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Influence of biofertilizers and plant growth regulators on growth, yield and alkaloid content of Ashwagandha (*Withania somnifera* Dunal.)

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Abstract
Ashwagandha (*Withania somnifera* Dunal.) is a medicinal plant of great values, which came into prominence in recent years. The commercial drug made out from ashwagandha consists of the dried roots, rich in several alkaloids, which have important medicinal values. Therefore, to augment the root yield and alkaloid content an experiment was carried out to study the Influence of biofertilizers and plant growth regulators on growth, yield and alkaloid content of ashwagandha. The treatments were comprised with the seedling root dip of biofertilizers (*Azospirillum* and Phosphobacteria) and combined application of biofertilizers along with the foliar spray of PGR's *viz.*, IBA @ 100 ppm, Ethrel @ 250 ppm, MH @ 500 ppm and CCC @ 2000 ppm at 85, 115 and 145 DAS. The study revealed that the growth, yield and alkaloid content of Ashwagandha were increased favourably by the combined application of biofertilizers along with CCC @ 2000 ppm.

Keywords: biofertilizers, *Withania somnifera* Dunal

Introduction
Ashwagandha (*Withania somnifera* Dunal.) known as Indian Zingseng belongs to the family solanaceae is an important cultivated medicinal crop of India and has been mentioned as an important drug in ancient ayurvedic literature. The root is the usable part (raw drug) having number of alkaloids, out of which withanine and somniferine are important. This crop is also mainly used as an ingredient of medicaments prescribed for curing disability and sexual weakness in the male. Hence it is drawing the attention of the industrialists and farmers because of the increasing demand for its root in pharmaceutical industry. At present, Ashwagandha is being cultivated in Farmer's field with out adopting any scientific technologies for the crop production. Therefore, the full potential of this economically valued medicinal plants could not be realized. Studies made in medicinal plants research in the last two decades have shown that use of PGR's and biofertilizers has resulted in positively. But the research works related to the role of PGR's and biofertilizers on Ashwagandha is scanty. Hence the present investigation was undertaken to study the efficacy of biofertilizers and PGR's on growth, yield and alkaloid content of Ashwagandha.

Materials and Methods
An experiment was carried out in the Department of Horticulture, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India. to study the effect of biofertilizers and plant growth regulators on the growth, yield and alkaloid content of ashwagandha. The trial was laid out in CRD along with treatments namely, the seedling root dip of biofertilizers (*Azospirillum* + Phosphobacteria) @ 500 g ha^{-1} alone (T1), biofertilizers (T1) along with foliar spray of plant growth regulators *viz*., indol-3-butyric acid (IBA) @ 100 ppm (T2), ethrel @ 250 ppm (T3), malic hydrazide (MH) @ 500 ppm (T4), 2(chloroethyl) triammonium chloride (CCC) @ 2000 ppm (T5) and control (T6). Foliar sprays of plant growth regulators were given at 85, 115 and 145 days after sowing (DAS). The concentrations of plant growth regulators have been fixed based on the findings of anon (1997). The spraying was done in the morning with knapsack sprayer till the leaves were adequately wet. The spray was continued on the plants until the excess solution started dripping. The observations were recorded for all the growth and yield parameters of ashwagandha.

Results and Discussion
Growth Components
The influence of biofertilizers and plant growth regulators on growth parameters were aptly

measured in terms of plant height, number of branches, internodal length, number of leaves, leaf area, total chlorophyll content, shoot diameter, herbage yield are presented in the table 1.

The plants treated with biofertilizers + IBA at the rate of 100 ppm recorded maximum plant height (104.11 cm) and internodal length (18.98 cm). This may be due to the effect of IBA, which promoted the vegetative growth by the enhanced cell division and cell elongation through auxin production. Similar observations were recorded by Saffari *et.al.* (2004) in *Rosa damascene* and Mary Haokip *et.al.* (2016) in Coriander. This may be also due to the production of growth substances like auxin in addition to the additional nitrogen fixed by biofertilizers as reported by Chezhyian *et.al.* (2003) in *Pyllanthus amarus.* But in the case of other treatments, the application of plant growth regulators gradually masked the favourable effect of biofertilizers. The application of ethrel, CCC and MH along with biofertilizers reduced the plant height (71.93 cm, 61.03 cm and 49.91 cm) and internodal length (13.74 cm, 11.57 cm and 9.39 cm) than that of untreated plants (78.22 cm and 15.11 cm). These growth retardants suppressed the vegetative growth by their anti-gibberelin action and reduction of apical dominance. This result was in conformity with the findings of Rajangam *et al.* (2006) in *Coleus forskohlii* and Kuncham (2013) in Ashwagandha.

The more number of 42.74 branches and 273.22 leaves with smaller leaf size of 10.30 cm^2 were seen in the biofertilizers + CCC at the rate 2000 ppm applied plants over the untreated plants (9.96,187.26 and 21.09 cm^2)respectively. This may be due to the suppression of shoot growth and increment of photosynthates with more meristametic activity through mobilization of nutrients for the production of more number of primary branches and leaves. This was in agreement with the reports of Saffari *et.al.*(2006) in *Rosa damascene* and Mary Haokip *et.al.* (2016) in Coriander. As well as the addition of biofertilizers fixed the atmospheric nitrogen and induced the growth substances like auxin. The earlier findings of Gopal and Paramaguru (2006) in Senna was in accordance to the present study. The next best in order was the biofertilizers + ethrel application (35.24, 252.42, 16.52cm^2). The MH arrested the apical dominance and triggered the lateral buds.

The higher total chlorophyll content of 2.278 mg per g of leaves was recorded in the treatments of biofertilizers along with CCC followed by ethrel (1.588 mg per g of leaves). This may be due to the higher deepening of green colour and the prevention of chlorophyll degradation by the growth retardants and the 'N' fixed by the bacteria in the rhizosphere was efficiently utilized by the plants through the addition of biofertilizers. Chezhyian *et.al.* (2003) saw similar results in in *Pyllanthus amarus*. Minimum amount of 0.531 mg per g of leaves total chlorophyll was seen in MH treated plants over the control (0.818 mg per g of leaves).

The high amount of herbage yield of 55.75 q ha^{-1} was recorded in biofertilizers +IBA treated plants. This may be due to the induction of apical dominance with more supply of photosynthates and production of auxin, carbohydrates *etc*. this result was in agreement with the reports of Saffari *et.al.* (2004) in *Rosa damascene* and Mary Haokip *et.al.* (2016) in Coriander. This may be also due to the production of phytohormones and increased supply of photosynthates by biofertilizers. This result was confirmed with the findings of Gopal and Paramaguru (2006) in Senna. The herbage yield was increased moderately when plants were treated with CCC (53.25 q ha^{-1}) followed by ethrel (50.68 q ha^{-1}). As MH retarded the shoot growth by inhibition of cell chromosomes, the plants treated with MH gave less herbage yield of 26.51 q ha^{-1} than the untreated plants (46.05 q ha^{-1}).

Table 1: Effect of biofertilizers and growth regulators on growth and herbage yield of ashwagandha

Treatment	Plant height (cm)	No. branches plant $^{-1}$	Inter nodal length (cm)	No. leaves plant $^{-1}$	Leaf area (cm^2)	Total Chlorophyll (mg g^{-1})	Herbage yield (q ha^{-1})
Control	78.22	9.96	15.11	187.26	21.09	0.818	46.05
Azospirillum + Phosphobacteria	92.97	15.33	17.62	213.60	28.13	0.946	48.68
Azospirillum + Phosphobacteria + IBA	104.11	22.20	18.98	232.31	33.21	1.244	55.75
Azospirillum + Phosphobacteria + Ethrel	71.93	35.24	13.74	252.42	16.56	1.588	50.68
Azospirillum + Phosphobacteria + MH	49.91	29.50	9.39	98.82	8.96	0.531	26.51
Azospirillum + Phosphobacteria + CCC	61.03	42.74	11.57	273.22	10.30	2.273	53.25
S.E±.	3.08	2.16	0.60	5.21	2.24	0.034	0.70
CD at 5%	6.21	4.34	1.21	10.42	4.49	0.071	1.49

Yield Components

The influence of biofertilizers and plant growth regulators were also seen in the yield attributed characters *viz.,* number of roots, root length, root diameter, root yield, total alkaloid content and alkaloid yield (table 2).

The application of ethrel at the rate of 250 ppm along with biofertilizers significantly produced with maximum effect of 9.42 number of roots. This may be due the reduction of shoot and root length and induction of phytohormones and increased supply of nutrients to the roots. Application of ethrel over the shoot led to the formation of a substance, which enhanced initiation of the laterals in the roots, in addition to the hormonal control of vascular cambium activation and of secondary vascular tissues of the roots. This has been confirmed by the influence of ethrel in high tuberisation of roots in *Coleus forskohlii* as reported by Rajangam *et al.* (2006) and Kuncham (2013) in Ashwagandha.As well as due to the production of growth substances by biofertilizers. This result coincided with the findings of Sathiyaraj *et al.* (2006) in *Coleus forskohlii*. The next in order was CCC in increasing 7.54 number of roots per plant.There was a reduction in the roots when the plants were treated with MH (1.09) over the untreated plant (2.40).

The higher root length was recorded in biofertilizers +IBA at the rate 100 ppm with the maximum effect of 63.99 cm, which may be due to the external application of auxin. Rajangam *et al.* (2006) in *Coleus forskohlii* and Kuncham (2013) in Ashwagandha observed similar findings. This may be also due to the production of enzymatic root exudate produced by biofertilizers associated with the crop roots. This was in agreement with the findings of Sathiyaraj *et al.* (2006) in *Coleus forskohlii*. The application of CCC and ethrel also moderately increased the root length of 54.58 and 45.19 respectively over the control (24.60).

Root diameter and root yield were more with the maximum effect of 2.58 cm and 9.72 q ha $^{-1}$ when the plants treated with biofertilizers+ CCC followed by ethrel at the rate of 250 ppm (1.63 cm and 7.83 q ha $^{-1}$). This may be due to the reduction of shoot growth and root length and higher uptake of nutrients, which aided in storage of carbohydrates in the roots. Stimulation of cell production in the cambium accompanied by a delay in cell differentiation and increased cell volume of parenchymatous cortical cell and more utilization of photosynthates and starch deposition occurred at a separate stage of development. This was in accordance with the findings of Rajangam *et al.* (2006) in *Coleus forskohlii* and Kuncham (2013) in Ashwagandha. As well as to the production of phytohormones by biofertilizers and more amount of "N" uptake by the tissues. These reports were confirmed with the finding of Sathiyaraj *et al.* (2006) in *Coleus forskohlii*. The MH applied plants decreased the root diameter of 0.77 cm and root yield of 1.86 q ha $^{-1}$ over the untreated plant (0.86 cm and 2.28 q ha $^{-1}$).

The maximum alkaloid content and alkaloid yield (1.402 per cent and 13.63 kg ha^{-1}) was registered when the plants were treated with biofertilizers and CCC. Higher rate of production of both carbohydrate and amino acid by CCC was likely to facilitate the higher production of alkaloids by providing necessary precursors. This result was in accordance with finding of Rajangam *et al.* (2006) in *Coleus forskohlii* and Kuncham (2013) in Ashwagandha and also the combination of biofertilizers, which favourably increased the activity of plant cells and produced the more amount of carbohydrates like substances. The application of MH was found to be reducing the total alkaloid content and alkaloid yield (0.431 per cent and 0.80 kg ha^{-1}) in roots over the control (0.452 per cent and 1.03 kg ha^{-1}) by inhibiting and interfering with the plant metabolic process. Application of other growth regulators had no effect on the alkaloid content in ashwagandha

It could be inferred from the study, among the application of biofertilizers and plant growth regulators treatments, biofertilizers + CCC at the rate 2000 ppm favourably influenced the growth and yield components of Ashwagandha. In terms of root yield, total alkaloid content and alkaloid yield, the application of CCC at the rate 2000 ppm along with Biofertilizers had very significant effect whereas ethrel at the rate 250 ppm had moderately enhanced the root yield.

Table 2: Effect of biofertilizers and growth regulators on root parameters, root and alkaloid yield of ashwagandha

Treatment	No. Roots plant^{-1}	Root length (cm)	Root Diameter (cm)	Root yield (q ha^{-1})	Total alkaloid content (%)	Alkaloid yield (kg ha^{-1})
Control	2.40	24.60	0.86	2.28	0.452	1.03
Azospirillum + Phosphobacteria	3.69	38.16	1.14	4.03	0.463	2.48
Azospirillum + Phosphobacteria + IBA	5.51	63.99	1.20	5.63	0.478	2.69
Azospirillum + Phosphobacteria + Ethrel	9.42	45.19	1.63	7.83	0.592	4.64
Azospirillum + Phosphobacteria + MH	1.09	19.22	0.77	1.86	0.431	0.80
Azospirillum + Phosphobacteria + CCC	7.54	54.58	2.58	9.72	1.402	13.63
S.Em.±	0.64	2.61	0.04	0.78	0.324	0.48
CD at 5%	1.29	5.23	0.08	1.55	0.651	1.01

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