



## RESPONSE OF BIO-ORGANIC NUTRITION ON GROWTH, YIELD AND QUALITY OF ASHWAGANDHA (*Withania somnifera* Dunal.)

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**ABSTRACT :** In an experiment conducted on ashwagandha (*Withania somnifera* Dunal), to study the response of different organic amendments with organic manure (FYM) and bio-fertilizers in relation to plant growth, root yield and quality parameters it was found that the seedlings (5-7 leaf stage) inoculated with *Azospirillum* @ 10<sup>5</sup> or 10<sup>6</sup> CFU resulted a significant increase in plant growth and biomass yield. However, the root and seed yields were observed higher in the plants planted in soil amended with vermi-compost and FYM @ 2 or 3 kg / 1.8 m<sup>2</sup> /plot. The plant height varied significantly among all the treated plots but remain taller (24.80 cm) in plots treated @ 2kg FYM having maximum stem diameter (0.48 cm) at 30 DAP (days after planting) and highest numbers of leaves per plant (438) after reaching 75 DAP followed by seedling treated with *Azospirillum* @ 10<sup>6</sup> CFU. However, the lowest number of leaves per plant (97.4) was observed in the plants grown in plots amended vermi-compost @ 2kg / plot. Number of branches per plant remained highest (51.0) with plants treated with *Azospirillum* @ 10<sup>6</sup> CFU, soil amended with vermi-compost @ 2kg and FYM @ 3 kg/ plot followed by plants grown with *Azospirillum* @ 10<sup>5</sup> CFU (46.0). Whereas, the plants grown in plots amended without FYM produced least number of branches (21.2) even at 75 DAP. Fresh root weight per plant was observed maximum (24.0 g) in the plants amended with vermi-compost @ 2kg and FYM @ 3kg/plot and inoculated with *Azospirillum* @ 10<sup>6</sup> CFU. However, the dry weight of the roots remained highest ( 7.6g /plant) in the plants treated with FYM @ 3kg , vermi-compost @ 2 kg / plot and inoculated with *Azospirillum* @ 10<sup>5</sup> CFU.

**Keywords :** *Withania*, seeds, root, alkaloid, withanolid.

India is known as a treasure house of medicinal and aromatic plants for ages. Medicinal plants have been classified as trees, shrubs, woody perennials, annuals and biennials, and climbers. Their distribution spreads all over India, Baluchistan, and Punjab and in Sind in the Indian sub-continent. Several of these grow abundantly in the drier parts of India ascentily to 5500 ft in Himalayas. Among the perennials, Ashwagandha is very well adapted to grow under subtropical and dry climate in well drained, sandy loam or light red soils having ph of 7.5 to 8.0 with an average rainfall of 600-750 mm. Ashwagandha is being cultivated on large scale as medicinal plant, especially on marginal lands in several districts of Madhya Pradesh, covering an area of more than 4000 hectares (Nigam, 8). In the recent years, cultivation of Ashwagandha has been extended to the areas of Kota in Rajasthan, foot-hills of Punjab and

Himachal Pradesh and Tarai regions of Uttarakhand and Uttar Pradesh. Commercial cultivation, being on priority for high returns needs a sustained and agronomic package for production of economically safe raw material for pharmaceutical industry on large scale. Owing to the increased demand for organic and safe products in the market as ashwagandha roots, leaves and seeds are used in formulation of various *Ayurvedic* and *Unani* medicines, there is prudent to cultivate this crop with an application of Vermicompost and FYM along with a beneficial free-living soil bacteria usually applied as plant growth promoting *Rhizobacteria* or PGPR in the formulation as strains of *Azospirillum*, which lives in close association of plant roots and enhance plant growth by its ability to fix atmospheric nitrogen, production of indole acetic acid, siderophore, nitrate and single molecules resulting in an

increased mineral uptake in the plant roots (Bashan and Holguin, 4).

It has been proven that various strains of *Azospirillum* are capable of promoting the yield of economically important medicinal and aromatic crops in different soils and climatic regions, using various strains of *A. brasilense* and *A. lipoferum* and cultivars of different species of plants (Bashan and Levanony, 3). Several field experiments have revealed a significant increase in different plant growth parameters including grain yield recorded under all levels of treatments of nitrogen due to *Azospirillum* inoculations over those un-inoculated and proved the importance of use of bio-fertilizer (Okon and Labandera-Gonzalez, 10). In *Azospirillum* application has also increased the nitrogen availability in soil through biological nitrogen fixation resulting in the promotion of cell division and synthesis of organic compounds in leaves, ultimately increasing the biomass and root yield (Arul, 2) and consequent increase in total alkaloid content in various plant parts including roots (Srivastava *et al.* 15).

The application of inorganic nutrients may not significantly influence the various economic traits in contradiction due to the fact that biosynthesis of secondary metabolites is under genetic control to influence plant growth and seed yield in various responsive crops including Ashwagandha as reported by Umrao *et al.* (16) but the development of a reliable and consistent inoculation technology determines that the application of *Azospirillum*, FYM and Vermi-compost, interaction beneficial with regards to a biological model for fundamental studies on symbiotic associations between them to have a significant impact in future agricultural production. Therefore, the present experiment was conducted to see and evaluate a response of bio-organic nutrition through a application of FYM, Vermi-compost and *Azospirillum* in Ashwagandha (*Withania somnifera* Dunal.).

## MATERIALS AND METHODS

The present experiment was carried out at the

experimental fields of Ch. Shivnath Singh Shandilya (P.G.) College, Machhra, Meerut (U.P) during the two consecutive years viz. 2005-06 and 2006-07 on Ashwagandha (*Withania somnifera*) cv. Jawahar-20 under the field conditions using of FYM (Farm Yard Manure), Vermi-compost and *Azospirillum*. The experiment was laid out in the factorial RBD under three replications. The ingredients of experimental field were kept in the combinations comprising of FYM, (0 kg/plot (F<sub>0</sub>), 2 kg/plot (F<sub>1</sub>) or 3 kg/plot (F<sub>3</sub>), Vermi-compost 0 kg/plot (V<sub>0</sub>), 2 kg/plot (V<sub>1</sub>) or 3 kg/plot (V<sub>2</sub>) and *Azospirillum* 0 CFU/plot (AZ<sub>0</sub>), 10<sup>5</sup> CFU/plot (AZ<sub>1</sub>) or 10<sup>6</sup> CFU/plot (AZ<sub>2</sub>).

Extraction of total alkaloid and withanaloid content was done employing the 'Gravimetric method taking a sample of 10 g of fine powdered roots in methanol immersed in 'Soxhlet Apparatus' for 6 h. After removal of the methanol from the flask a residue was then filtered with N/2 H<sub>2</sub>SO<sub>4</sub> consequently, five times and brought to 7 pH by adding 20% KOH solution @ 11.5 pH. The resultant residue was washed out to diluted chloroform to purify available alkaloid content and finally the left over chloroform layer was water-distilled by rejecting the aqueous solution. Alkaloid and withanaloid (%) were pre-weighed and dried to a constant weight; and finally recorded as total withanaloid content. The package and practices of cultivation under experimental field were followed as per standard recommendations. The data on plant growth, yield and quality parameters were analyzed for least errors by the methods as suggested by Panse and Sukhatme (11).

## RESULTS AND DISCUSSION

The results obtained from the investigations carried out on ashwagandha cv Jawahar-20 to study the response of bio-organic nutrition employing an admixture of Vermi-compost and FYM @ 2, 3 kg/plot, respectively and plants inoculated with *Azospirillum* @ 10<sup>5</sup> and 10<sup>6</sup> CFU before transplanting revealed that all the plant growth, root yield and quality parameters differed significantly among plots amended with bio-organic

amendments. The plant height was recorded maximum (84.4 cm) in the plants treated with Vermi-compost @ 3 kg/plots, FYM @ 2 kg/plot and inoculated with *Azospirillum* @  $10^6$  CFU and minimum (58.9 cm) in the plants applied with Vermi-compost @ 3 kg and FYM @ 2 kg per plot without inoculation of *Azospirillum* (Table 1). Stem diameter was maximum (0.815 cm) in the plants treated with Vermi-compost @ 3 kg per plot and FYM @ 3 kg/plot and inoculated with *Azospirillum* @  $10^5$  CFU whereas, minimum (0.54 cm) applied with Vermi-compost @ 2 kg/plot without FYM applied and inoculation of *Azospirillum*. Number of leaves/plant was recorded maximum (384.4) in the plants treated with Vermi-compost @ 3 kg/plot, FYM @ 2 kg/plot and an inoculation of plant with *Azospirillum* @  $10^6$  and minimum (160.1) in the plants inoculated with *Azospirillum* @  $10^5$ . However, untreated plants recorded (124.2 leaves), significantly higher than the lower value. Number of branches recorded was maximum (45.6) in the plants treated with Vermi-compost @ 3 kg/plot, FYM @ 2 kg per plot and *Azospirillum* @  $10^5$  inoculation and minimum (21.1) in the plant applied with Vermi-compost @ 3 kg/plot and an inoculation of *Azospirillum* @  $10^6$  CFU without FYM application. Plant canopy ( $\text{cm}^2$ ) was recorded maximum ( $4.165 \text{ cm}^2$ ) in the plants grown after inoculation with *Azospirillum* @  $10^6$  CFU alone and minimum ( $2.075 \text{ cm}^2$ ) in the plants raised in the plots amended with FYM @ 3 kg/plot and an inoculation of plants with *Azospirillum* @  $10^5$ . Leaf area ( $\text{cm}^2$ ) recorded was maximum ( $67.43 \text{ cm}^2$ ) in the plants treated with Vermi-compost @ 3 kg, FYM @ 3 kg each per plot and inoculated with *Azospirillum* @  $10^5$  and minimum ( $33.36 \text{ cm}^2$ ) in plants grown with plots amended with Vermi-compost @ 3 kg/plot and inoculated with *Azospirillum* @  $10^6$  CFU without FYM application. Number of berries/plant was recorded maximum (115.4) in the plants grown in the plots amended with Vermi-compost @ 3 kg/plot, FYM @ 2 kg/plot and inoculated with *Azospirillum* @  $10^5$  CFU whereas, minimum (19.5) was recorded in untreated plants which remained at par (21.2) with

those inoculated with *Azospirillum* @  $10^5$  alone. Number of seeds/berry was recorded maximum (46.8) in plants treated with *Azospirillum* @  $10^5$  alone without the application of admixture of Vermi-compost and FYM and minimum (27.6) in the plants raised in the plots amended with Vermi-compost @ 3 kg/plot, FYM @ 2 kg/plot and inoculation with *Azospirillum* @  $10^5$ . Main root length per (Table 2) plant was noted maximum (20.9 cm) in the plots treated with FYM @ 3 kg/plot and plants inoculated with *Azospirillum* @  $10^6$  CFU without an amendment of the soil plots with Vermi-compost and minimum (12.6 cm) in the plant treated with FYM @ 3 kg/plot and inoculation of plants with *Azospirillum* @  $10^5$ . Number of primary roots/plant was recorded maximum (5.3) in the plants grown in the beds admixed with Vermi-compost @ 3 kg/plot, FYM @ 2 kg/plot and inoculated with *Azospirillum* @  $10^5$  whereas, minimum (1.55) in the plants treated with FYM @ 2 kg/plot and inoculated with *Azospirillum* @  $10^6$ . Number of secondary roots was noted maximum (6.6) in the plants treated with Vermi-compost applied @ 2 kg/plot and inoculated with *Azospirillum* @  $10^6$  without the application of FYM. However, a minimum secondary roots (3.7) were noted for the plants grown in the beds amended with Vermi-compost @ 2 kg/plot and FYM @ 2 kg/plot. Secondary root length was recorded maximum (7.07 cm) in the plants treated with Vermi-compost @ 3 kg/plot and FYM 2 kg/plot and inoculated with *Azospirillum* @  $10^5$  and minimum (3.41 cm) in the plants treated with Vermi-compost @ 2 kg/plot and FYM @ 2 kg/plot.

Fresh root weight/plant (Table 2) was obtained maximum (28 g) in the plants treated with Vermi-compost @ 3 kg/plot and FYM @ 3 kg/plot and an inoculation of *Azospirillum* @  $10^5$  and minimum (11.75 g) with plants administered soil beds with Vermi-compost @ 2 kg/plot alone. Dry root weight/plant was obtained maximum (7.47 g) from the plants grown in beds applied with Vermi-compost @ 3 kg/plot and FYM 2 kg/plot and inoculation of *Azospirillum* @  $10^5$  CFU. Whereas,

**Table 1: Plant growth, flowering and seed yield parameters of Ashwagandha (*Withania somnifera* Dunal.) as influenced by different bio-organic treatments.**

Treatment	Plant height (cm)	Stem diameter (cm)	Number of leaves/plant	Number of branches/plant	Plant canopy (WxL, cm <sup>2</sup> )	Leaf area (cm <sup>2</sup> )	No. of berries/plant	No. of seeds/berry
V <sub>0</sub> F <sub>0</sub> AZ <sub>0</sub>	59.50	0.635	124.20	37.00	3.465	51.53	19.50	29.90
V <sub>0</sub> F <sub>0</sub> AZ <sub>1</sub>	61.50	0.675	160.10	27.80	2.550	59.04	21.20	46.80
V <sub>0</sub> F <sub>0</sub> AZ <sub>2</sub>	64.00	0.740	189.70	36.10	4.165	59.30	43.60	37.30
V <sub>0</sub> F <sub>1</sub> AZ <sub>0</sub>	67.20	0.660	201.00	38.50	3.875	52.43	62.70	33.90
V <sub>0</sub> F <sub>1</sub> AZ <sub>1</sub>	66.80	0.700	239.20	22.70	3.190	50.84	45.70	28.80
V <sub>0</sub> F <sub>1</sub> AZ <sub>2</sub>	67.00	0.675	257.20	41.60	2.980	56.30	32.50	37.20
V <sub>0</sub> F <sub>2</sub> AZ <sub>0</sub>	60.00	0.660	227.60	36.30	3.080	54.22	35.65	33.20
V <sub>0</sub> F <sub>2</sub> AZ <sub>1</sub>	64.40	0.670	236.40	27.80	2.075	40.57	62.00	34.50
V <sub>0</sub> F <sub>2</sub> AZ <sub>2</sub>	65.80	0.615	262.40	26.40	2.955	56.46	80.80	35.80
V <sub>1</sub> F <sub>0</sub> AZ <sub>0</sub>	59.00	0.540	266.30	24.00	2.620	44.93	72.60	28.90
V <sub>1</sub> F <sub>0</sub> AZ <sub>1</sub>	63.50	0.640	267.80	29.00	2.590	51.45	21.30	28.10
V <sub>1</sub> F <sub>0</sub> AZ <sub>2</sub>	69.00	0.690	276.80	30.10	3.806	47.10	35.80	42.10
V <sub>1</sub> F <sub>1</sub> AZ <sub>0</sub>	74.70	0.615	290.20	41.10	2.380	62.28	49.00	28.10
V <sub>1</sub> F <sub>1</sub> AZ <sub>1</sub>	79.10	0.675	315.10	30.40	3.175	54.34	41.50	30.50
V <sub>1</sub> F <sub>1</sub> AZ <sub>2</sub>	80.00	0.730	370.90	30.80	3.455	50.23	37.30	34.20
V <sub>1</sub> F <sub>2</sub> AZ <sub>0</sub>	72.00	0.705	280.20	34.90	3.660	57.73	40.40	37.20
V <sub>1</sub> F <sub>2</sub> AZ <sub>1</sub>	77.50	0.730	365.60	25.70	3.505	52.30	65.60	28.60
V <sub>1</sub> F <sub>2</sub> AZ <sub>2</sub>	72.00	0.685	383.10	21.20	3.560	42.31	46.50	31.60
V <sub>2</sub> F <sub>0</sub> AZ <sub>0</sub>	72.40	0.685	299.30	29.80	3.410	54.52	107.40	29.80
V <sub>2</sub> F <sub>0</sub> AZ <sub>1</sub>	69.90	0.590	302.80	29.00	2.415	40.22	45.00	31.30
V <sub>2</sub> F <sub>0</sub> AZ <sub>2</sub>	71.00	0.635	311.70	21.10	2.085	33.36	88.90	31.50
V <sub>2</sub> F <sub>1</sub> AZ <sub>0</sub>	58.90	0.620	321.90	22.20	2.885	56.22	52.30	35.10
V <sub>2</sub> F <sub>1</sub> AZ <sub>1</sub>	81.00	0.730	378.10	45.60	2.670	62.82	115.40	27.60
V <sub>2</sub> F <sub>1</sub> AZ <sub>2</sub>	84.40	0.780	384.40	40.90	2.755	51.33	90.70	33.50
V <sub>2</sub> F <sub>2</sub> AZ <sub>0</sub>	72.60	0.740	335.00	39.90	4.155	50.86	97.60	32.00
V <sub>2</sub> F <sub>2</sub> AZ <sub>1</sub>	80.20	0.815	365.70	35.40	3.720	67.43	98.50	35.80
V <sub>2</sub> F <sub>2</sub> AZ <sub>2</sub>	79.20	0.740	341.70	36.50	3.150	44.57	75.40	32.00
Mean	70.096	0.680	287.200	31.918	3.123	52.025	58.698	33.159
LSD (5%)	4.899	0.055	40.174	5.342	0.641	6.331	6.821	4.430
P (0.001)	**	**	**	**	**	**	**	**

V<sub>0</sub> = Vermi-compost (Control)V<sub>1</sub> = Vermi-compost (2 kg/plot)V<sub>2</sub> = Vermi-compost (3 kg/plot)F<sub>0</sub> = FYM (Control)F<sub>1</sub> = FYM (2 kg/plot)F<sub>2</sub> = FYM (3 kg/plot)AZ<sub>0</sub> = *Azospirillum* (Control)AZ<sub>1</sub> = *Azospirillum* (10<sup>5</sup> CFU)AZ<sub>2</sub> = *Azospirillum* (10<sup>6</sup> CFU)

minimum (2.9 g) in the plants treated with Vermi-compost @ 3 kg/plot and inoculation of *Azospirillum* @  $10^6$ , without FYM application. Fresh root yield/plot (kg) was recorded maximum (0.534 kg) in the plants treated with application of Vermi-compost 3 kg/plot, alone and minimum (0.344 kg) in the plants applied with FYM @ 2 kg/plot and *Azospirillum* @  $10^6$  CFU. However, untreated plant recorded (0.268 kg) which was significantly higher than the lowest value obtained in the present investigation. Dry root yield/plot was recorded highest and maximum (0.12 kg) in the plants raised in the soil plots amended with Vermi-compost @ 2 kg/plot along with an inoculation of roots with *Azospirillum* @  $10^6$ . However, a minimum (0.05 kg) was obtained in the plants treated with Vermi-compost @ 3 kg/plot and inoculated with *Azospirillum* @  $10^5$  CFU, without applying FYM. Fresh root yield (kg/ha) was obtained maximum (2150 kg/ha) in plants grown in plots treated with FYM @ 2 kg/plot alone and minimum (1075 kg/ha) in the plants in the soil beds amended with Vermi-compost @ 2 kg/plot, alone. Dry root yield/plot (kg/ha) was obtained maximum (690 kg/ha) in the plants grown in the treatments composed of Vermi-compost @ 3 kg/plot and FYM 2 kg/plot inoculated with *Azospirillum* @  $10^6$ . Whereas, a minimum (283 kg/ha) in the plants treated with FYM @ 2 kg/plot and inoculated with *Azospirillum* @  $10^5$ . Fresh and dry root ratio was recorded highest and maximum (5.95) in the plants grown in the plots amended with Vermi-compost @ 3 kg/plot and plant roots inoculated with *Azospirillum* @  $10^6$  and minimum (2.5) in the plants raised with admixing the Vermi-compost @ 2 kg/plot, alone. These findings are in close agreement with those obtained by and Nigam *et al.* (9) and Patidar *et al.* (13) in ashwagandha. It is evident from the findings that the higher application of bio-organic nutrients might have led to the improved production potential of crops determining the plant growth and development (Marschner, 5). The elemental response of available nutrients leading to the complementary effect, might have increased the plant growth

attributes indirectly by taking part in the chlorophyll bio-synthesis process after its association into chlorophyll precursor's glycine as glutamine (Mishra and Srivastava, 6) production in the treated plants as reported by Singh *et al.* (14).

The varying data recorded on root growth and quality parameters were noted significant in our findings. The accumulation of total alkaloid content and withananoid in the roots were significant with an application of vermi-compost, FYM and *Azospirillum* inoculation, expressed remarkable complimentary effects on the traits, which might be due to slower release of nutrients and a long duration of crop. Although a transitional change in the chemical constituents is reported at different harvesting dates (Patel *et al.* 12) but in our results it is harvested once and only on full maturity of the crop owing to the recorded higher values that might be due to an oxidation of starch and its conversion into alkaloids and production of more precursory compound to accumulate more withanaloids in the thicker plants and heavy roots under the different treatments composed of Vermi-compost and FYM @ 3 kg each per plot and growth an inoculation of *Azospirillum* @  $10^5$  CFU in the current investigation. Contrary to these findings, the nitrogen amendments through inorganic source of fertilizers are known to responds poorly as reported by Muthumanickam and Balakrishnamurthy (7). It is however, expressed (Agarwal *et al.*, 1), that a significant influence on increased root length and yield were due to favourable environmental conditions, prevailing during the initial growth, flowering and fruiting stage extending the longer growing period of the crop.

The total alkaloid content (%) in the plant roots was recorded maximum (0.759%) in the plots treated with a mixture of Vermi-compost @ 3 kg/plot and FYM @ 2 kg per plot along with an inoculation with *Azospirillum* @  $10^6$ . However, the minimum alkaloid content (0.39 %) was noted in the plant roots raised with Vermi-compost @ 2 kg/plot, applied alone. However, the untreated plant recorded a content (0.387 %) that remained

**Table 2: Root yield and quality parameters of Ashwagandha (*Withania somnifera* Dunal.) as influenced by different bio-organic treatments.**

Treatment	Main root length (cm)	No of Primary roots/plant	No of secondary roots	Secondary root length (cm)	Fresh root weight /plant (g)	Dry root weight /plant (g)	Fresh root yield /plot (kg)	Dry root yield /plot (kg)	Alkaloid content (%)	With-naloid content (%)
V <sub>0</sub> F <sub>0</sub> AZ <sub>0</sub>	14.30	2.60	6.10	4.18	19.50	4.960	0.268	0.080	0.387	0.308
V <sub>0</sub> F <sub>0</sub> AZ <sub>1</sub>	15.00	2.55	5.20	5.09	20.50	5.195	0.405	0.115	0.457	0.330
V <sub>0</sub> F <sub>0</sub> AZ <sub>2</sub>	16.70	3.90	5.70	6.05	21.50	5.165	0.329	0.095	0.450	0.342
V <sub>0</sub> F <sub>1</sub> AZ <sub>0</sub>	16.30	4.30	6.40	5.43	23.00	5.050	0.470	0.085	0.554	0.368
V <sub>0</sub> F <sub>1</sub> AZ <sub>1</sub>	16.90	4.80	5.35	6.24	17.70	3.250	0.347	0.055	0.584	0.378
V <sub>0</sub> F <sub>1</sub> AZ <sub>2</sub>	16.70	1.55	5.90	4.18	16.50	5.090	0.344	0.085	0.571	0.392
V <sub>0</sub> F <sub>2</sub> AZ <sub>0</sub>	17.20	2.70	5.45	5.12	21.00	5.115	0.397	0.085	0.595	0.418
V <sub>0</sub> F <sub>2</sub> AZ <sub>1</sub>	12.60	2.20	4.90	4.27	14.50	4.725	0.359	0.075	0.621	0.452
V <sub>0</sub> F <sub>2</sub> AZ <sub>2</sub>	20.90	2.90	4.45	6.30	17.00	5.250	0.460	0.095	0.648	0.468
V <sub>1</sub> F <sub>0</sub> AZ <sub>0</sub>	15.60	2.35	4.60	3.53	11.75	4.905	0.430	0.095	0.390	0.322
V <sub>1</sub> F <sub>0</sub> AZ <sub>1</sub>	14.60	3.30	5.15	5.06	18.50	6.090	0.351	0.095	0.433	0.348
V <sub>1</sub> F <sub>0</sub> AZ <sub>2</sub>	17.70	2.65	6.60	6.49	21.50	6.390	0.425	0.120	0.533	0.397
V <sub>1</sub> F <sub>1</sub> AZ <sub>0</sub>	14.80	2.95	3.70	3.41	21.50	5.015	0.424	0.105	0.571	0.404
V <sub>1</sub> F <sub>1</sub> AZ <sub>1</sub>	16.30	2.15	6.30	5.83	20.50	5.285	0.439	0.095	0.624	0.440
V <sub>1</sub> F <sub>1</sub> AZ <sub>2</sub>	14.80	2.30	5.05	4.39	17.50	4.780	0.410	0.085	0.642	0.453
V <sub>1</sub> F <sub>2</sub> AZ <sub>0</sub>	14.70	2.44	4.30	4.25	20.50	4.515	0.456	0.095	0.583	0.470
V <sub>1</sub> F <sub>2</sub> AZ <sub>1</sub>	14.50	4.20	5.05	6.04	15.75	4.670	0.431	0.115	0.645	0.481
V <sub>1</sub> F <sub>2</sub> AZ <sub>2</sub>	15.40	2.10	5.40	4.52	18.50	4.540	0.441	0.075	0.647	0.519
V <sub>2</sub> F <sub>0</sub> AZ <sub>0</sub>	16.50	5.10	4.35	5.91	20.00	5.075	0.534	0.095	0.531	0.330
V <sub>2</sub> F <sub>0</sub> AZ <sub>1</sub>	17.30	2.50	5.25	4.26	19.75	5.125	0.365	0.050	0.585	0.362
V <sub>2</sub> F <sub>0</sub> AZ <sub>2</sub>	14.10	1.75	4.85	6.06	14.75	2.900	0.449	0.085	0.601	0.387
V <sub>2</sub> F <sub>1</sub> AZ <sub>0</sub>	17.30	2.30	4.50	5.30	17.50	4.755	0.472	0.095	0.552	0.449
V <sub>2</sub> F <sub>1</sub> AZ <sub>1</sub>	14.00	5.30	5.70	7.07	17.75	7.470	0.450	0.075	0.725	0.475
V <sub>2</sub> F <sub>1</sub> AZ <sub>2</sub>	15.50	1.70	6.55	5.02	20.50	4.145	0.450	0.085	0.759	0.579
V <sub>2</sub> F <sub>2</sub> AZ <sub>0</sub>	14.56	1.70	5.45	3.62	22.50	5.320	0.507	0.075	0.640	0.469
V <sub>2</sub> F <sub>2</sub> AZ <sub>1</sub>	18.80	2.70	4.80	5.81	28.00	6.130	0.501	0.095	0.688	0.505
V <sub>2</sub> F <sub>2</sub> AZ <sub>2</sub>	15.70	2.30	4.80	3.85	19.50	5.190	0.446	0.075	0.657	0.478
Mean	15.880	2.862	5.253	5.087	19.164	5.040	0.421	0.088	0.580	0.419
LSD (5%)	2.523	0.765	1.156	0.822	3.517	0.732	0.041	0.018	0.118	0.095
P(0.001)	**	**	**	**	**	**	**	**	**	**

V<sub>0</sub> = Vermi-compost (Control)F<sub>0</sub> = FYM (Control)AZ<sub>0</sub> = *Azospirillum* (Control)V<sub>1</sub> = Vermi-compost (2 kg/plot)F<sub>1</sub> = FYM (2 kg/plot)AZ<sub>1</sub> = *Azospirillum* (10<sup>5</sup> CFU)V<sub>2</sub> = Vermi-compost (3 kg/plot)F<sub>2</sub> = FYM (3 kg/plot)AZ<sub>2</sub> = *Azospirillum* (10<sup>6</sup> CFU)

significantly higher than the lowest value among all the treatments in the experiment. The maximum withanoloid content (0.579 %) was observed in the plants grown in the plots amended with an admixture of Vermi-compost @ 3 kg/plot, FYM @ 2 kg per plot and an inoculation of plant roots with *Azospirillum* @  $10^6$  CFU. Whereas, the lowest contents (0.308 %) of withanoloid was obtained from the untreated plants. The quantitative determination of total alkaloid and withanoloid content in the roots have also been worked out.

### REFERENCES

1. Agarwal, Manish., Singh, P. and Agarwal, M.K. (2004). Effect of sowing dates and spacing on yield attributes and root yield of ashwagandha, *Withania somnifera*. *J. Med. and Arom. Plant Sci.*, **26**: 473-474.
2. Arul, Navamani V.J. (2002). Integrated nutrient management of Ashwagandha (*Withania somnifera* Dunal.) for growth, yield and quality. *M.Sc. (Hort.) Thesis*, AC and RI, TNAU, Madurai.
3. Bashan, Y. and Levanony, H. (1990). Current status of *Azospirillum* inoculation technology: *Azospirillum* as a challenge for agriculture. *Can. J. Microbiol.*, **36**: 591-608.
4. Bashan, Y. and Holguin, G. (1997). *Azospirillum* plant relations environmental and physiological advances (1990-1996). *Can. J. Microbiol.*, **43**: 103-121.
5. Marschner, M. (1995). *Mineral nutrition of higher plants*. Edn 2, London Academic Press Ltd.
6. Mishra, S.N. and Srivastava, H.S. (1983). Role of inorganic nitrogen in the synthesis and degradation of chlorophyll and carotenoids in maize leaves. *Biol Plant*, **25**: 21-27.
7. Muthumanickam, D. and Balakrishnamurthy, G. (1999). Optimum stage of harvest for extraction of total Withanolides in ashwagandha (*Withania somnifera* Dunal.). *J. Spices and Arom. Crops*, **8**: 95-96.
8. Nigam, K.B., Rawat, G.S. and Prasad, Bhagwat (1984). Effect of methods of sowing, plant density and fertility level on ashwagandha (*Withania somnifera* Dunal.). *South Indian Hort.*, **32**(6): 356-359.
9. Nigam, K.B., Patidar, H., Kandalkar, V.S. and Pathan, M.A. (1991). A new, promising, pre-release variety, 'WS 20', of ashwagandha (*Withania somnifera*). *Indian J. Agric. Sci.*, **61**(8): 581-82.
10. Okon, Y. and Labandera-Gonzalez, C.A. (1994). Agronomic applications of *Azospirillum*: an evaluation of 20 years worldwide field inoculation. *Soil Biol. Biochem.*, **26**: 1591-1601.
11. Panse, V.G. and Sukhatme, P.V. (1979). Genetics and quantitative characters in relations to plant breeding. *Indian J. Genet.*, **17**: 318-328.
12. Patel, K.V., Patel, D.H., Sushila, Patel, A. and Sriram, S. (2003). Effect of seed rate and crop duration on root yield and quality of ashwagandha (*Withania somnifera*). *J. Med. and Arom. Plant Sci.*, **25**: 54-57.
13. Patidar, H., Kandalkar, V.S. and Nigam, Smita (1990). Estimation of leaf area in asgandh (*Withania somnifera*). *Indian J. Agric. Sci.*, **60**(4): 263-264.
14. Singh, Gopal, Kumar, Ashok, Kumar, Kamalesh, Singh, L.R. and Ram, B. (2005). Management of Seedling and Collar Rot Disease by Fungal Bioagent and Chemical Fungicide in Nursery of Ashwagandha (*Withania somnifera*). In: *Proceedings of National Seminar on Recent Advances in MAPs*. February 15-17, Meerut. P. 83-84.
15. Srivastava, S.K., Iyer, S.S., and Ray, G.K., (1960). Estimation of the total alkaloids of *Withania somnifera* (Dunal). *Indian J. Pharmacy*, **22**(4): 94-95.
16. Umrao, V.K., Tyagi, A.K., Sharma, Vishal, m Malik, Faem, Akbar, P.I. and Ali, Sher (2005). Effect of various levels of FYM & SOM (Sugar Organic Manure) on the vegetative growth and seed yield of Ashwagandha. (*Withania somnifera* Dunal.). In: *Proceedings of National Seminar on Recent Advances in M & APs*. February 15-17, Meerut. P. 78.