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

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ABSTRACT

The study revealed that the combination of Azospirillum along with CCC at the rate of 2000 ppm retarded the plant height (59.23 cm) and simultaneously increased the root growth such as root diameter (2.54 cm) and root yield (9.18 q ha^{-1}). Similarly, the ethrel at the rate of 250 ppm retarded the plant height (69.04 cm) and produced more number of roots (8.98) it had resulted in moderate root yield (7.68 q ha^{-1}). IBA at the rate of 100 ppm increased the plant height (102.98 cm) and root length (60.21 cm) with moderate root yield (5.59 q ha^{-1}) whereas; MH at the rate of 500 ppm had the detrimental effect on the plant growth and root yield. The total alkaloid content and alkaloid yield of 1.362 per cent and 12.50 kg ha^{-1} were significantly enhanced by the application of CCC at the rate of 2000 ppm.

Keywords: Ashwagandha (*Withania somnifera* Dunal.), *Azospirillum*, plant growth regulators

Introduction

Ashwagandha is an important medicinal crop used in different medicinal preparations since ancient times. The pharmacological activity of root is attributed to the presence of several alkaloids such as withanine and somniferine. The dried roots of ashwagandha are widely used in various preparations of Indian System of Medicine and Homeopathy to cure several diseases like leprosy, nervous disorders, and sexual weakness in the male. Due to the demand for ashwagandha roots in recent times and considering the future demand, there exists much scope for extensive cultivation of this crop. 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. Among the advanced production technologies, biofertilizers and plant growth regulators are more effective in recent days.

So far no work has been made to study the effect of biofertilizers and plant growth regulators on this crop. Hence, the present investigation was undertaken to study the effect of *Azospirillum* and plant growth regulators 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 *Azospirillum* @ 500 g ha^{-1} alone (T_1), *Azospirillum* (T_1) along with foliar spray of plant growth regulators *viz*., indol-3-butyric acid (IBA) @ 100 ppm (T_2), ethrel @ 250 ppm (T_3), malic hydrazide (MH) @ 500 ppm (T_4), 2(chloroethyl) tri ammonium chloride (CCC) @ 2000 ppm (T_5) and control (T_6). 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) [1]. 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 *Azospirillum* 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.

Table 1: Effect of *Azospirillum* 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	88.46	14.82	17.19	206.42	26.94	0.926	48.66
Azospirillum + IBA	102.98	20.84	18.63	230.67	33.07	1.236	55.72
Azospirillum + Ethrel	69.04	34.69	13.59	249.20	16.13	1.563	50.72
Azospirillum + MH	47.27	27.48	9.06	95.94	8.34	0.526	26.33
Azospirillum + CCC	59.23	41.92	11.43	270.39	10.24	2.268	52.81
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

The plants treated with *Azospirillum* + IBA at the rate of 100 ppm recorded maximum plant height (102.98 cm) and internodal length (18.63 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. Saffari *et al.* (2004) [5] had recorded similar observation in *Rosa damascena*. This may be also due to the production of growth substances like auxin in addition to the additional nitrogen fixed by *Azospirillum* as reported by Chezhyian *et al.* (2003) [2] in *Phyllanthus 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 *Azospirillum* reduced the plant height (69.04 cm, 59.23 cm and 47.27 cm) and internodal length (13.59cm, 11.43 cm and 9.06 cm) than that of untreated plants (78.22 cm and 15.11 cm). These growth retardants suppressed the vegetative growth by their anti-gibberellin action and reduction of apical dominance. This result was in conformity with the findings of Rajangam *et al.* (2006) [4] in *Coleus forskohlii*.

The more number of 41.92 branches and 270.39 leaves with smaller leaf size of 10.24 cm^2 were seen in the *Azospirillum* + 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 meristematic 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.* (2004) [5] in *Rosa damascena*. As well as the addition of *Azospirillum* fixed the atmospheric nitrogen and induced the growth substances like auxin. The earlier findings of Gopal and Paramaguru (2006) [3] in Senna was in accordance to the present study. The next best in order was the *Azospirillum*+ethrel application (39.69, 249.20, 16.13 cm^2). The MH arrested the apical dominance and triggered the lateral buds.

The higher total chlorophyll content of 2.268 mg per g of leaves was recorded in the treatments of *Azospirillum* along with CCC followed by ethrel (1.563 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 *Azospirillum*. Chezhyian *et al.* (2003) [2] saw similar results in in *Phyllanthus amarus*. Minimum amount of 0.526 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 55.72 q ha^{-1} was recorded in *Azospirillum* +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) [5] in *Rosa damascena*. This may be also due to the production of phytohormones and increased supply of photosynthates by *Azospirillum*. This result was confirmed with the findings of Gopal and Paramaguru (2006) [3] in Senna. The herbage yield was increased moderately when plants were treated with CCC (52.81 q ha^{-1}) followed by ethrel (50.45 q ha^{-1}). As MH retarded the shoot growth by inhibition of cell chromosomes, the plants treated with MH gave less herbage yield of 26.33 q ha^{-1} than the untreated plants (46.05 q ha^{-1}).

Yield Components

The influence of *Azospirillum* 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).

Table 2: Influence of *Azospirillum* and growth regulators on root parameters and root yield of ashwagandha.

Treatment	No. roots plant $^{-1}$	Root length (cm)	Root dia meter (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	3.76	29.96	0.95	3.54	0.458	1.62
Azospirillum + IBA	5.30	60.21	1.16	5.59	0.476	2.66
Azospirillum + Ethrel	8.98	41.28	1.59	7.68	0.589	4.52
Azospirillum + MH	1.06	16.47	0.75	1.58	0.429	0.67
Azospirillum + CCC	7.03	51.28	2.54	9.18	1.362	12.50
S.E.±	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

The application of ethrel at the rate of 250 ppm along with *Azospirillum* significantly produced with maximum effect of 8.98 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) [4]. As well as due to the production of growth substances by *Azospirillum*. This result coincided with the findings of Sathiyaraj *et al* (2006) [6] in *Coleus forskohlii*. The next in order was CCC in increasing 7.03 number of roots per plant. There was a reduction in the roots when the plants were treated with MH (1.06) over the untreated plant (2.40).

The higher root length was recorded in *Azospirillum* +IBA at the rate 100 ppm with the maximum effect of 60.21 cm, which may be due to the external application of auxin. Rajangam *et al.* (2006) [4] in *Coleus forskohlii* observed similar findings. This may be also due to the production of enzymatic root exudate produced by *Azospirillum* associated with the crop roots. This was in agreement with the findings of Sathiyaraj *et al* (2006) [6] in *Coleus forskohlii*. The application of CCC and ethrel also moderately increased the root length of 51.28 and 41.28 respectively over the control (24.60).

Root diameter and root yield were more with the maximum effect of 2.54 cm and 9.18 q ha $^{-1}$ when the plants treated with *Azospirillum*+ CCC followed by ethrel at the rate of 250 ppm (1.59 cm and 7.68 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) [4] in *Coleus forskohlii*. As well as to the production of phytohormones by *Azospirillum* and more amount of "N" uptake by the tissues. These reports were confirmed with the finding of Sathiyaraj *et al* (2006) [6] in *Coleus forskohlii*. The MH applied plants decreased the root diameter of 0.75 cm and root yield of 1.58 q ha $^{-1}$ over the untreated plant (0.86 cm and 2.28 q ha $^{-1}$).

The maximum alkaloid content and alkaloid yield (1.362 per cent and 12.50 kg ha^{-1}) was registered when the plants were treated with *Azospirillum* 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) [4] in *Coleus forskohlii* and also the combination of *Azospirillum*, which favorable 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 (0.429 per cent and 0.68 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 *Azospirillum* and plant growth regulators treatments, *Azospirillum* + CCC at the rate 2000 ppm favourably influence 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 *Azospirillum* had very significant effect whereas ethrel at the rate 250 ppm had moderately enhanced the root yield.

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