763	15,776
Mean B	7,907	14,402	18,835	16,035	13,559	22,698	
Effect	B	F	BxF				
S.Em.±	216	125	305				
lsd	637	368	NS				
CV%	2.74						

Table.8 Dry root yield (kg/ha)

Fertilizer dose (F)	Bacterial treatment (B)						
	B0	B1	B2	B3	B4	B5	Mean F
F0	244	327	373	368	353	436	350
F1	316	353	420	410	412	534	407
Mean B	280	340	397	389	382	485	
Effect	B	F	BxF				
S.Em.±	20	12	29				
lsd	60	35	NS				
CV%	13.15						

Table.9 Dry foliage yield (kg/ha)

Fertilizer dose (F)	Bacterial treatment (B)						
	B0	**B1**	**B2**	**B3**	**B4**	**B5**	**Mean F**
F0	5,595	5,958	7,565	8,281	6,839	8,187	7,071
F1	5,813	6,373	7,913	8,773	7,085	8,960	7,486
Mean B	5,704	6,165	7,739	8,527	6,962	8,574	
Effect	**B**	**F**	**BxF**				
S.Em.±	216	125	305				
lsd	637	368	NS				
CV%	7.26						

Table.10 Chlorophyll content of leaves (µg/cm^2)

Fertilizer dose (F)	Bacterial treatment (B)						
	B0	**B1**	**B2**	**B3**	**B4**	**B5**	**Mean F**
F0	64.16	63.74	65.23	65.61	62.79	64.69	64.37
F1	64.77	64.16	66.43	66.57	64.34	65.35	65.27
Mean B	64.47	63.95	66.09	65.83	63.56	65.01	
Effect	**B**	**F**	**BxF**				
S.Em.±	0.805	0.465	1.139				
lsd	NS	NS	NS				
CV%	3.04						

Table.11 Active constituent's estimation

		Active constituents mg/g of dry root		
Sr. No.	**Treatment**	**Withanolide**	**Withaferin-A**	**12-Deoxywithastramonolide**
1	F0B0	0.130	0.020	0.209
2	F0B1	0.151	0.032	0.237
3	F0B2	0.190	0.038	0.302
4	F0B3	0.203	0.022	0.336
5	F0B4	0.174	0.035	0.270
6	F0B5	0.248	0.030	0.396
7	F1B0	0.146	0.023	0.229
8	F1B1	0.159	0.035	0.253
9	F1B2	0.203	0.053	0.320
10	F1B3	0.226	0.024	0.338
11	F1B4	0.187	0.038	0.294
12	F1B5	0.282	0.068	0.434

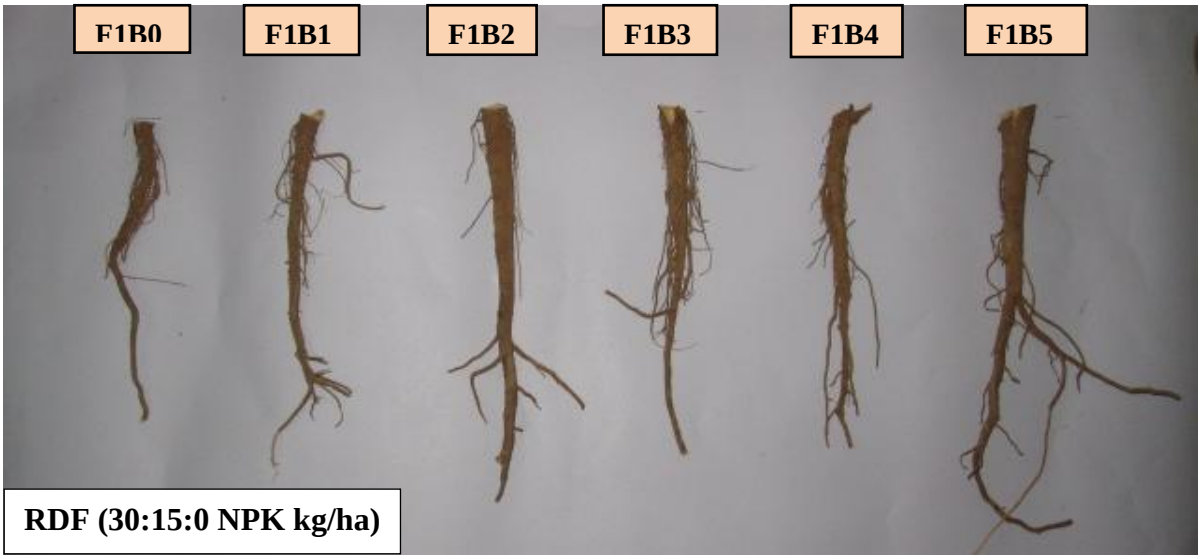
Table.12 Starch content (%)

Fertilizer dose (F)	Bacterial treatment (B)						
	B0	B1	B2	B3	B4	B5	Mean F
F0	10.23	13.20	14.40	16.10	13.83	18.37	14.36
F1	10.57	13.60	15.13	16.27	14.10	18.83	14.75
Mean B	10.40	13.40	14.77	16.19	13.97	18.60	
Effect	B	F	BxF				
S.Em.±	0.10	0.06	0.15				
lsd	0.30	0.17	0.43				
CV%		1.73					

Table.13 Soil bacterial population (log cfu/g)

Fertilizer dose (F)	Bacterial treatment (B)						
	B0	B1	B2	B3	B4	B5	Mean F
F0	5.50	6.41	6.62	6.54	6.51	6.79	6.39
F1	5.56	6.44	6.63	6.57	6.52	6.82	6.42
Mean B	5.53	6.43	6.63	6.65	6.52	6.81	
Effect	B	F	BxF				
S.Em.±	0.005	0.008	NS				
lsd	0.024	0.014	NS				
CV%	0.311						

Fig.1 Effect of endophytic bacterial isolates on dry root of Ashwagandha



Dry root yield (kg/ha)

Data (Table 8) indicated that dry root yield was found significant. Dose F1 gave significantly the highest dry root yield (407 kg/ha) as compared to F0 (350 kg/ha). Among various bacterial treatments, B5 showed significantly the highest yield (485 kg/ha) when compared with all the other treatments. Effect of FxB interaction was found non-significant (Fig. 1). Shrivastava and Sahu (2014) studied the yield and quality parameters of *W. somnifera* using integrated nutrient management with the use of organic manures in combination with inorganic fertilizers and micro-nutrient improved the quality parameters of root of ashwagandha crop. Among all the treatments, T11 treatment receiving 100% NPK and 2.5 t/ha vermi-compost as well as 5 t/ha FYM with 20 kg $ZnSO_4$ excelled to all the treatments followed by T14, T10 and T13 in terms of root length, girth and dry root yield. It was minimum under T2 where 50 % RDF was applied through fertilizers.

Kumar *et al.,* (2009) studied the yield parameters and cost economics of *W. somnifera* using dual inoculation of *Azotobacter chroococcum* and *Pseudomonas putida.* Inoculation with the bacteria generated encouraging results wherein, fresh root yield (1185.6 kg ha^{-1}), seed yield (208.13 kg ha^{-1}), number of shoots per plant (6.07), and plant height (108.4 cm) were maximum with treatment T6 (organic manure [OM] 20 t ha^{-1}+ both bacteria), followed by T9 (OM 10 t ha^{-1}+ both bacteria), T4 (OM 20 t ha^{-1} + *A. chroococcum*), and T5 (OM 20 t ha^{-1} + *P. putida*), as compared to T1 (control), T2 (OM 10 t ha^{-1}), and T3 (OM 20 t ha^{-1}).

Dry foliage yield (kg/ha)

Data (Table 9) indicated that dry foliage yield was found significant. Dose F1 gave significantly the highest dry foliage yield (7486 kg/ha) as compared to F0 (7071 kg/ha). Among various bacterial treatments, B5 showed the highest dry foliage yield (8574 kg/ha) which was at par with B3 (8527 kg/ha). Effect of interaction was found non-significant.

Chlorophyll content of leaves (µg/cm^2)

Data (Table 10) indicated that chlorophyll content of leaves was found non-significant.

Withanolide

Three important bioactive compounds namely withaferin-A (WA), 12-deoxy withastramonolide (WO) and withanolide-A (WD) were determined by the liquid chromatography mass spectrometry (LC-MS) method in dry roots of *W. somnifera*. Withanolide content ranged from 0.130 to 0.282 mg/g of dry root.

Treatment B5 gave the highest withanolide content as compared to un-inoculated control. Withaferin content ranged from 0.020 to 0.068 mg/g of dry root. Treatment B5 gave the highest withaferin content as compared to un-inoculated control. 12-Deoxy withastramonolide content ranged from 0.209 to 0.434 mg/g of dry root. Treatment B5 gave the highest 12-Deoxy withastramonolide content as compared to un-inoculated control (Table 11).

Gajbhiye *et al.,* (2015) studied accumulation of three important bioactive compounds in different plant parts of *W. somnifera* and its determination by LC-ESI-MS-MS (MRM) method. The accumulation of WA was the highest in leaves (8.84 ± 0.37 mg/g) and it was 2.23, 5.85 and 27.26 times higher than its concentration in fruits, stems and roots, respectively. WO and WD contents were the highest (0.44 ± 0.016 and 0.72 ± 0.016 mg/g, respectively) in root.

Starch estimation

Starch estimation was carried out from the dry root samples. Data (Table 12) indicated that starch content was found significant. Dose F1 gave significantly the highest starch content (14.75 %) as compared to F0 (14.36 %). Among various bacterial treatments, B5 showed the highest starch content (18.60 %). Effect of interaction was found significant. Treatment F1B5 was found at par with treatment F0B5. Also treatment F1B3 was found at par wih treatment F0B3.

Kakaraparthi *et al.,* (2013) studied Effect of sowing dates on morphological characteristics, root yield and chemical composition of the root of *W. somnifera* grown in the semi-arid regions of Andhra Pradesh, India. Among the varieties tested, Poshita produced significantly high starch content (19.55%) compared to variety Nagore (15.33%). Early sowing (June-July) resulted in higher root yield. The starch content showed decrease-increase-decrease pattern with delay in sowings. By and large on an average there is a steady decrease in starch content with advancement in sowings from June to October.

Kumar *et al.,* (2011) studied the root textural quality in ashwagandha as influenced by crop growth periods and morphotypes. They observed that the pattern of starch and fiber accumulation varied with different crop growth periods. The pattern of root starch accumulation at different growth intervals followed a trend of decrease–increase–decrease. Starch content was high at early stage of the crop growth 45 DAS (17.44%), decreased gradually up to middle of crop growth 105 DAS (11.10%), thereafter replenished (120 DAS – 14.45%; 135 DAS – 15.37%) and then depleted 150 DAS (14.18%) and at 165 DAS (13.12%).

Khanna *et al.,* (2006) studied the biochemical composition of *W. somnifera* (L.) Dunal. Starch contents were measured in five selective accessions viz. AGB-002, AGB-009, AGB-015, AGB-025 and AGB-030 of *W. somnifera* (L.) Dunal. Measured activity of starch in roots was found to be involved in root development and composition showing increased trend in activities measured in selected five accessions at maturity stage *i.e.,*

210 Days after planting (DAP) ranging from 7.98±0.20 to 9.46±0.08 mg g^{-1}. Young roots at 150 DAP showed low amount of starch ranging from 6.09±0.13 to 8.87±0.25 mg g^{-1}.

Soil bacterial population

Initial soil population of bacteria was 3.53 log cfu/g. Data (Table 13) indicated that soil bacterial count was found significant. Dose F1 gave significantly the highest bacterial count (6.42 log cfu/g) as compared to F0 (6.39 log cfu/g). Among various bacterial treatments B5 showed significantly the highest bacterial count (6.81 log cfu/g) when compared with all the other treatments. Interaction was found to be non-significant.

The efficacy of bacterial endophytes was tested on Ashwagandha (*Withania somnifera* L. Dunal) along with FYM and with/without recommended dose of fertilizer in field. The results indicated soil application of B5 *P. aeruginosa* showed significantly higher growth parameters *viz*. plant height, no. of branches, root length, root girth, green leaves yield, dry root yield and dry foliage yield when compared with uninoculated control. Similarly, amongst two levels of fertilizer doses, F1 was superior to F0. The results indicated that soil application of endophytic bacteria @ 1L/ha as well as RDF (30:15:0 NPK kg/ha) increased yield and yield attributing parameters with improved quality namely starch and withanolides of Ashwagandha dry root. Endophytic *Pseudomonas aeruginosa* strain AAU K5 Accn MF034737 proved to be the best plant growth promoting endophytic bacteria for Ashwagandha.

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