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International Journal of Current Research in Life Sciences
Vol. 07, No. 04, pp.1467-1468, April, 2018

ISSN: 2319-9490

RESEARCH ARTICLE

STUDIES ON THE EFFECT OF BIOFERTILIZERS AS SINGLE AND CONSORTIUM INOCULANT ON THE PROTEIN CONTENT OF *CATHARANTHUS ROESUS*

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Received 24th February, 2018; Accepted 30th March, 2018; Published Online 06th April, 2018

ABSTRACT

Periwinkle, Catharanthus *roseus* (L.) G.Don, Synonym (*Vinca rosea)* is a tropical perennial plant, belonging to apocynaceae family. The main reason of great interest in periwinkle is its ability to synthesize a wide range of terepenoid indole al kaloids (TIAs) which are valued for their wide spectrum of pharmaceutical effects. The plant contains more than 100 alkaloid, distributed in all parts of plant but in different proportions. Total alkaloid concentrations in root amount to 2-3% and sometimes reaches upto 9% in fibrous roots. The root alkaloid is ajmalicine. Use of biofertilizers containing beneficial microorganisms are known to improve plant growth through the supply of plant nutrients and releasing growth regulators and pathogen inhibitor compounds and also increases the yield . A pot culture experiment was conducted to study the inoculation of Biofertilizer as single and consortium inoculant (*A.lipoferum, A.chrococcum, B.megaterium, P.fluorescens*) shoot weight, root yield, protein content, of C.*roseus roesea.*

Key words: Catharanthus roseus, Biofertilizer, Protein.

Citation: Rajasekar, S. and Thaiyalnayagi, S. 2018. "Studies on the effect of bio fertilizers as single and consortium inoculant on the protein content of *catharanthus roesus*" *International Journal of Current Research in Life Sciences*, 7, (04), 1467-1468.

INTRODUCTION

Periwinkle *Catharanthus roseus* (L.) G. Don, synonym, (*Vinca rosea* L., *Lochnera rosea* Reich.) is a tropical perennial plant, native to Madagascar, belonging to apocynaceae family. Presently, it is grown in many tropical and subtropical regions. The main reason of a great interest in *Catharanthus roseus* is its ability to synthesize a wide range of terepenoid indole alkoloids (TIAs) which are valued for their wide spectrum of pharmaceutical effects. Three types of actions are particularly important, diuretic (vindoline, catharanthine), anti hypertensive (ajmalicine, serpentine) and anticancer (Vinblastine and vincristine). The plant contains more than 100 alkaloids, distributed in all parts of plant, but in different proportions. Total alkaloid concentration in root amounts to 2-3% and sometimes reaches upto 9% in fibrous roots, whereas leafs contains 1 per cent. A very small amount of these compounds was found in flowers (Barabara Lata, 2007).

MATERIALS AND METHODS

A pot culture experiment was conducted in Sribharathi Arts and Science College, Kaikkurichi pudukkottai.

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To study the effect of inoculant as consortium of plant growth promoting bacteria on plant growth and root alkaloid of *C. roseus* rosea. The plant growth promoting bacterial isolates of *A. lipoferum* CAZS-4, *A. chroococcum* CAZB-1, *B. megaterium* CPB-18, and *P.fluorescens* CPF-14 were prepared as carrier based inoculants and used in this study.

Preparation of pots and seed inoculation

The cement pots of size 1' x 2' x2' filled with land soil and sand in the ratio of 1:1. The seeds of *C. roseus* var. 'rosea and alba' varieties were surface sterilized with 80 per cent ethanol and 0.1 per cent mercuric chloride and washed the seeds with sterile distilled water for 3 to 4 times. The seeds were mixed with carrier based plant growth promoting bacteria, either as individual organisms and consortium of organisms separately having a cell load of $10x10^9$ cells m^{-1} for 30 min. and shade dried. After shade drying, the seeds were sown @ 25 seeds per pot and finally five seeds were maintained. A control pot without inoculation was also maintained. The experiment was conducted in completely randomized block design with three replications.

The treatments are as follows

- T_1-*Azospirillum lipoferum* - CAZS-4
- T_2-*Azotobacter chroococcum* - CAZB-1
- T_3-*Bacillus megaterium* – CPB-18

- T_4-*Pseudomonas fluorescens* - CPF-14
- T_5-Consortium ($T_1 + T_2 + T_3 + T_4$)
- T_6-Uninoculated control

Biometric observations like shoot dry weight, root dry weight and biochemical properties like total chlorophyll content, protein content, alkaloid content, were recorded at 90, 120, 150 and 180 DAS were recorded.

Estimation of protein content

Bradford's reagent

One hundred mg Coomassie Brilliant Blue G250 was dissolved in 50 ml of 95 per cent ethanol and mixed with 100 ml of concentrated phosphoric acid. The volume was made up to 200 ml with distilled water. The reagent was stored at 4ºC. This was diluted five times with distilled water prior to use. One gram fresh leaf sample was extracted with 2 ml of 0.1 M phosphate buffer (pH 7.0). The extract was centrifuged at 5000 g for 10 min. at room temperature. To 0.1 ml of clear supernatant, 5.0 ml of Bradford's reagent was added. The blue colour developed was read at 595 nm in Elico-Spectrophotometer. From the standard graph prepared using known quantities of Bovine serum albumin over a concentration ranging from 1 to 100 µg ml^{-1}, the protein concentration of leaf sample was estimated and expressed as mg g^{-1} fresh weight of sample.

Table 4. Effect of PGPB consortium inoculation on the Protein content of *Catharanthus roseus*

Treatments	*Protein content of *Catharanthus roseus*)			
	90 DAS	120 DAS	150 DAS	180 DAS
T_1 – *Azospirillum*	42.63	46.84	42.66	39.46
T_2 – *Azotobacter*	41.50	44.00	41.96	38.14
T_3 – *Bacillus*	40.50	42.86	40.60	37.54
T_4 – *Pseudomonas*	42.00	45.50	41.56	38.76
T_5 – Consortium ($T_1 + T_2 + T_3 + T_4$)	46.36	51.42	49.44	46.84
T_6 - Uninoculated control	40.00	41.20	39.00	36.00
SEd	0.273	0.617	0.499	0.272
CD (P=0.05)	1.549	1.241	1.004	0.547

RESULTD AND DISCUSSION

Pot culture experiment to assess the effectiveness of PGPB consortium versus single inoculant preparations on the growth of periwinkle revealed that the consortium effect was better than the single inoculant effect. protein content, of *Catharanthus roseus* significantly increased with seed inoculation of PGPB strains as single inoculant and as consortium preparations. The increase in growth parameters is directly related to increased content The previous study conducted with *P. fluorescens* showed positive effect on the root initiation and root proliferation due to phytohormones, produced (IAA and GA_3) in the rhizosphere of black pepper (Dibypaul and Sarma, 2006). Co-culture of microbes performed better than their individual microbes. The combination of bacteria interacts with each other synergistically, provide nutrients, remove inhibitory substances and stimulate each other through physical and biochemical activities. Co-inoculation of PGPB with different beneficial properties may be the future trend for biofertilizer application to enable sustainable production (Han and Lee, 2005).

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