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International Journal of Current Research in Life Sciences
Vol. 07, No. 02, pp.1006-1008, February, 2018

ISSN: 2319-9490

RESEARCH ARTICLE

EFFECT OF RHIZOBACTERIAL INOCULATION ON YIELD AND QUALITY OF ASHWAGANDHA

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Received 08th December, 2017; Accepted 24th January, 2018; Published Online 28th February, 2018

ABSTRACT

The present study indicated higher shoot and root length, dry matter, yield and alkaloid content of ashwagandha, when the mixed inoculant of *Azospirillum, Azotobacter, Bacillus* and *Pseudomonas* was applied. The free-living plant growth-promoting rhizobacteria (PGPR) can be used in a variety of ways to increase the plant growth. The addition of PGPR increased the germination rate, root growth, leaf area, chlorophyll content, magnesium content, nitrogen content, protein content, hydraulic activity, tolerance to drought, shoot and root weights, and delayed leaf senescence which reflected in higher grain yield.

Key words: Ashwagandha, *Azospirillum, Azotobacter, Bacillus* and *Pseudomonas.*

Citation: Rajasekar, S., Elango, R., Vinoth Kumar P. and Udhayaraja, P. 2018. "Isolation and Purification of Water Soluble Polysaccharide from *Pleurotus djamor* var. roseus corner" *International Journal of Current Research in Life Sciences*, 7, (02), 1006-1008.

INTRODUCTION

In India, the use of medicinal plants to cure specific ailments has been in vogue from ancient times. More than two thousand medicinal plant species have been reported in India and it occupies the top position in the export of medicinal plants. In spite of the ever growing demand for medicinal plants in pharmaceutical and phytochemical industries they are still collected from their natural habitats. (Gilani and Atta-ur-Rahman; 2005; Mukherjee and Wahile, 2006). The natural resources how-so-ever large are bound to diminish and time has therefore come to bring these plants under plough to meet the rising demand of the resultant product. Mediculture, the scientific cropping of important medicinal plants has become the need of the day to improve the productivity and quality of these medicinal plants. The Chinese book on roots and grasses "Pen T'Sao," written by Emperor Shen Nung circa 2500 BC, treats 365 drugs (dried parts of medicinal plants), many of which are used even nowadays such as the following:

Rhei rhisoma**,** camphor, Theae folium, Podophyllum**,** the great yellow gentian, ginseng, jimson weed, cinnamon bark, and ephedra (Bottcher, 1965; Wiart, 2006).The Indian holy books Vedas mention treatment with plants, which are abundant in that country.

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Numerous spice plants used even today originate from India: nutmeg, pepper, clove, etc (Tucakov, 1971). Ashwagandha (Latin: *Withania somnifera*), also known as winter cherry or Indian ginseng is both a tonic and a sedative due to its adaptogenic properties. Withania refers to the plants primary extract and somnifera literally means "sleep-inducing" (Ven Murthy *et al*., 2010). Ashwagandha is a dryland medicinal crop having tremendous marketing potential owing to demand of its roots to the tune of 7000 tonnes and estimated production of 1500 tonnes (Umadevi *et al.,* 2012). The most important pharmacological use of ashwagandha is as adaptogen with antistress antioxidant, antitumor, anti-inflammatory, mind boosting and has rejuvenating properties (Khanna *et al.,* 2006; Kulkarni and Dhir, 2008). Ashwagandha is a traditional medicinal plant is cultivated in different parts of Tamil Nadu. Because of its medicinal value and alkaloid content there is a great demand for the crop leading to an intensification of its cultivation. The knowledge on the use of various agrotechniques to increase the yield and quality of ashwagandha is inadequate. The rhizobiocoenosis is an important biological process that plays a major role in satisfying the nutritional requirement of these crops. Therefore, this study was aimed at investigating the potential of bioinoculants to sustain the cultivation of ashwagandha in a scientific way.

MATERIALS AND METHODS

Preparation of Pots and Seed Inoculation

A pot culture experiment was conducted at the Department of Microbiology, Annamalai University, and Chidambaram to study the effect of combined inoculation of rhizobacteria on growth, yield and quality of ashwagandha (var. Jawahar 20). The rhizobacterial isolates *viz*., *Azospirillum lipoferum*-AAz-1, *Azotobacter*-AAt-13, *Bacillus*-APb-19 and *Pseudomonas fluorescens*-APf-5 were prepared as carrier based inoculants used for this study. The pots were filled with potting mixture (soil + sand + FYM) and the rhizobacteria treated seeds were sown at 25 seeds per pot and finally 5 seedlings were maintained. The experiment was conducted in completely randomized block design with three replications.

Collection and Processing of Plant Material

The total number of fruits produced by the plant was counted and fruit yield was expressed in number plant^{-1}. The fully ripe berries were harvested and dried in the shade. The seeds were extracted from the dried berries by gently rubbing against cement floor. The extracted seeds were cleaned by winnowing, and expressed as number of seeds per fruit.

Preparation of Plant Extract

The total alkaloid content of roots was estimated by adopting the method suggested by Srivastava and Iyer (1960). One gram of powdered root material was extracted with a mixture of ether: ethanol (4:1) for 24 h. The extract was shaken with 25 ml of 5.0 per cent H_2SO_4 thrice (3 x 25 ml). Diluted ammonia solution (1:10) was added to the pooled extract until it became alkaline. It was then extracted with 20 ml of chloroform thrice until extraction of alkaloids was complete. The chloroform extract was washed with 5.0 ml of distilled water and filtered through pre weighed filter paper. Two ml of absolute alcohol was added to the residue and evaporated. The dry weight of the filter paper with residue was estimated and the total alkaloid was estimated by subtracting the filter paper weight and expressed as percentage on dry weight of sample.

RESULTS AND DISCUSSION

In various treatments the number of fruits per plant and number of seeds per fruit in ashwagandha varied from 64.54 to 95.36 and 17.00 to 22.25 respectively. Among the individual inoculations, *Azospirillum lipoferum*-AAz-1 recorded maximum number of fruits and seeds (81.66 and 18.92 respectively), whereas the uninoculated control recorded only 64.54 and 17.00 number of fruits and seeds respectively. Combined application of all the rhizobacterial isolates *viz*., *Azospirillum lipoferum*-AAz-1, *Azotobacter*-AAt-13, *Bacillus*-APb-19 and *Pseudomonas fluorescens*-APf-5 increased the number of fruits plant^{-1} (95.36) and seeds fruit^{-1} (22.25) (Table-1). In contrast to the chlorophyll and protein content, the alkaloid content increased with the age of the crop. Inoculation with rhizobacteria either alone or in various combinations significantly increased the alkaloid content of ashwagandha roots (Table- 2). Among the treatments, combined inoculation of *Azospirillum lipoferum*-AAz-1, *Azotobacter* -AAt-13, *Bacillus*-APb-19 and *Pseudomonas fluorescens*-APf-5 recorded maximum total alkaloid content (1.42 per cent) in ashwagandha roots followed by the triple inoculation of *Azospirillum lipoferum*-AAz-1, *Bacillus*-APb-19 and *Pseudomonas fluorescens*-APf-19 (1.29 per cent) on 180 DAI.

Table 1. Effect of rhizobacterial inoculation on fruit and seed yield of ashwagandha (var. Jawahar -20)

Treatments	Fruits (No. Plant^{-1})	Seeds (No. Fruit^{-1})
T_1 – *Azospirillum* (AAz-1)	81.66	18.92
T_2 – *Azotobacter* (AAt-13)	68.76	18.18
T_3 – *Bacillus* (APb-19)	66.85	18.00
T_4 – *Pseudomonas* (APf-5)	79.33	18.73
T_5 – T1 + T2	84.34	19.00
T_6 – T1 +T3+T4	90.65	20.63
T_7 – T2 +T3+T4	88.76	19.83
T_8 – T1+T2+T3	86.00	19.16
T_9 – T1+T2+T3+T4	95.36	22.25
T_{10} – Uninoculated control	64.54	17.00
SEd	7.14	1.72
CD (P=0.05)	14.89	3.59

Table 2. Effect of rhizobacterial inoculation on total alkaloid content of ashwagandha (var. Jawahar 20) roots

Treatments	Total alkaloid (%)	Total alkaloid yield (mg plant^{-1})
T_1 – *Azospirillum* (AAz-1)	1.18	56
T_2 – *Azotobacter* (AAt-13)	1.12	48
T_3 – *Bacillus* (APb-19)	1.13	49
T_4 – *Pseudomonas* (APf-5)	1.15	51
T_5 – T1 + T2	1.20	59
T_6 – T1 +T3+T4	1.29	71
T_7 – T2 +T3+T4	1.26	67
T_8 – T1+T2+T3	1.23	62
T_9 – T1+T2+T3+T4	1.42	87
T_{10} – Uninoculated control	1.10	44
SEd	0.10	5.33
CD (P=0.05)	0.22	11.13

In the present investigation, the inoculation of rhizobacteria enhanced the growth and yield parameters of ashwagandha. Inoculation with various rhizobacteria enhanced the root elongation and proliferation and this might be due to the production of IAA and GA by these organisms. Okon and Kapulnik (1986) demonstrated that *Azospirillum* inoculation enhanced the root elongation; root branching, root surface area and root dry weight in crops. *Pseudomonas* strains promoted wheat growth in terms of root and shoot length and weight (Srivastava *et al*., 1999). The inoculated PGPR strains usually have been found to increase the root length and root biomass (Yan *et al.,* 2003; Chakraborty *et al*., 2003 and Khalid *et al*., 2004) and this better developed root system may increase the mineral uptake in plants. The stimulatory effects of this PGPR strains on the yield and growth of these crops were attributed to the N_2 fixation ability, plant growth regulator production and phosphate solubilizing capacity (Cakmakci *et al.,* 2007; Lucy *et al*., 2004).

Summary

The rhizobacterial inoculation positively influenced the germination, vigour index, shoot and root length, biomass, dry matter production, root and alkaloid yield of ashwagandha. Inoculation of *Azospirillumlipoferum*-AAz-1, *Azotobacter*-AAt-13, *Bacillus*-APb-19 and *Pseudomonasfluorescens*-APf-5 as combined inoculant recorded the maximum growth, fruit, seed and alkaloid yield of ashwagandha.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest: The authors declare that there is no conflict of interest.

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