



RESEARCH ARTICLE

The enhancement of root yield and quality of ashwagandha [*Withania somnifera* (L.) Dunal] by weeds leaves extracts

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ABSTRACT

Ashwagandha, having multiple therapeutic uses, is a highly valuable medicinal plant for pharmaceutical industry. In order to meet the industrial demand, both quality as well as yield of ashwagandha needs to be improved agronomically. In this study, effect of weed leaves extracts (WLE) as bio-stimulants to improve yield and quality of ashwagandha roots were studied in pot experiments during 2020-21. The treatments consisted of combinations of four commercial preparations with microorganisms (Pusa zinc solubilizing biofertilizer, Pusa Azotobacter liquid biofertilizer, Pusa PSB liquid biofertilizer, Pusa Potash solubilizing liquid biofertilizer) with four weeds [*Cyperus rotundus* L., *Amaranthus viridis* L., *Echinochloa colona* (L.) Link, *Digera arvensis* Forsk.] leaves extracts. The treated plants exhibited stimulatory responses in growth and physiology, leading to enhanced dry root yield of ashwagandha compared to control. Yield enhancing effects of different treatments, when used separately, without combination was the highest in case of *Amaranthus viridis* WLE, followed by *Digera arvensis* WLE and PSB solubilizing biofertilizer, however co-application resulted in synergistic effect. Among different combination of treatments, Pusa PSB liquid biofertilizer + *Amaranthus viridis* WLE recorded the highest whole ashwagandha plant dry matter production (157.3 g/plant), root fresh weight per plant (65.0 g) and root dry weight (23.0 g). Different bioactive compounds in ashwagandha roots (withanoloides A, withanosides IV and withanone) were also enhanced with this treatment indicating the potentiality of weed leaves extracts as biostimulants, with a possibility to use as a novel eco-friendly approach for enhancing root yield and quality of ashwagandha.

Keywords: Ashwagandha, Bio-stimulants, Weed leaves extracts, Bio-fertilizers, Root yield and quality, *Withania somnifera*

INTRODUCTION

Ashwagandha [*Withania somnifera* (L.) Dunal] is highly valuable medicinal plant having immense pharmaceutical uses. Ashwagandha is grown on an estimated area of 10,780 hectares in India, with a total dry root yield of 8,429 tonnes. India exported a total amount of about 132.72 tons of *W. somnifera* valuing 8.17 crores (USD 1,202,740) during 2014–2016 (Srivastava *et al.* 2018). It is widely used in more than 100 formulations in traditional medicine systems like Siddha, Unani, and Ayurveda, since over 3000 years. *W. somnifera* possess diverse pharmacological properties such as anti-carcinogenic, anti-apoptotic, anti-tumor, bone healing, neuroprotective, cardioprotective, anti-tumor, anti-oxidant, immunomodulatory, anti-stress, and anti-inflammatory (Sangwan *et al.* 2017). Because of its therapeutic applications, antioxidants, and anticancerous activities, products

like herbal tea, powders, tablets, and syrups are prepared through its extracts (Leyon and Kuttan 2004). Ashwagandha owes its medicinal benefits to the presence of distinct group natural steroidal lactones called withanolides of alkaloid metabolites, mainly in the roots (Chaurasiya *et al.* 2000). Withanolides, which are polyoxygenated C28 steroidal lactones, are the major pharmacologically active constituents of *W. somnifera* roots.

In order to meet the current industrial demand, researchers are focusing towards enhancing yield and quality by low-cost organic inputs. Weeds may also act as potential biostimulant, because of having higher nutrient concentration in their respective biomass as they accumulate these nutrients from the cropping soil. They usually absorb mineral nutrients, both macro and micro with a faster rate as compared to several other crops and are able to accumulate them in larger amounts in their tissues (Mahajan and Jha 2009, Rao and Matsumoto 2017). The weeds (*Tephrosia vogelii* and *Tithonia diversifolia*) leaves extracts (WLE) were reported to significantly increased chlorophyll content, the number of pods per plant and overall seed yield of beans (Mkindi *et al.* 2020). Hence, a study was conducted to quantify the biostimulant

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potential of leaves extracts of common weeds, viz. *Cyperus rotundus*, *Amaranthus viridis*, *Echinochloa colona* and *Digera arvensis* to enhance yield and quality of ashwagandha.

MATERIALS AND METHODS

Experimental site and weather conditions

The experimental site was the research farm of CSIR–Central Institute of Medicinal and Aromatic Plants (CIMAP), located at 26°5′ N latitude 80°5′ E longitude with an elevation of about 120 m above mean sea level Lucknow, Uttar Pradesh, India which is classified as a subtropical region of north Indian plains. The climate of the site is characterized with hot summers, fairly cool winters and with an average annual precipitation of 1000 mm. The soil of experimental field was categorized as loamy sand having pH 8.03 with organic carbon of 3.21 g/kg soil and N, P, K as 202.1, 52.06, and 152.24 kg/h soil, respectively. Pot experiment was conducted during October, 2020 to April, 2021.

Crop raising

Quality seed material of *Withania somnifera* was procured from the gene bank of CSIR-CIMAP, Lucknow. During the cropping periods, standard agronomic procedures were performed for the cultivation of crops. In all pots required amount of applicable bio-fertilizer recommended dose were applied through microbial cultures. The amount of different microbial cultures according to the treatments was applied and weed leaf extract sprayed treatment wise as recommended duration after sowing. All the recommended cultural practices like irrigation, weeding and foliar applications etc. were followed according to the requirement during crop growth period.

Treatment and experimental design

The main pot experiment was designed in completely randomized block design (CRBD) with three replications comprising of 17 treatments including control. The treatments were combinations of four commercial preparations with micro-organisms (Pusa zinc solubilizing biofertilizer, Pusa Azotobacter liquid biofertilizer, Pusa PSB liquid biofertilizer, Potassium solubilizing bacteria) with four weeds (*Cyperus rotundus* L., *Amaranthus viridis* L., *Echinochloa colona* (L.) Link, *Digera arvensis* Forsk.) leaves extracts. The treatments include: Pusa zinc solubilizing biofertilizer + *Cyperus rotundus* weed leaf extracts (WLE); Pusa zinc solubilizing biofertilizer + *Amaranthus viridis* WLE; Pusa zinc solubilizing biofertilizer + *Echinochloa colona* WLE; Pusa zinc solubilizing biofertilizer + *Digera arvensis*

WLE; Pusa azotobacter liquid biofertilizer + *Cyperus rotundus* WLE; Pusa azotobacter liquid biofertilizer + *Amaranthus viridis* WLE; Pusa azotobacter liquid biofertilizer + *Echinochloa colona* WLE; Pusa azotobacter liquid biofertilizer + *Digera arvensis* WLE; Pusa phosphate solubilizing bacteria (PSB) liquid biofertilizer + *Cyperus rotundus* WLE; Pusa PSB liquid biofertilizer + *Amaranthus viridis* WLE; Pusa PSB liquid biofertilizer + *Echinochloa colona* WLE; Pusa PSB liquid biofertilizer + *Digera arvensis* WLE; Potash solubilizing liquid biofertilizer + *Cyperus rotundus* WLE; Potash solubilizing liquid biofertilizer + *Amaranthus viridis* WLE; Potash solubilizing liquid biofertilizer + *Echinochloa colona* WLE; Potash solubilizing liquid biofertilizer + *Digera arvensis* WLE and control. Control plants were sprayed with distilled water to maintain the same moisture content.

Another set of pot experiment was also conducted to compute individual effect of these four biofertiliser's and four weed leaves extracts with one control (total nine treatments) on root yield of ashwagandha. The effective microorganism (EM) culture, commercially known as Pusa zinc solubilizing biofertilizer, Pusa Azotobacter liquid biofertilizer, Pusa PSB liquid biofertilizer and potash solubilizing liquid biofertilizer were obtained from Indian Agricultural Research Institute, New Delhi, India. Potash solubilizing liquid biofertilizer contains *Bacillus decolorationis*. Pusa Azotobacter liquid formulation have highly efficient nitrogen fixing *Azotobacter chroococcum*. Pusa zinc solubilizing biofertilizer, a liquid formulation contains highly efficient zinc solubilizing bacteria (*Bacillus endophyticus*). Pusa PSB liquid biofertilizers are liquid formulations of P-solubilizing bacteria containing *Paenibacillus tylophilus*.

Fresh solution of biofertilizers for use was prepared from stock. Tap water was added to the stock to prepare a 0.2% solution. Liquid biofertilizers were administered during the time of sowing via seed treatment and soil application in each respective pot. Foliar applications of the different weed leaves extracts were done twice consecutively at an interval of 60 and 75 days after sowing. Graduated atomizer or sprayer (5 ml) was used for the careful foliar application of weed leaves extracts (WLE).

Preparation of weed leaves extracts

The leaves of selected weeds were collected individually from research farm of CSIR-CIMAP, Lucknow. One kg leaves of each weed were air-dried under shade for two weeks and subsequently grinded using pestle mortar. For the preparation of 1% stock extract, 10 g leaf powder was soaked in 1-liter

distilled water and kept on shaker for 24 hrs at temperature ($25 \pm 1\%$ °C). The extract was purified by filtering twice through (Whatman No. 1) filter paper. The extracts were used within five hours from cutting and extracting (Mahdavia and Saharkhiz 2015). The mineral content and chemical composition in weed leaf extracts were analyzed (Table 1).

Plant growth related observations and harvesting of crop

Plant height (cm), number of leaves, plant canopy, LAI, branches per plant, root girth (mm), and root length (cm) were measured from the plants in each pot. 200 days after sowing, the roots were dug, rinsed with plain water, and oven dried. The roots were dried till they had 7-8% of the original moisture content following which, they were stored in air-tight packs for further chemical analysis.

Chemical profiling of roots

The percentages of total alkaloid, withaferin A, withanolide, withanolide A, and 12 deoxywithastramonolide were also estimated in the properly dried root samples of each treatment using high-performance liquid chromatography (HPLC) as per the standard method (API 2010). Fresh plant root samples were collected and kept for dry in oven. The dried roots were grinded for making fine powder. The root powder (2 gm) was soaked in methanol in 50 ml and sonicated for 30 minutes. The solution was filtered twice through Whatman No.1 filter paper after cooling. In a 100-ml volumetric flask, the filtrates were mixed, concentrated, and the volume was made up. 2 ml of each sample were filtered using a 0.45- μ m membrane filter and subjected into the High-performance liquid chromatography (HPLC) for the analysis of bioactive compounds. HPLC analysis was performed on a SHIMADZU (Nexera XR, autosampler), Phenomenex C18 column (250×4.6 mm i.d.; 5 μ m) were used. The gradient elution was carried out using solvent system A) phosphate buffer (prepared by dissolving 0.14 g of potassium dihydrogen orthophosphate in 500 ml of water, adding 0.5 ml of orthophosphoric acid and diluting up to 1000 ml) and B) only acetonitrile (100%). Injection volume was 20 μ l and flow rate 1.5 ml/min. Run time of injection was 45min and absorbance recorded at 227 nm.

Statistical analysis

The data recorded were analyzed statistically using the techniques described by Panse and Sukhatme (1985). LSD values at 5% level of probability were calculated for comparing the treatment means where the “F” test was found significant at $p=0.05$.

RESULTS AND DISCUSSION

Plant growth characteristics

Ashwagandha crop growth parameters at 90 DAS were significantly influenced by combined application of biofertilizers and WLE (Table 2). Among the different treatments, Pusa PSB liquid biofertilizer + *Amaranthus viridis* WLE recorded significantly highest plant height (53.33 cm), closely followed by T 10 (51.0) and potash solubilizing liquid biofertilizer + *Echinochloa colona* WLE (48.5) as compared to untreated control (30.33). Leaf area index and number of leaves per plant also followed similar trend. At harvest stage, plant growth attributes as well as root and shoot yield was significantly higher in Pusa PSB liquid biofertilizer + *Amaranthus viridis* WLE, closely followed by Pusa PSB liquid biofertilizer + *Cyperus rotundus* and Potash solubilizing liquid biofertilizer + *Echinochloa colona* WLE than control (Table 3). Root biomass per pot increased with Pusa PSB liquid biofertilizer + *Amaranthus viridis* WLE application, as compared to control. Similar results were reported in Senna (Anisuzzaman *et al.* 2014) and *Thymus vulgaris* (Yadegari *et al.* 2012), upon treatments with different microbial biostimulants.

Root yield and quality

Quality of root (length and girth) of ashwagandha forms an important quality parameter. Marked variation was observed on both individual and combined effect of different biofertilizer and WLE treatments on ashwagandha whole plant and root dry matter production (Table 4 and 5). Among all the nine treatments of secondary pot experiment, yield enhancing effects of individual treatments was the highest in case of *Amaranthus viridis* WLE, followed by *Digera arvensis* WLE and PSB solubilizing biofertilizer (Table 5). Among the combined application of biofertilizer and WLE treatments (main pot experiment), Pusa PSB liquid

Table 1. Major nutrient content found in weeds leaves extracts

Selected weed	N mg/L	P mg/L	K mg/L	Cu mg/L	Fe mg/L	Mn mg/L	Zn mg/L
<i>Amaranthus viridis</i>	381	79.89	206	0.77	2.345	-	0.05
<i>Digera arvensis</i>	274	62.16	132	1.32	4.88	0.415	0.025
<i>Echinochloa colona</i>	40	20.1	307	5.1	2.975	0.085	0.085
<i>Cyperus rotundus</i>	-	16.84	350	0.4	1.505	0.005	0.08

biofertilizer + *Amaranthus viridis* WLE recorded the highest whole plant dry matter production (157.3 g per plant), root fresh weight/plant (65.0 g) and root dry weight (23.0 g) while the minimum values were reported in control. Taiwo and Makinde (2005) have reported stimulatory effects of foliar application of *Tithonia diversifolia* extract on growth and yield of *Vigna unguiculata*. Mkindi *et al.* 2020 reported that foliar application of plant extracts from *Tephrosia vogelii* and *Tithonia diversifolia* contributed to plant nutrition as a foliar fertilizer, by enhancing growth and yield of common bean plant. Mkindi *et al.* 2020 further suggested that this contribution to growth and yield was related to the addition of nitrogen as *T. diversifolia* and *T. vogelii* were known to produce

nitrogen-rich green biomass. Our results were also supported by weed leaf extracts helping in indirect physiological assistance by acting as a topical green fertilizer (Jama *et al.* 2000), bio-stimulant (Pretali *et al.* 2016), or foliar feed (Shaaban 2001). The consortia of biostimulants (biofertilisers and WLE), having beneficial soil microbes, and plant nutrients resulted in improved root yield in the present investigation. The synced administration of biostimulants (biofertilizers and WLE) could have dramatically triggered the soil processes and increased the bioavailability of nutrients as leguminous weeds like *Amaranthus viridis* and *Digera arvensis* contained large amount of N, P, K nutrients and their extract contain these nutrients in

Table 2. Effect of biofertilizers and weeds leaves extracts (WLE) on ashwagandha plant height, number of leaves per plant and Leaf area index at 90 DAS

Treatment	Plant height (cm)	No. of leaves	Leaf area index
Control (sprayed with distilled water)	30.33	18.0	0.41
Pusa zinc solublizing biofertilizer + <i>Cyperus rotundus</i> WLE	40.33	23.3	0.56
Pusa zinc solublizing biofertilizer + <i>Amaranthus viridis</i> WLE	38.00	25.6	0.67
Pusa zinc solublizing biofertilizer + <i>Echinochloa colona</i> WLE	39.33	20.6	0.49
Pusa zinc solublizing biofertilizer + <i>Digera arvensis</i> WLE	41.00	26.3	0.79
Pusa Azotobacter liquid biofertilizer + <i>Cyperus rotundus</i> WLE	45.33	24.3	0.68
Pusa Azotobacter liquid biofertilizer + <i>Amaranthus viridis</i> WLE	47.00	26.0	0.72
Pusa Azotobacter liquid biofertilizer + <i>Echinochloa colona</i> WLE	42.66	22.3	0.56
Pusa Azotobacter liquid biofertilizer + <i>Digera arvensis</i> WLE	42.66	23.6	0.62
Pusa PSB liquid biofertilizer + <i>Cyperus rotundus</i> WLE	51.00	36.3	1.05
Pusa PSB liquid biofertilizer + <i>Amaranthus viridis</i> WLE	53.33	38.0	1.19
Pusa PSB liquid biofertilizer + <i>Echinochloa colona</i> WLE	42.66	27.6	0.85
Pusa PSB liquid biofertilizer + <i>Digera arvensis</i> WLE	45.33	30.6	0.85
Potash solublizing liquid biofertilizer+ <i>Cyperus rotundus</i> WLE	40.66	25.0	0.66
Potash solublizing liquid biofertilizer+ <i>Amaranthus viridis</i> WLE	40.15	25.6	0.73
Potash solublizing liquid biofertilizer+ <i>Echinochloa colona</i> WLE	48.50	35.40	1.00
Potash solublizing liquid biofertilizer+ <i>Digera arvensis</i> WLE	48.00	32.33	0.92
LSD (p=0.05)	2.97	2.61	0.13

Table 3. Effect of biofertilizers and foliar applied weeds leaves extracts (WLE) on ashwagandha crop growth parameters of at harvest stage

Treatment	Ashwagandha			
	Plants height (cm)	Plants canopy (cm)	No. of branches	Plant biomass per plant (gm)
Control (sprayed with distilled water)	55.0	36.7	9.0	77.7
Pusa zinc solublizing biofertilizer + <i>Cyperus rotundus</i> WLE	68.6	55.0	13.0	100.3
Pusa zinc solublizing biofertilizer + <i>Amaranthus viridis</i> WLE	66.6	57.7	10.0	117.0
Pusa zinc solublizing biofertilizer + <i>Echinochloa colona</i> WLE	61.5	58.3	11.0	110.7
Pusa zinc solublizing biofertilizer + <i>Digera arvensis</i> WLE	75.0	63.3	11.3	120.3
Pusa Azotobacter liquid biofertilizer + <i>Cyperus rotundus</i> WLE	71.7	60.0	10.0	107.3
Pusa Azotobacter liquid biofertilizer + <i>Amaranthus viridis</i> WLE	76.7	59.0	9.7	100.0
Pusa Azotobacter liquid biofertilizer + <i>Echinochloa colona</i> WLE	68.7	61.7	11.0	111.6
Pusa Azotobacter liquid biofertilizer + <i>Digera arvensis</i> WLE	71.3	67.0	11.6	131.0
Pusa PSB liquid biofertilizer + <i>Cyperus rotundus</i> WLE	78.0	57.0	11.5	132.7
Pusa PSB liquid biofertilizer + <i>Amaranthus viridis</i> WLE	73.3	66.7	11.7	157.3
Pusa PSB liquid biofertilizer + <i>Echinochloa colona</i> WLE	69.0	56.7	8.0	124.0
Pusa PSB liquid biofertilizer + <i>Digera arvensis</i> WLE	62.7	53.3	10.3	83.3
Potash solublizing liquid biofertilizer+ <i>Cyperus rotundus</i> WLE	70.7	55.3	10.7	97.3
Potash solublizing liquid biofertilizer+ <i>Amaranthus viridis</i> WLE	71.0	53.0	9.0	107.7
Potash solublizing liquid biofertilizer+ <i>Echinochloa colona</i> WLE	76.3	68.3	11.0	131.3
Potash solublizing liquid biofertilizer+ <i>Digera arvensis</i> WLE	67.7	53.3	11.3	109.0
LSD (p=0.05)	10.99	NS	NS	NS

available form, (Table 1) which significantly influenced the ashwagandha plant growth and yield. Similar effects of combined application of vermicompost and biofertilizers have been reported previously in rosemary (*Salvia rosmarinus* L.), thyme (*Thymus vulgaris* L.) (Sudhakar 2005) and *Withania somnifera* (Basak et al. 2020).

Bioactive compounds and their content

The highest withanolide A content was detected in Pusa Azotobacter liquid biofertilizer + *Amaranthus viridis* WLE (0.268 mg/g) closely followed by Pusa PSB liquid biofertilizer + *Amaranthus viridis* WLE (0.268 mg/g) while it was detected 1.48 mg/g in control. Withanone content in control 0.034 mg/g whereas 0.061 mg/g found in Pusa PSB liquid biofertilizer + *Cyperus rotundus* WLE treatment (Table 5). Highest content of withanoside IV was found in Pusa Azotobacter liquid biofertilizer + *Cyperus rotundus* WLE and Pusa Azotobacter liquid biofertilizer + *Amaranthus viridis* WLE treatment (0.096 mg/g). Pusa Azotobacter

liquid biofertilizer + *Amaranthus viridis* WLE treatment has given highest content of withanoloides A and withanoside IV in comparison to the control and other treatments, while Pusa PSB liquid biofertilizer + *Cyperus rotundus* WLE treatment recorded the highest withanone content. In the present investigation, the increased content of withanoloides A, withanosides IV and withanone post biostimulant application might be due its beneficial role in improving soil health and quality and increasing nutrient bioavailability. Increased nutrient availability has been associated with enhanced production of bioactive compounds in several medicinal plants like *Centella asiatica* (Jat and Gajbhiye 2017), Kalmegh (Jat and Gajbhiye 2019), and ashwagandha (Rajasekar and Elango 2011).

The spraying of microbial bio-elicitors along with foliar application of weed leaves extracts improved plant growth, root yield and quality in ashwagandha in this study, indicating the potentiality of weeds as a valuable ‘resource’ rather than ‘waste’.

Table 4. Effect of biofertilizers and foliar applied weeds leaves extracts (WLE) on ashwagandha growth and yield at harvest stage

Treatment	Ashwagandha			
	Root length (cm)	Root diameter (mm)	Root fresh weight / plant (gm)	Root dry weight / plant (gm)
Control (sprayed with distilled water)	14.66	19.26	38.33	11.66
Pusa zinc solubilizing biofertilizer + <i>Cyperus rotundus</i> WLE	17.33	24.53	55.00	15.00
Pusa zinc solubilizing biofertilizer + <i>Amaranthus viridis</i> WLE	15.00	23.40	58.00	16.88
Pusa zinc solubilizing biofertilizer + <i>Echinochloa colona</i> WLE	16.00	23.10	48.30	13.25
Pusa zinc solubilizing biofertilizer + <i>Digera arvensis</i> WLE	18.66	22.93	56.76	15.80
Pusa Azotobacter liquid biofertilizer + <i>Cyperus rotundus</i> WLE	17.00	23.33	47.33	12.80
Pusa Azotobacter liquid biofertilizer + <i>Amaranthus viridis</i> WLE	19.00	20.76	41.25	12.00
Pusa Azotobacter liquid biofertilizer + <i>Echinochloa colona</i> WLE	17.33	23.10	51.00	15.33
Pusa Azotobacter liquid biofertilizer + <i>Digera arvensis</i> WLE	19.00	21.566	52.00	15.66
Pusa PSB liquid biofertilizer + <i>Cyperus rotundus</i> WLE	19.33	25.76	62.00	21.33
Pusa PSB liquid biofertilizer + <i>Amaranthus viridis</i> WLE	16.66	27.56	65.00	23.00
Pusa PSB liquid biofertilizer + <i>Echinochloa colona</i> WLE	16.33	23.45	64.00	22.20
Pusa PSB liquid biofertilizer + <i>Digera arvensis</i> WLE	18.00	22.26	41.00	11.90
Potash solubilizing liquid biofertilizer+ <i>Cyperus rotundus</i> WLE	18.00	25.43	54.45	16.40
Potash solubilizing liquid biofertilizer+ <i>Amaranthus viridis</i> WLE	20.33	23.2	50.60	14.85
Potash solubilizing liquid biofertilizer+ <i>Echinochloa colona</i> WLE	23.66	24.86	54.00	16.20
Potash solubilizing liquid biofertilizer+ <i>Digera arvensis</i> WLE	17.00	22.70	42.00	12.50
LSD (p=0.05)	NS	NS	NS	NS

Table 5. Effect of biofertilizers and foliar applied weeds leaves extracts (WLE) (applied alone, without combination) on ashwagandha growth and yield at harvest stage

Treatment	Ashwagandha			
	Root length (cm)	Root diameter (mm)	Root fresh weight/plant (gm)	Root dry weight/plant (gm)
Control (sprayed with distilled water)	14.02	18.79	37.57	11.20
<i>Amaranthus viridis</i> WLE	16.75	23.49	54.52	15.23
<i>Digera arvensis</i> WLE	19.75	24.05	51.21	14.99
<i>Echinochloa colona</i> WLE	17.42	22.19	47.90	13.95
<i>Cyperus rotundus</i> WLE	18.08	22.84	50.26	14.59
Pusa zinc solubilizing biofertilizer	13.39	18.79	43.61	12.18
Pusa Azotobacter	16.09	19.75	42.64203	12.41
Pusa PSB liquid biofertilizer	17.11	22.44	50.31	14.33
Potassium solubilizing bacteria)	15.79	19.23	40.21	11.99
LSD (p=0.05)	NS	NS	NS	NS

Table 6. HPLC profiling for analysis of withanolide content in dry root powder of *Withania somnifera*

Treatment	Withanoside IV (mg/g)	Withanolide A (mg/g)	Withanone (mg/g)
Control (sprayed with distilled water)	ND	0.148	0.038
Pusa zinc solublizing biofertilizer + <i>Cyperus rotundus</i> WLE	ND	0.125	0.024
Pusa zinc solublizing biofertilizer + <i>Amaranthus viridis</i> WLE	ND	0.141	0.018
Pusa zinc solublizing biofertilizer + <i>Echinochloa colona</i> WLE	0.050	0.129	0.022
Pusa zinc solublizing biofertilizer + <i>Digera arvensis</i> WLE	0.007	0.115	0.010
Pusa azotobacter liquid biofertilizer + <i>Cyperus rotundus</i> WLE	0.096	0.181	0.038
Pusa azotobacter liquid biofertilizer + <i>Amaranthus viridis</i> WLE	0.096	0.268	0.044
Pusa azotobacter liquid biofertilizer + <i>Echinochloa colona</i> WLE	0.064	0.168	0.046
Pusa azotobacter liquid biofertilizer + <i>Digera arvensis</i> WLE	0.046	0.134	0.030
Pusa PSB liquid biofertilizer + <i>Cyperus rotundus</i> WLE	0.011	0.141	0.061
Pusa PSB liquid biofertilizer + <i>Amaranthus viridis</i> WLE	0.000	0.118	0.010
Pusa PSB liquid biofertilizer + <i>Echinochloa colona</i> WLE	ND	0.183	0.032
Pusa PSB liquid biofertilizer + <i>Digera arvensis</i> WLE	ND	0.258	0.054
Potash solublizing liquid biofertilizer+ <i>Cyperus rotundus</i> WLE	0.010	0.171	0.015
Potash solublizing liquid biofertilizer+ <i>Amaranthus viridis</i> WLE	0.040	0.200	0.022
Potash solublizing liquid biofertilizer+ <i>Echinochloa colona</i> WLE	ND	0.246	0.028
Potash solublizing liquid biofertilizer+ <i>Digera arvensis</i> WLE	0.017	0.218	0.014

* ND- Not Detected

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