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Research Article

Growth, Phytochemicals And Antioxidant Evaluation Of Bioinoculants Treated Sarcostemma Brevistigma

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ABSTRACT

Globally, human population in the rural areas and tribals are dependent on medicinal plants for their health care as they are very closely associated with nature and is cost effective. Medicinal herbs contribution to health care among urban population is also significant because of the least or no side effects. *Sarcostemma* commonly called as Somalatha or Moon plant is a rare plant which is used in herbal medicine. The study was conducted to evaluate the effects of bioinoculants on the growth, phytochemicals and antioxidant content of the plant. Combined inoculation of all the bioinoculants, T1 (Compost + *Glomus mossae* + *Glomus fasciculatum* + *Trichoderma viride* +*Aspergillus awamori* +*Azotobacter vinelandii*) fared better for growth, all the phytochemicals and antioxidant content evaluated.

Keywords: *Sarcostemma,* Growth parameters, Phytochemicals, Antioxidants

Introduction:

Sarcostemma a member of the family Asclepiadaceae is a perennial joint shrub with a small scaly leaves. Stem is greenish, cylindrical, twining branched and having latex. Scale leaves are opposite and plant can be propagated by seeds and stem cuttings. It is also called moon plant, moon creeper in English, soma or somavalli in Sanskrit, Somalata or Hambukalli in tamil..

The plant is acrid, narcotic, carminative, antiphlogistic, thermogenic expectorant, antiviral and rejuvenating. It is found to be active as an anti-rheumatic, anti allergic and bronchodilater. (Kirtikar and Basu, 1980; Oberoi *et al.* 1985; Khare *et al.* 1987; Khare *et al.* 1980). The plant is also used as an anti-inflammatory topical agent in Ayurveda. It has been prescribed for joint pains after delivery by bathing with water with the plant boiled gives relief (Indraji 1998). Tincture of the plant is also used in homoeopathy. The latex is applied on eczema, boils, wounds etc. It is useful in vitiated conditions of pitta, asthma, viral infection, hydrophobia, psychopathy, general debility, swellings, coughs, fever etc. The formulations are somasava, somachurna and tincture of *Sarcostemma*

Lalitha *et al.* (2002) evaluated the analgesic activity of the plant on rats and reported a relatively high analgesic activity in rats.
The approach of phytochemicals in medicinal plants has attracted a great deal of attention mainly concentrated on their role in preventing diseases which may otherwise cause as a result of oxidative stress. Oxidative stress, which releases free oxygen radicals or reactive oxygen species in the body, has been implicated in a number of disorders including cardiovascular malfunction, cataracts, cancers, rheumatism and many other autoimmune diseases. Antioxidants do not themselves become free radical by donating electrons because they are stable in either form. They act as scavengers and neutralize the free radicals before they could cause damage to the cell.

The curative efficacy of herbal formulations could be due to the antioxidant principles of the constituent herbs. The phytochemicals which act as antioxidants against oxidative stress scavenge free radicals and act as saviours of cell. High content of polyphenolic compounds, ascorbic acid, carotenoids, tocopherols, flavonoids, anthocyanins in different parts of various medicinal plants have the antioxidant properties.

There is an ever-increasing demand for herbal medicines in recent years due to the side effects of synthetic drugs, and in turn there is a need for conservation and alternate methods of propagation of medicinal plants in vivo without using chemicals. Propagation techniques Because of the huge demand, there is indiscriminate extraction of the plants from wild which may result in extinction of the plant. Hence there is an urgent need to develop alternate propagation techniques with good herbal biomass yield, high level of phytochemicals and antioxidants contents to meet the market demand and avoid extraction from the wild.
Free living nitrogen fixer viz., *Azotobacter vinelandii* naturally fixes atmospheric nitrogen in the rhizosphere. In addition to nitrogen fixation, *Azotobacter* has the capacity to produce aminoacids and growth hormones like Thiamin, Riboflavin, Nicotin, Indole acetic acid and gibberlin.
Aspergillus awamori is an efficient phosphate mobilizing fungus, which has been widely used as biofertiliser to supplement phosphorus.
Frateuria aurentia is a newly defined gram negative, motile rod type, aerobic and acid tolerant potassium solubilising bacteria and can grow at pH 3.5-8.
Trichoderma viride is an aggressive, toxin producing mycoparasite which grow rapidly in field under favourable moisture, soil and temperature. It is a very potent biopesticide and used extensively as a biocontrol agent against soil borne plant pathogens.
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. This vitamin C even metabolizes cholesterol which means it may help lower cholesterol levels.
Vitamin E is the lipid soluble, chain breaking antioxidant, scavenges free radicals and prevents lipid peroxidation, thus stabilising the cell membrane.
Natural polyphenols have also been reported to be promising in the treatment of lymphocyte malignancy. Phenolic compounds have also been shown to exhibit cellular defence mechanism in atherogenesis and cancer.

Materials and methods

Compost preparation

Dried leaves of Pongamia and Neem were collected from nearby Jnanabharathi campus and composted at Botany department garden, Bangalore University, Bangalore. They were dumped into compost bins and mixed with soil and cow dung. The cement compost bins were provided with two holes to facilitate turning with a long stick during decomposition. Turning was done manually once in 3 days to facilitate aeration. This was allowed to decompose and used for the test plants .

Maintenance of bioinoculants:

Azotobacter vinelandii* (Azo):* Cultures of *Azotobacter vinelandii* was maintained on Azotobacter medium.

Mycorrhizal inoculum: The inoculum consisted of sand and soil mixture containing chlamydospores and root segments of finger millet infected with *Glomus mossae* (G.m) and *Glomus fasciculatum* (G.f). About three grams was inoculated to the root zone soil

Fungal bioinocunts: *Trichoderma viride*(T.v) and *Aspergillus awamori* (A.a) were inoculated into Potato Dextrose Agar medium for 7 days. Uniform fungal discs were cut using a sterile cork borer (8mm diameter). The fungal discs were inoculated to the sterilized potato dextrose broth at the rate of one disc per flask and inoculated flasks were incubated for 7bdays.After incubation, fungal biomass along with the medium was collected, 4ml of the above said medium was added to each pot (10^{6cfu}) as per the treatment.

Potassium mobiliser: *Frateuria aurantia* (F.a) (RCOF) cultures were maintained on nutrient broth medium for three days.4ml of the medium with the inoculum at the rate of 10^{9cfu} was added to each pot as per the treatment plan.

Bioinoculants enriched compost treatments: Stem cuttings with one to two internodes were planted in pots containing sand, soil mixture and compost in the ratio 1:1:1. After 5 days of planting, the liquid bioinoculants at 5 ml/plant and mycorrhizal inoculum at 3 g/plant per plant were added to the root zone. Each bioinoculants treatment was repeated at 30, 60 and 90 days. The treatment given were:
T_1 = G.m + G.f + Azo + A.a + F.a + T.v
T_2 = G.m + Azo + A.a + F.a + T.v
T_3 = G.f + Azo + A.a + F.a + T.v
T_4= G.m alone
T_5= G.f alone
T_6= F.a alone
C=Control

Growth parameters
Growth parameters like shoot length, root length, number of branches, number of leaves, fresh and dry weight were recorded at 30,60 and 90 days of plants growth.

Plant fresh weight
Fresh weight of the plant material was recorded after depotting.

Plant dry weight
Dry weight of the plants was recorded by drying the plants in oven at 60^{o} C.

Nutrient studies
Nitrogen was estimation from the plant samples by micro-kjeldahl method (Amma, 1990**)**

Total phosphate in the plant samples was estimated by Jackson (1971).
Estimation of potassium in plant samples was done as outlined by Toth and Prince, 1949
Dewise and Freitas (1970) method was followed to estimate of Calcium and Magnesium
For estimation of sodium Toth *et al.* (1948) method was followed.
The protein content in the extract was estimated by the method outlined by Lowry *et al.* (1951).
Nitsch and Nitsch, (1962) method was used to estimate of phenolics
Quantitative estimation of carbohydrates and reducing sugars were done by anthrone method (Mahadevan and Sridhar, 1986)

Antioxidants
Estimation of vitamin (Total carotenoids) A and was calculated by Sadasivam and Manickam, (1996) method
Vitamin C (Ascorbic acid) was determined by the method given by Varley *et al.* (1984).
Estimation of Vitamin E **(α-tocopherol) by** Dadlani and Agarwal method:
The total antioxidants or antioxidant capacity of medicinal plants was estimated using the FRAP assay (Connor *et* al. 2002).

Statistical Analysis:
All data were analyzed by two-way Anova. Significant 'F' ratios between groups means were further subjected to least significant differences (LSD) probability (P) values < 0.05 were considered significant using Graphpad Prism Software (www.graphpad.com).

Results and Discussion

The investigation was an attempt to study the effect of natural resources enriched with bioinoculants on the growth, phytochemical and antioxidant properties of *Sarcostemma brevistigma*. Rural waste like pongamia and neem leaves was decomposted to yield fine compost. Neem protects the plants from various pathogens, at the same time pongamia is a good nitrogen manure. The compost of these plants were used along with soil, sand enriched with bioinoculants which proved to be the best for the medicinal plants studied.
Maximum height was recorded in combined inoculation with T_1 followed by T_4, T_3 and T_5. Reduced height was recorded in T_6 treated plants. Maximum number of nodes was observed in T_1, T_3 and T_4 plants than other treatments and control (Table1). Several reports indicated that there is synergistic interaction with beneficial soil microorganisms and AM fungi (Meyer and Lindermann 1986; Earanna, 2001; Chandrika, 2002; Hiremath 2006) which might have enhanced the growth. The shoot length and number of nodes in *Sarcostemma* treated with G.m was significantly higher than control plants however *Glomus fasciculatum* treated plants also recorded higher plant growth. This indicated that G.m is a better symbiont for *Sarcostemma R.Br*. This report coincides with the report of Chandrika (2002) in *Bacopa monnieri* .Enhanced plant growth due to AM inoculation could be due to improved P uptake recorded in these treatments which is attributed to increased per cent root colonization.
T1 recorded highest NPK content followed by T_2 and T_3 for N and P whereas for K T_6 was second best. Least NPK was recorded in control (Table 2). Calcium, Magnesium and Sodium content were significantly higher in T1 Plants and minimum was recorded in control (Table 3). . *Frateuria aurentia* was proved to be second best for potassium uptake which showed that *Frateuria aurentia* is a good K mobiliser.The above observations upholds the observations made by earlier workers that Phosphorus uptake by AM fungus is increased when it is coinoculated with PGPR such as *Azospirillum* (Tilak and Singh,1988), *Azotobacter* (Meenu and Saralabhai ,2001), *Trichoderma* (Jayanthi and Bagyaraj, 2000). Earanna (2001) in the medicinal plants *Withania somnifera and phyllanthus amarus* reported that VAM fungi when coinoculated with *Bacillus coagulans* and *Trichoderma harzianum* recorded enhanced P uptake compared to single inoculations and uninoculated control. Chandrika (2002) reported enhanced P uptake in *Acalypha indica* when VAM was coinoculated with *Trichoderma harzianum* and *Bacillus coagulans*. Veeraswamy *et al.* (1992) reported that increased uptake of nitrogen and phosphorus by Sorghum plants inoculated with *Glomus intraradices* and *Azospirillum lipoferum*. Higher P content was recorded in *Vigna unguiculata* treated with AM and *Bacillus coagulans* (Caroline

Machado, 1994). Increase in N content due to combined inoculation of *Glomus macrocarpum, Bacillus polymixa* and *Azotobacter chroococcum* inoculation in chilli compared to uninoculated control was reported by Hiremath *et al*.(2006). Arpana (2001) reported increased P uptake in kalmegh inoculated with *Glomus mosseae* and *Trichoderma harzianum*

Calcium content varied from 0.433 to 0.505 per cent. Ca content was highest in T_1 treated plants followed by T_3. In T_2, T_5 and T_6 treated plants calcium content showed similar values. Control plants showed the least. T_1 treated plants showed maximum Magnesium and Sodium content also Least Mg content was observed in control plants. Control recorded minimum magnesium and sodium content (Table 3).Srinivasa and Krishnaraja, (1992) reported that the dual inoculation of *Glomus macrocarpum* and *Pseudomonas striata* significantly increased plant nutrients that is uptake of P, Cu, Zn, Mn, and Fe and plant dry weight. This may be due to synergistic interaction occurred among bioinoculants in the rhizosphere of these medicinal plants which might have increased the efficiency of Ca and Mg absorption.

Significant increase in the protein content was recorded in the plants treated with combined inoculation than uninoculated control. Table 4 shows that T_1 recorded maximum protein content followed by T_2, T_3 and T_6. It is evident from the Table 4 Maximum carbohydrate content was recorded in T_1 and T_2 plants followed by T_3, T_5 and T_6. Minimum was observed in uninoculated control plants. There was not much significant difference in the reducing sugar content in both treated plants and uninoculated control plants. However reducing sugar was maximum in T1 treated plants whereas T2 and T3 recorded similar values (Table 4).

Vitamin A content ranged between a minimum of 0.014mg g^{-1} to a maximum of 0.0237mg g^{-1}. In T_1 and T_2 inoculated plants two fold increase in carotene content was recorded followed by T_3 and T_6 whereas T_5 and control resulted in least carotenoids content. It is evident from the Table 5 Ascorbic acid content varied between 8.57 mg g^{-1} to 17.1mg g^{-1}. T_1 and T_3 treated plants recorded appreciable increase in Vitamin C content than other treatments and control plants.There was not much appreciable increase in Vitamin E content among treated and control plants and it was maximum in T_1 followed by T_3. All other treatments showed the least. Significant increase in the total phenolics content was observed in T_1 (30µg g^{-1}) plants than control plants (16µg g^{-1}). T_1 and T_3 also showed a significant increase in total phenol content than control. In T_4, T_5 and T_6 plants total phenolic content recorded was 22-23 µg g^{-1}.The total antioxidants ranged between 0.38 Ascorbic acid equivalents to 0.62 Ascorbic acid equivalents. There was considerable increase in total antioxidant content in T_1 treated plants than control. Next best was T_2 and T_3 which recorded significant increase in FRAP values followed by T_5, T_6 and T_4 plants. Control plants showed minimum values. (Table 6)

The impact of several diseases may be prevented by improving the intake of natural nutrients with antioxidant properties, such as Vitamin E, Vitamin C and carotenoids etc. (Gajera *et al*. 2005). In *Sarcostemma* R.Br. Combined inoculation of T1 fared better for all the antioxidant content studied. The efficiency of biofertilizer, vermicompost, farmyard manure and combined treatment on the levels of antioxidants, catalase and peroxidase which involved in the detoxication of reactive oxygen species in *Adathoda vasica* at different time intervals were studied by Shubha *etal* (2021), in which highest quantity of carotenoids (mg/g) was recorded in combined treatment consisting of *Azospirillum brasileins, Bacillus megaterium,* vermicompost and farmyard manure

Conclusion

Growth, NPK, nutrient contents and antioxidant potential of *Sarcostemma brevistigma* were significantly higher in compost with all the bioinoculant treatments which could be extended to other medicinal plants that may enhance the therapeutic power and better yield with respect to the phytrochemicals studies

Use of bioinoculants technology is considered to be important in farming practices aiming at sustainability in soil fertility and crop productivity which protects the environment from harmful chemicals and cost effective nature.

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Table-1: Synergistic effect of bioinoculants on growth parameters of *Sarcostemma* R.Br. (90 days)

Treatments	**Height** (cm)	**No.of nodes**
T_1	25.5*	5
T_2	16	4
T_3	23.5*	5
T_4	24*	5
T_5	18*	4
T_6	15	4
C	16	4

*Significant at $P < 0.05$

T_1= G. m+ G. f + Azo + A.a + F.a +T. v
T_2= G. m+ Azo +A.a + F.a+T. v
T_3= G. f + Azo +A.a + F.a+T. v

T_4= G. m
T_5= G. f.
T_6= F.a
C=Control

Table-02: Influence of bioinoculants on NPK content of *Sarcostemma* R.Br. (90 days)
(Values expressed in per cent)

Treatments	Nitrogen	Phosphorus	Potassium
T_1	1.25*	0.122*	0.54*
T_2	1.125*	0.112*	0.43*
T_3	1.125*	0.118*	0.41*
T_4	1	0.11*	0.42*
T_5	1	0.11*	0.42*
T_6	1	0.08	0.51*
C	1	0.08	0.39

*Significant at $P < 0.05$

T_1= G. m + G. f + Azo +A.a + F.a + T. v
T_2= G. m + Azo +A.a + F.a+T. v
T_3= G. f + Azo +A.a + F.a+T. v
T_4= G. m
T_5= G. f.
T_6= F.a
C = Control

Table-03: Influence of bioinoculants on Calcium, Magnesium and Sodium content of *Sarcostemma* R.Br (90 days)
(values expressed in per cent for Calcium, Magnesium and ppm for Sodium)

Treatments	Calcium	Magnesium	Sodium
T_1	0.505*	0.2254*	8.8*
T_2	0.4649*	0.2205*	8.4*
T_3	0.48096*	0.2151*	8*
T_4	0.44086*	0.2107*	8*
T_5	0.4649*	0.2151*	8*
T_6	0.4649*	0.2107*	8*
C	0.43286	0.1911	7.8

*Significant at $P < 0.05$

T_1= G. m + G. f + Azo + A.a + F.a (K mobiliser) + T. v
T_2= G. m + Azo +A.a + F.a+T.v
T_3= G. f + Azo +A.a + F.a+T. v
T_4= G. m
T_5= G. f.
T_6= F.a

C = Control

Table- 04: Influence of bioinoculants on Protein, Total carbohydrates and Reducing sugar of *Sarcostemma* R.Br. (90 Days)

(Values expressed in mg g^{-1})

Treatments	Proteins	Total Carbohydrates	Reducing Sugar
T_1	3.4*	6.2*	0.28
T_2	3.2*	6.2*	0.27
T_3	3.2*	5.8	0.27
T_4	3.1*	5.6	0.25
T_5	2.9	5.8	0.25
T_6	3.2*	5.8	0.25
C	2.4	5.5	0.24

*Significant at $P < 0.05$

T_1= G. m + G. f + Azo + A.a + F.a (K mobiliser) + T.v
T_2= G. m + Azo + A.a + F.a+T.v
T_3= G. f + Azo + A.a + F.a+T.v
T_4= G. m
T_5= G. f.
T_6=F.a
C=Control

Table- 05: Influence of bioinoculants on antioxidant content of *Sarcostemma* R.Br. (90days)

(Values expressed in mg g^{-1} for Vitamin A, Vitamin C, Vitamin E, Ascorbic acid equivalents for FRAP and µg g^{-1} for Total Phenols)

Treatments	Vitamin A	Vitamin C	Vitamin E	FRAP**	Total Phenols
T_1	0.0237*	17.1*	27*	0.62*	30*
T_2	0.0237*	8.57	25	0.56	28*
T_3	0.0221*	12.8	26*	0.56	28*
T_4	0.021	8.57	25	0.5	23*
T_5	0.014	8.57	25	0.52	23*
T_6	0.0221	8.57	25	0.52	22*
C	0.014	8.57	25	0.38	16

*Significant at $P < 0.05$

** **FRAP**=Ferrous Reducing Ability of Plasma
T_1= G. m + G. f + Azo + A.a + F.a + T. v
T_2= G. m + Azo +A.a + F.a+T.v
T_3= G. f + Azo +A.a + F.a+T. v
T_4= G. m
T_5= G. f.
T_6=F.a
C=Control