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ENVIRONMENT FRIENDLY STRATEGY TO INCREASE ANTIOXIDANT CONTENT AND PRODUCE OF *SAUROPUS ANDROGYNUS*

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ABSTRACT
Sauropus androgynus is an important green leafy vegetable used in South India and is a rich source of antioxidants. A field trial was conducted wherein Sauropus androgynus was treated with biofertilisers Azospirillum, Bacillus megaterium, farm yard manure (FYM), vermicompost, combined treatment of all and compared with the control. The results obtained were analyzed for growth, enzymic and nonenzymic antioxidants.The study revealed that FYM treated plants showed significant increase in shoot length and number of leaves. There was a significant increase in non-enzymic antioxidants viz., carotenoids, ascorbic acid and total phenolics in FYM treated plants. Among enzymic antioxidants highest peroxidase activity and catalase activity was recorded in combined treatment. FYM treated plants also recorded maximum catalse activity.

INTRODUCTION

Sauropus androgynus belonging to family Phyllanthaceae is commonly called as multivitamin plant, tropical asparagus, star gooseberry, sweet leaf bush, chakkerumuni. It is also a rich source of antioxidants. In Karnataka it is called as Chakramuni soppu and is used in making many delicacies and leaves are eaten raw as it has a nutty taste.Reactive oxygen species are produced continuously in the cells as by-products of metabolism and cause several diseases like diabetes, cancer, inflammation etc (Josh and Janardhanan, 2000; Gomes *et al.,* 2001). Dietary antioxidants found in fruits and vegetables can be used to protect cells against oxidative damage caused by reactive oxygen species and prevent chronic diseases such as cancer, cardiovascular disease and diabetes (Podsedek, 2005).The fruit and vegetable antioxidant help in reducing the risk of these degenerative diseases (Ames *et al.,* 1993). The major antioxidants of plants are compounds like vitamin C, vitamin E, carotenoids and phenols which are non-enzymic antioxidants. Enzymic antioxidants may include catalase, peroxidase, superoxide dismutase etc. These antioxidants scavenge free radicals and inhibit the chain initiation or break the chain propagation (Shi *et al*., 2001). Carotenoids are one of the important antioxidants and have been shown to reduce the risk of cancer and protect against heart disease. Carotenoids trap reactive oxygen species from sunlight, break free radical chain reactions and prevent oxidative damage. Ascorbic acid lowers the chances of developing high blood pressure, cataracts, heart disease and even cancer. Phenolic compounds exhibit cellular defense mechanism in atherogenesis and cancer. As with the chemical antioxidants, cells are protected against oxidative stress by an interacting network of antioxidant enzymes (Sies and Helmut, 1997; Baillie *et al.,* 2009). As *Sauropus androgynus* is rich in antioxidants (Padma and Anitha, 2005; Naruman *et al.*, 2008) and very meager report on the cultivation practices of the plant. The objective was to explore the possibilities of supplementing chemical fertilizers with organic inputs, biofertilizers of microbial origin to grow and increase the antioxidant content of *Sauropus androgynus*.

MATERIALS AND METHODS

The medicinal plant *Sauropus androgynus* was procured from Dhanvanthri Vana, Bangalore University, Bangalore and three months old cuttings were planted in Vedic Bio-farm located near Bangalore. On land trials were conducted in replicates of five. Biofertilisers viz., *Azospirillum*, *Bacillus megaterium*, vermicompost were brought from Biotechnology Department, Government of Karnataka, Hulimavu, Bangalore. FYM containing decomposed farm waste and cow dung was taken from the farm. The cuttings were planted at a distance of 1.5 ft.Then the cuttings were treated with a free nitrogen fixer, *Azospirillum*, phosphate solubiliser, *Bacillus megaterium*, vermicompost, Farm Yard Manure, combined treatment of all and compared with control. Treatment was given once in 30

days for 90 days.

Growth parameters

At 90 days, shoot length, number of branches and number of leaves were recorded. Mean of five replicates was tabulated.

Non-enzymic anti-oxidants

Estimation of Vitamin A (Total carotenoids)

Fresh leaf tissue was homogenized using acetone adding a pinch of clean, fine sand. The extract was centrifuged and supernatant was collected. Finally absorbance was read at 440nm and total carotenoids content was calculated following the method given by Ikan (1969).

Estimation of Vitamin C (Ascorbic acid)

Vitamin C was determined by the method given by Varley *et al*. (1984). Required quantity of tissue was homogenized in distilled water and filtered. To the filtrate glacial acetic acid was added and titrated against DCPIP (Dichlorophenol indophenol). Standard titration was done by using ascorbic acid and distilled water as blank. Then quantity of ascorbic acid was determined

Estimation of total phenols

Total phenol content was estimated by FC (Folin-Ciocalteu) method (Malick and Singh, 1980). A known quantity of the sample was ground in mortar and pestle in 80% ethanol. Then the homogenate was centrifuged. Supernatant was evaporated to dryness. Residue was dissolved in distilled water. Then FC reagent was added. After two minutes 20% sodium carbonate solution was added. Absorbance was read at 650nm against reagent blank.

Enzymic antioxidants

Catalase activity

Fresh leaf sample was ground with 0.1M phosphate buffer, pH 7.0 in a prechilled mortar and pestle.The homogenate was centrifuged at 15,000g at 4ºC. Supernatant was collected. To a mixture of phosphate buffer and hydrogen peroxide enzyme extract was added and incubated at 20 degree C for 1 minute. Then reaction was stopped by adding 0.7N sulphuric acid. The reaction mixture was titrated against 0.01N potassium permanganate (Barber, 1980).

Peroxidase activity

Peroxidase activity was assayed using o-dianisidine as hydrogen donor and hydrogen peroxide as electron acceptor. In a prechilled mortar and pestle the fresh leaf sample was homogenized in 0.1M phosphate buffer, pH 6.0. with a pinch of clean white sand. Homogenate was filtered and centrifuged at 60,000g for 20min at 4ºC. Supernatant was used as enzyme source. To a mixture of o-dianisudine, hydrogen peroxide, phosphate buffer and distilled water enzyme source was added. After 5 minutes reaction was stopped by adding sulphuric acid. Then absorbance was read at 430nm (Summer and Gjessing, 1943).

Statistical analysis

Data obtained were analysed by two way ANOVA Significant F ratios between groups were further subjected to least significant difference (LSD) probability (p) values < 0.05 were considered significant using graphpad prism software (www.graphpad.com)

RESULTS AND DISCUSSION

Plants treated with farm yard manure (T4) showed significant increase in shoot length, and number of leaves followed by vermicompost treated plants compared to other treatments and control (Table 1). In T3, T2, T5, and T1 there was increase in shoot length compared to control.This is in accordance with Shubha and Anusuya (2009) in *Andrographis paniculata*. Not much difference was observed with respect to the number of leaves among other different treatments. T4 recorded maximum number of leaves followed by T3. Other biofertiliser applications viz., *Bacillus megaterium* (T2), *Azospirillum* (T1) and combined inoculation (T5) also showed increase in number of leaves compared to control. These results are in accordance with the reports of medicinal plants inoculated with PGPR improved plant growth and biomass in *Piper nigrum* (Anandraj and Sharma, 1994), Neem (Mohan *et al.,* 1994)

Table 1: Influence of biofertlisers, vermicompost, FYM and combined treatment on growth parameters of Sauropus androgynus

Treatment	Shoot length(in)	No.of branches	No.of leaves
Azospirillum(T1)	23.92	2	29.6
Bacillus megaterium(T2)	26.5	2	35.6*
Vermicompost (T3)	29.58*	3	37.2*
Farm yard manure(FYM) (T4)	36.76*	3	38*
T1+ T2 + T3 + T4 (T5)	25.7	2	34
Control	23.6	2	27

*Significant at $p < 0.05$

Table 2: Influence of biofertlisers, vermicompost, FYM and combined treatment on antioxidant content of Sauropus androgynus

Treatment	Carotenoids (mg/g)	Ascorbic acid(mg/g)	Total phenols(%)	Catalase (units/min/ mg of the sample)	Peroxidase(mmol)
Azospirillum(T1)	1.07	5.3	4.0	0.3	22.37
Bacillus megaterium(T2)	1.50	5.3	4.0	0.4	21.36
Vermicompost (T3)	1.62	6.3	4.3	0.4	22.32
Farm yard manure(FYM) (T4)	2.10*	6.9*	4.7*	0.5	22.67
T1+ T2 + T3 + T4 (T5)	1.90	6.6	4.2	0.5	23.12
Control	1.50	5.1	3.8	0.3	21.22

*Significant at $p < 0.05$

and Turmeric (Sena and Das, 1998). According to Table 2, there was a significant increase in non-enzymic antioxidant content viz., carotenoids, ascorbic acid and total phenols of T4 plants compared to other treatments and control. Plants given treatments such as vermicompost, *Bacillus megaterium* and *Azospirillum* also recorded higher non-enzymic antioxidant content compared to control. Shubha and Anusuya, (2010) reported increase in antioxidant content in biofertiliser treated micropropagated *Costus pictus*. Not much difference was recorded with respect to enzymic antioxidant, catalase activity among different treatments. But enzymic antioxidant, Peroxidase activity was maximum in combined treatment (T5) followed by *Azospirillum* treated plants. Control plants recorded the least.Herbal medicines are a rich source of antioxidants, which are responsible for the therapeutic property of these plants. Our study has reflected the fact that the farm yard manure application comparatively enhanced the leaf produce and antioxidant level of *Sauropus androgynus*. As *Sauropus androgynus* has high therapeutic value and used as green leafy vegetable, this is a better cultivation strategy to grow plants having higher antioxidant content and better growth. It is this kind of technology which is useful for planning future strategies in medicinal plants to save chemical fertilizers as costly inputs and to formulate multiple benefits to the plant.

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