

## Growth response of *Basella alba*. Linn to *Azotobacter* species isolated from marine sediments and garden soil

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P.Nirmala\* and P.Aruna

PG and Research Department of Microbiology, Mohamed Sathak College of Arts and Science, Sholinganallur, Chennai.

### Abstract

In the development and implementation of sustainable agricultural techniques, biofertilization is of great importance. One of the most interesting non-symbiotic bacteria that have great potential for use in production of biofertilizers is *Azotobacter chroococcum*, due to its ability to fix atmospheric nitrogen. The present work was carried out to study the effect of *Azotobacter* species on growth of *Basella alba*. Totally 10 *Azotobacter* isolates (5 from marine sediments and 5 from garden soil) were isolated. A pot experiment was conducted in glass house for 90 days under green house conditions. Each *Azotobacter* isolate was separately added to the pot as per the treatment allocation. *B. alba* seeds were sown in each pot including control. The influence of *Azotobacter* isolates on the growth and biomass of *B. alba* has been reported. The physical parameters of *B. alba* was significantly increased in the inoculated treatments at 30, 60 and 90 days after treatment compared to uninoculated control plants. On the 90<sup>th</sup> day after treatment, the isolate MS-1 and GS-1 recorded maximum plant height, number of leaves, number of branches, fresh and dry weight which is followed by MS-4 and GS-4 isolate. Plants inoculated with different isolates of *Azotobacter* species revealed increase in chlorophyll content and NPK content compared to uninoculated control plants. The highest amount of chlorophyll (1.94±0.01, 1.92±0.09 SD), nitrogen (6.50±0.01, 6.42±0.006 SD), phosphorus (0.200±0.001, 0.192±0.006 SD) and potassium (2.98±0.08, 2.94±0.04 SD) content was observed in the plants treated with MS-1 and GS-1 isolate. Comparatively MS-1 isolate was more efficient than GS1. Results of the present study revealed enhanced growth, biomass, chlorophyll, nitrogen, phosphorus and potassium content of *B. alba* due to inoculation with *Azotobacter* strains.

**Keywords:** *Azotobacter chroococcum*, *Basella alba*, Biofertilizers, Nitrogen fixation.

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### INTRODUCTION

Biofertilizers has been used as alternative to chemical fertilizers to increase soil fertility and crop production in sustainable agriculture (Wu *et al.*, 2005). Biofertilizer is a large population of a specific or a group of beneficial microorganisms for enhancing the productivity of soil either by fixing the atmospheric nitrogen or by solubilizing soil phosphorus or by stimulating plant growth through synthesis of growth promoting substances (Narayanamma *et al.*, 1985). Use of microorganisms beneficial to agriculture started more than 60 years ago because of their capacity to convert unavailable and nutritionally important elements into available ones, and as an alternative to increase plant resistance to adverse environments (Narula *et al.*, 2000; Vessey, 2003). Among the microorganisms that have had a beneficial effect on crop growth and yield, and found associated with plant rhizosphere, are species

of the genera: *Azospirillum*, *Azotobacter*, *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Rhizobium*, *Serratia*, and *Trichoderma* (Sturz and Nowak, 2000). These microorganisms benefit plant development owing to the fact that they increase nitrogen absorption, phytohormone synthesis, mineral solubilization, and iron chelates. Some can also induce resistance against pests and inhibit soil pathogens through the production of antimicrobial metabolites. Furthermore, they improve soil structure and reduce erosion (Nelson, 2004; Bostock, 2005; Kohlar *et al.*, 2006).

Diverse studies report that inoculation with only one beneficial microorganism generally increases plant growth and decreases pathogenic agents (Chandanie *et al.*, 2006; Raimam *et al.*, 2007). Each microorganism has different beneficial effects.

*Azotobacter* is one of the most important microorganisms, which is widely used as biofertilizer. *Azotobacter* spp. are Gram negative, aerobic, asymbiotic free living nitrogen fixing bacterium belonged to family

\*Corresponding Author :  
email: [nirmala\\_dineshkumar@yahoo.com](mailto:nirmala_dineshkumar@yahoo.com)

Azotobacteriaceae and play an important role in improving plant growth and yield by producing plant hormones and anti microbial substances. Their size ranges from 2-3  $\mu\text{m}$  in diameter and of various lengths. *Azotobacter* spp. are sensitive to acidic pH, high salts and temperature above 35°C (Hoflich *et al.*, 1994). It is widely distributed in different types of soils as a nitrogen fixer and thus it enhances the plant development. The genus *Azotobacter* has role in plant growth promotion due to the production of hormonal substances - indole-3-acetic acid (IAA), gibberellic acid (GA3) and cytokinin (Mirkovacki and Milic, 2001), improvement of nutrient uptake by plants (Subba Rao, 1982), involved in vitamins production (Martinez-Toledo *et al.*, 1996) and act as biocontrol agent (Fatima *et al.*, 2009).

Zahir *et al.* (2000) reported efficient growth of maize seedlings by the application of IAA producing *Azotobacter* sp. The application of *Azotobacter* and arbuscular mycorrhiza on plants also enhanced the survival percentage of *Morus alba* on salt affected lands (Kashyap and Sharma, 2006). Kizilkaya (2008) found that strains of *Azotobacter chroococcum* exerted a positive effect on nitrogen yield and concentration in wheat. Suman *et al.* (2001), found substantial genetic diversity in some strains of *Acetobacter* for future research as microorganisms promoting plant growth, and suggested that strains of *Acetobacter* and *Azospirillum* can be used as the basic source for the development of sugarcane biofertilizers.

Over the years, medicinal plants have been found useful in the treatment and management of various health problems. Traditional medicine is undoubtedly a reliable alternative approach to health care delivery in the metropolis because it is cheap, easily accessible and efficacious. In spite of millions of chemically synthesized drug for a number of diseases; natural products of plant origin has got its own importance and has remained the most important source of new drugs. One such medicinal herb is *Basella alba*. *B. alba* is a widely cultivated and cool season vegetable with climbing growth habit. It has high medicinal property. *B. alba* can grow under conditions of moderate soil fertility and the production can be enhanced with application of biofertilizers.

*Basella alba* is belonged to the family *Basellaceae*. *B. alba* is a fast growing vegetable, native to tropical Asia (India or Indonesia) and extremely heat tolerant (Grubben and Denton, 2004). It is commonly known as Chinese spinach (Facciola, 1990). It is fast growing perennial plant. It is an easy growing plant propagated by seed, root or long tip cuttings. *B. alba* is commonly grown for its leaves and young shoots, which are high in Vitamin A, B9, C, iron and calcium (Grubben and

Denton, 2004). Due to its mucilaginous nature of leaves and stems, the juice of leaves has been prescribed against constipation especially for children and pregnant women (Duke and Ayensu, 1985). Its thick, semi-succulent, heart- shaped leaves have a mild flavour and mucilaginous texture. Daily consumption of Indian spinach has been shown to provide vitamin A especially in populations at high risk of vitamin A deficiency (Haskell *et al.*, 2004).

The main objective of the present study is to provide a cheaper source of nitrogen to the agriculture industry and to meet the needs of crops as there is spread of inflation day by day due to high prices of petroleum which influences the prices of chemical nitrogenous fertilizers. Also for a sustainable agriculture system, it is imperative to utilize renewable input that can maximize the ecological benefits and minimize the environmental hazards.

Therefore, realizing the importance of this, the work was carried out to study the effect of *Azotobacter* species on growth of *B. alba* and to determine the importance of biofertilizer application in order to improve the yield quality and productivity and avoid environmental pollution.

## MATERIALS AND METHODS

### Collection of soil samples

Two different localities were selected for the collection of soil sample. Marine sediments were collected from Marina Beach, Chennai and Garden soil from Sholinganallur, Chennai. Totally 10 samples were collected in sterile polyethylene bags and placed properly in the ice boxes and transported safely to the laboratory for the further studies. The samples collected were dried inside the laboratory at 28°C. The samples collected from each site were mixed well to get a pooled sample. Each sample was sieved through 1000 mesh to remove the bigger particles and debris. The sieved samples were used for the isolation of the *Azotobacter* species.

### Isolation of *Azotobacter* species (Sandeep *et al.*, 2011)

*Azotobacter* species were isolated using *Azotobacter* isolation agar by employing serial dilution plate technique. The samples were diluted upto  $10^{-7}$  dilution with sterile distilled water. The dilutions of  $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$  were plated on to *Azotobacter* isolation agar by spread plate technique. Then the plates were incubated for 72 hours in an inverted position at 30° C. After incubation, the plates were checked for *Azotobacter* isolates. The well isolated colonies were re-streaked for purification and the pure isolates thus obtained were maintained on the agar slants prepared with *Azotobacter* isolation medium.

### Identification of *Azotobacter* Species

The pure isolates obtained from the serial dilution technique were identified based on the standard morphological and biochemical properties according to Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994). The tests employed were Gram's staining, Capsular staining, Motility and various biochemical tests such as Starch hydrolysis, catalase, oxidase, Indole, Methyl red, Voges-proskauer, citrate and Carbohydrate fermentation tests (glucose, lactose and sucrose).

### Inoculum Preparation

The pure isolates of *Azotobacter* species were inoculated separately into 250 ml conical flask containing 100ml of *Azotobacter* isolation broth and incubated at 30°C under shaking at 100 rpm for six days. The grown cultures were homogenized and 5ml of each culture ( $12.4 \times 10^6$  cfu/ml) was used for inoculation in the pot experiment.

### Plant growth response under green house conditions

A pot experiment was conducted in glass house for 90 days. The pots measuring 2 kg capacity were filled with sand:soil mixture in the ratio of 1:1 v/v upto  $\frac{3}{4}$ th of the volume. The pots were watered one day prior to planting. A planting hole was made at the center of the pots to enable the inoculation of *Azotobacter* isolates (5ml inoculum representing each zone). *Azotobacter* isolate from each site was separately added to the pot as per the treatment allocation. There were three replicates for each treatment. The treatment of experiments includes:

C – Control (uninoculated control), MS1 – *Azotobacter* isolate from Marine sediment 1, MS2 – *Azotobacter* isolate from Marine sediment 2, MS3 – *Azotobacter* isolate from Marine sediment 3, MS4 – *Azotobacter* isolate from Marine sediment 4, MS5 – *Azotobacter* isolate from Marine sediment 5, GS1 – *Azotobacter* isolate from Garden soil 1, GS2 – *Azotobacter* isolate from Garden soil 2, GS3 – *Azotobacter* isolate from Garden soil 3, GS4 – *Azotobacter* isolate from Garden soil 4, GS5 – *Azotobacter* isolate from Garden soil 5.

In this study, the influence of *Azotobacter* isolates on growth and biomass of *Basella alba* was reported. *B. alba* seeds were sown in each pot including control. These pots were watered regularly until harvest. The observations for growth parameters like plant height, number of leaves and number of branches were recorded at 30, 60, 90 days intervals. The plants were harvested after 90<sup>th</sup> day and the plant biomass (dry weight - after drying the harvested plants at 60°C in a

hot air oven for 7 days to reach constant weight and fresh weight - at the time of harvest) was determined. Biochemical analysis was made to determine total chlorophyll content, nitrogen, phosphorus, potassium content.

### Determination of Chlorophyll by DMSO method (Hiscox and Israelstam, 1979)

500 mg of dry leaf tissue was taken, cut into small pieces and suspended in test tubes containing 2 mL of dimethyl sulphoxide (DMSO). Test tubes were incubated at 60° C for 20 min in a water bath. The supernatant was decanted and another 3 mL of DMSO was added to the residue and incubated at 60° C for 20 min. The supernatants were pooled and the volume was made up to 10 mL by adding DMSO. The chlorophyll extract was transferred to a cuvette and the absorbance was read in a Spectrophotometer (Genesys, UK) at 645 and 663 nm against DMSO.

### Determination of Nitrogen by Micro-Kjeldahl Method (AOAC -Official methods of Analysis, 1980)

The dry leaf tissue and 1.1g of salt/catalyst mixture was added in standard Kjeldahl digestion tube. Digest blanks contained only reagents with each set of sample. 3mL of concentrated sulphuric acid was added and slowly heated upto 200° C. Once the frothing was subsided, brought the temperature upto 350-375°C and heated till the digest clears. The froth was heated at 350 to 375°C for an additional 35 minutes to 1 hour past clearing. The digest was cooled and 20 mL of deionized water was added. On solidification within the digest, the contents were mixed using a vortex mixer so as to dissolve the solid. 5mL of  $H_3BO_3$  indicator solution to 50-mL flask was added. The flask was kept under the condenser with the condenser tube below the surface of the indicator solution. 20mL of 10 M NaOH was added to the digested sample. The digested sample was transferred to the Kjeldahl distillation apparatus and distilled. The distillate was collected till it reached the level of  $H_3BO_3$  solution using standard 0.01 M HCL or 0.005 M  $H_2SO_4$ . The end point was the turning of the solution to a pink colour.

### Determination of Phosphorus by Vanadomoly Bdophosphoric Acid Method (Jackson, 1973)

2.5mL of plant digest was taken into a 50mL plastic beaker with Oxford pipette, and 20mL of distilled water was added with automatic pipette. The reagent was delivered quickly as it was added. The color was developed within 10 minutes. The per cent transmittance was determined with the colorimeter. The optimum wavelength was 430 nm, however, the

420nm filter was used with the probe colorimeter. The blank was also carried through the procedure.

#### Determination of Potassium by Flame Photometer (Jackson, 1973)

0.200 ± .001 g of oven-dried plant material was weighted and transferred into a 50 mL Erlenmeyer flask. The check was included in every other set of 12. 20 mL of extracting solution was added to each flask with a buret. The solution was shaken for 10 minutes at 200 rpm. It was filtered through the Whatman No.2 filter paper.

5mL of the aliquot extract was transferred with an Oxford macropipetter into a clean 50mL flask and diluted with 25mL of deionized water. The speed was sufficient for mixing action. The standards and samples were run on the AA in the flame emission mode. The meter reading was recorded. The recommended dilutions were obtained.

#### Statistical Analysis

All the experiments were conducted in triplicates and data shown in results are mean and standard deviation.

### RESULTS

Five samples of marine sediments and five samples of garden soil were collected from different parts of Chennai for the isolation of *Azotobacter* species. All the samples showed positive for *Azotobacter* species based on their morphology and biochemical characteristics.

#### Isolation of *Azotobacter* isolates

Totally ten isolates of *Azotobacter* were isolated on *Azotobacter* isolation agar medium (one from each sample). Observations for growth characters of 10 isolates were recorded and presented in Table 1. All the isolates showed moderate to good growth, flat entire slimy colony with light brown to dark brown pigmentation.

**Table.1.** Growth characters of *Azotobacter* isolates

Azotobacter isolates	Cultural characters	Pigmentation
MS-1 isolate	Good growth, flat entire slimy colony	Light Brown
MS-2 isolate	Moderate growth, flat entire slimy colony	Dark Brown
MS-3 isolate	Good growth, flat entire slimy colony	Pale Brown
MS-4 isolate	Good growth, flat entire slimy colony	Dark Brown
MS-5 isolate	Good growth, flat entire slimy colony	Light Brown
GS-1 isolate	Good growth, flat entire slimy colony	Dark Brown
GS-2 isolate	Good growth, flat entire slimy colony	Dark Brown
GS-3 isolate	Moderate growth, flat entire slimy colony	Dark Brown
GS-4 isolate	Good growth, flat entire slimy colony	Dark Brown
GS-5 isolate	Moderate growth, flat entire slimy colony	Light Brown

MS- Marine sediments, GS- Garden soil.

#### Identification of *Azotobacter* isolates

The morphological characters of the colonies were oval to round in shape while some were blunt ended long cells. Based on the colony characters, cell shape, presence of capsule, gram reaction and utilization of different biochemical test, the isolates were confirmed as *Azotobacter* (Table 2).

**Table.2.** Confirmatory tests for *Azotobacter* species

Biochemical characteristics	<i>Azotobacter</i> isolates
Gram staining	Gram negative rods
Motility	Motile
Capsular staining	Capsulated
Catalase test	Positive
Oxidase test	Positive
Indole test	Positive
Methyl red test	Positive
Voges-proskauer test	Positive
Citrate test	Positive
Starch hydrolysis test	Positive
Glucose fermentation test	Acid production
Lactose fermentation test	Acid production
Sucrose fermentation test	Acid production

#### *Basella Alba* growth response to *Azotobacter* isolates

The influence of *Azotobacter* isolates on growth and biomass of *Basella alba* is reported. The growth parameters such as height, number of leaves, and branches, fresh and dry weight of the plant were recorded 30, 60, 90 days treatment after (Table 5).

**Table.3.** Growth parameters of *Basella alba* influenced by *Azotobacter* isolates

Zones	Plant height after 90 days (DAT) in cm	No. of leaves after 90 days (DAT) in cm	No. of branches after 90 days (DAT) in cm	Plant fresh weight/gm	Plant dry weight/gm
MS-1	46±1	18±1	25±0.6	76±1	18.27±0.01
MS-2	6±0.6	3±0.5	4±0.5	12±0.5	2.05±0.01
MS-3	17±0.07	12±0.2	14±1	33±0.3	6.30±0.05
MS-4	20±0.20	17±0.05	19±1	62±0.5	17.00±1
MS-5	19±0.9	13±0.03	18±1	52±1	16.00±0.6
GS-1	16±0.06	18±1	19±1	64±1	10.00±0.01
GS-2	11±1	8±1	8±1	36±1	4.00±0.5
GS-3	14±0.1	12±0.5	18±1	50±1	8.00±0.01
GS-4	15±1	16±1	19±0.9	60±1	8.50±0.05
GS-5	13±1	9±0.5	10±0.9	49±0.5	7.00±1
Control	7±1	5±0.5	5±0.5	8±1	2.59±0.05

Mean ± Standard deviation MS- Marine sediments, GS- Garden soil.

### Physical Parameters

The height, number of leaves and branches of *B. alba* were significantly increased in the inoculated treatments at 30, 60 and 90 days after treatment (DAT), compared to uninoculated control plants. Among the 5 marine sediment isolates, isolate MS-1 recorded maximum height, maximum number of leaves and branches which is followed by MS-4 and MS-5. Among the 5 garden soil isolates, isolate GS-1 showed maximum height, maximum number of leaves and maximum number of branches, which is followed by GS-4 and GS-3. The uninoculated control plant showed least height compared to inoculated plant. The increased growth could be attributed to the nitrogen fixation and production of growth hormones by *Azotobacter* species.

The plants (*B. alba*) showed increase in fresh and dry significant weight in response to at 30, 60 and 90 days after treatment (DAT), compared to un-inoculated control plants. Among the 5 marine sediment isolates, isolate MS-1 showed maximum fresh and dry weight per gram which is followed by MS-4 and MS-5 respectively. Among the 5 garden soil isolates, isolate GS-1 recorded higher fresh and dry weight per gram followed by GS-4 and GS-3, which were higher when compared to the untreated control.

Among the 10 *Azotobacter* isolates, MS-1 and GS-1 showed highest rate of plant growth parameters when compared to other isolates.

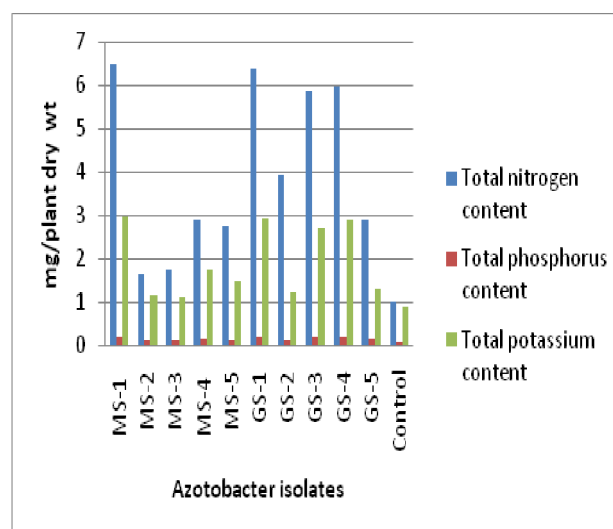
### Biochemical Parameters

The chlorophyll, nitrogen, phosphorus and potassium content of *Basella alba* were significantly increase in the treated plants at 30, 60 and 90 days after treatment (DAT), compared to un-inoculated control plants. Among the 5 marine sediment isolates, isolate MS-1 recorded relatively highest quantity of chlorophyll, nitrogen, phosphorus and potassium content which is followed by MS-4 and MS-5. Among the 5 garden soil isolates, isolate GS-1 showed highest quantity of chlorophyll, nitrogen, phosphorus and potassium content which is followed by GS-4 and GS-3. The uninoculated control plant showed least quantity of chlorophyll, nitrogen, phosphorus and potassium content (Table 4 and Figure 4)

The comparison of the isolation of *Azotobacter* obtained from the Marine sediments and garden soil showed that the isolates from marine influenced the growth of *Basella alba*.

**Table.4. Biochemical parameters of *Basella alba* influenced by *Azotobacter* isolates**

Zones	Total nitrogen content
MS-1	1.94±0.01
MS-2	1.25±0.02
MS-3	1.29±0.04
MS-4	1.82±0.09
MS-5	1.74±0.03
GS-1	1.92±0.09
GS-2	1.30±0.01
GS-3	1.72±0.08
GS-4	1.88±0.09
GS-5	1.20±0.06
Control	1.11±0.01



**Fig.1. Biochemical parameters of *Basella alba* influenced by *Azotobacter* isolates**

### DISCUSSION

With the increasing demand for the agriculture products, it has become important to increase the productivity by using various fertilizers, insecticides and pesticides. But with the tremendous use of these products, the soil has been affected very badly because of the depletion in the essential minerals in the soil. This resulted in associated environmental health problem. Hence it becomes necessary to overcome these problems through evolving alternate strategy. The best alternative to agrochemicals is biofertilizers. It has been widely recognized that is also necessary to understand the beneficial role played by biofertilizer in crop production and regaining the soil fertility through sustainable agricultural practices.

Biofertilizers are the carrier-based preparations containing mainly effective strains of microorganisms in sufficient number, which are useful for nitrogen fixation (Mahato *et al.*, 2009). Nitrogen is the only nutrient, which play a major role in synthesis of chlorophyll, aminoacids and protein building blocks, which is ultimately responsible for higher source to sink ratio. Amongst biofertilizers, *Azotobacter* strains play a remarkable role in harnessing the atmospheric nitrogen through its nitrogen fixation. (Kennedy *et al.*, 2004). Free-living nitrogen fixing bacterium *Azotobacter* plays a remarkable role, being broadly dispersed in different environments, such as soil, water and sediments (Palleroni, 1984). The different species of *Azotobacter* can be categorized in the group of the plant growth promoting rhizobacteria (PGPR) and have the potential to occupy the root system of the plants (Vessey, 2003). They play an important role in improving plant growth and yield by producing the hormones and antimicrobial substances.

*Basella alba* is a medicinal herb, and it is the most important source of new drugs. It has been used for many of its useful product since ancient times. Now-a-days, it has been utilized for the extraction of some useful constituents used in the formulation plant based drugs. (Adhikari *et al.*, 2012).

Therefore, the purpose of the present study was to evaluate the *Azotobacter* effect of on growth and yield of *B. alba*. As the strains of *Azotobacter* widely distributed in soil and water, it was attempted to isolate the drains of *Azotobacter* different types of soil.

Totally 10 *Azotobacter* isolates were isolated from marine sediments and garden soil, samples collected from Chennai. Similarly, Islam *et al.* (2008) also studied on *Azotobacter* species from different soil samples. Sandeep *et al.*, (2011) and Naik *et al.*, (2007) also isolated and characterized the *Azotobacter chroococcum* from different agro – climatic zones of Karnataka. Kalaigandhi *et al.*, (2010) observed the *Azotobacter* population in sediments of Tondi coast. The research of Ellis *et al.*, (2003) and Grandlic *et al.*, (2006) indicated abundance, diversity, and function of microbial communities in the different soil environments.

A pot experiment was conducted in green house for 90 days. The experiment was conducted in sterilized soil so that the effect of the inoculant could be studied without the interference of normal flora and fauna of the soil. In this study, the influence of *Azotobacter* isolates on growth and biomass of *Basella alba* showed that the growth parameters such as height, number of leaves, and branches, fresh weight and dry weight and biochemical parameters such as chlorophyll, nitrogen, phosphorus and potassium content of the plant

increased in the plants treated with *Azotobacter* compared to uninoculated control plants. Among the 10 isolates, Marine Sediment isolate –I showed highest activity. The increased growth could be attributed to the nitrogen fixation and production of growth hormones by *Azotobacter* species as reported by Awasthi *et al.*, 1996 and Naik, 2006.

The present results are similar to that of earlier findings, which showed improved yields of Banana varieties by using biofertilizers (Sreeramulu and Srikantaiah, 2003). Barik and Goswami (2003) reported that seed inoculation with *A.chroococcum* strains significantly influenced the growth and yield attributes. A better response was observed by Kumar *et al.*, (2001) in wheat varieties upon inoculation with *A.chroococcum*. These results were in agreement with the earlier findings (Gopal *et al.*, 2000) which showed increased Nitrogen content in *Azotobacter* inoculated plants. It is also reported earlier that there way increased growth, biomass, nitrogen and phosphorus in *Ocimum sanctum* and *Ocimum kilimandascharicum* inoculated with *G.fasciculatum*, *A.chroococcum* and *Aspergillus awamori* singly and in combinations (Vinutha, 2005). The present results are in agreement with the earlier findings of Sandeep *et al.*, (2011), Naik *et al.*, (2007) and Rahimi *et al.*, (2012), who reported that native *A.chroococcum* strains improved the growth and uptake of nitrogen and phosphorus in wheat plants.

Nitrogen level has a direct effect on chlorophyll content and many times chlorophyll content is used to determine the Nitrogen content of the plant (Eghball and Power, 1999). The effect could be due to the fact *Azotobacter sp.* has the ability to synthesize and secrete B-vitamins, growth hormones and antifungal antibiotics into its environment (Bakulin *et al.*, 2007).

The results of the present study confirm by the results of many previous investigations, that which showed biofertilization activated the microbiological processes in the soil and the part of nitrogen fertilizers could be replaced by microbiological fertilizers as suggested by Ozurk *et al.*, (2003).

## CONCLUSION

It is also concluded that the marine sediments and garden soil enhanced the growth, biomass, chlorophyll, nitrogen, phosphorus and potassium content of *B.alba*, because they enhanced the absorption of nitrogen by plant. In addition to natural abilities of plant uptake potential, they enhanced and improved the soil biodiversity and developed the biological activity and thus it is recommended as a strategy for the sustainable agricultural practices.



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