

## **Influence of Nitrogen Fixing Bacteria Incorporation with Organic and/or Inorganic Nitrogen Fertilizers on Growth, Flower Yield and Chemical Composition of *Celosia argentea***

<sup>1</sup>Rawia A. Eid, <sup>2</sup>S.A. Abo-Sedera and <sup>2</sup>M. Attia

<sup>1</sup>Ornamental Plant and Woody Trees Department,  
<sup>2</sup>Agriculture Microbiology Department,  
National Research Centre, Dokki, Cairo, Egypt

**Abstract:** Pot experiment study was conducted to ascertain the effectiveness of associative nitrogen fixing bacteria *Azospirillum* application with organic and inorganic nitrogen fertilizers on the growth parameters and marketable cut flowers of *C. argentea* during the growth seasons of 2004 and 2005. The results revealed that the population of *Azospirillum* significantly increased in all the inoculated treatments, when compared to uninoculated control. The maximum population of *Azospirillum* harbored in 75% N and *Azospirillum* treated plant, which was significantly more than all other treated plants. The same results were obtained when soil amended with FYM, which produce highest increase over unamended treatments. *Azospirillum* inoculation significantly increased the plant growth, flower yield and nutrient concentration of *C. argentea* when compared to uninoculated plants. Application of nitrogen at a rate of 75% from recommended doses to inoculated plants with *Azospirillum* further significantly increased the plant growth, yield parameters and nutrient concentration. The highest values of soluble, non-soluble sugars, indoles and phenols were obtained from leaves and flower of plants amended with FYM plus *Azospirillum* + 75% N compared with the plant amended with 75% N plus *Azospirillum* without FYM. Our results show that inflorescence marketability based on stem length is promising for *C. argentea* grown under FYM plus 75% N + *Azospirillum*. The 100% of the recommended N dose showed statistically same flower yield as that inoculation with *Azospirillum* in combination with application of 75% from recommended doses of nitrogen indicating the possibility of reducing input cost of N-fertilizers. Based on the present study it could be concluded that biofertilization of *C. argentea* with *Azospirillum* substitutes 25% of nitrogen application to soil and reduce the input cost and sustain the soil fertility.

**Keywords:** *Celosia argentea* • nitrogen fixing bacteria • *Azospirillum* • nitrogen fertilizer • organic fertilizer • growth and flower yield • chemical composition

### **INTRODUCTION**

Ornamental plants became an important crop, where the farmers began to diversify into high-value specialty crops; retail florists began asking for new and unusual flowers; consumers had more disposable income and were willing to spend some of it on flowers; more farmers' markets were established throughout the country and flowers became an integral part of those markets. The genus *Celosia* consists of about 60 species belong to family Amaranthaceae (Caryophyllales) is a native plant in subtropical and temperate zones of Africa, South America and South East Asia. *Celosia argentea* is a cultivated

annual plant that is mainly used for planting flowerbeds in different types of gardens that has become an economically important floral crop. *Celosia* grown under full light and warm conditions, reach heights up to 71 cm, tolerate a wide range of soil conditions and high levels of organic matter are required for good yields, particularly for the green type. *C. argentea* is a well known medicinal plant for treating dysentery, diarrhea, acute abdominal pain, inflamed stomach, skin eruptions [1] and exhibited antibacterial activity against, *Bacillus subtilis*, *S. aureus*, *Salmonella typhi*, *Escherichia coli*, *Agrobacterium tumefaciens* and *Mycobacterium tuberculosis* [2]. *Celosia* is also one of the main sources of natural pigments used

in several industries and in the bird-feed for poultry production.

The practical way to improve the quality of soils with a low organic matter content is of the addition of organic materials to the soil either fresh, or composted [3, 4] or farmyard manures (FYM). These materials are rich in labile carbon fractions which act as a source of energy for microorganisms also increasing soil microbial populations and their activities [5]. Organic materials also increase soil water-holding capacity and aggregation and improve the nutrient status [6]. In addition, organic matter is a necessary food for the soil organisms. The combination of organic matter and mineral fertilizers provides the ideal environmental conditions for the crop while alone is not sufficient (and often not available in large quantities) for the level of crop production the farmer is aiming at.

The genus *Azospirillum* are nitrogen-fixing organisms that live in close association with plants in the rhizosphere. The *Azospirillum*-plant association leads to the enhanced development and yield of different plants under appropriate growth conditions [7, 8]. This plant stimulatory effect exerted by *Aspergillum* has been attributed to several mechanisms, including nitrogen fixation and production of plant growth promoting substances [9]. Upon *Azospirillum* inoculation an alter in root morphology was observed which has been ascribed to the bacterial production of plant growth regulating substances [10]. An increase number of lateral roots and root hairs enlarge the root surface available for nutrients. This result in a higher nutrient uptake by inoculated roots and an improved water status of the plant, which in turn could be the nail factor enhancing plant growth [11]. Inoculation of plants with *Azospirillum* can result in a significant change in various plants growth parameters, which may affect crop yield. Worldwide data accumulated in the field over the past 30 years indicated that *Azospirillum* is capable of promoting the yield of agriculturally important crops in different soils and climatic regions. The reviewed data show statistically significant increases in yield on the order of 5-30% in 60-70% of published reports [12]. Most studies of the *Azospirillum* plant association have been conducted on cereals and grasses [13] and only a few other plants familiars have been investigated so far [14]. Although, not much information is available on the isolates from ornamental plants and their possible role in the N economy of these plants. Hence, the purpose of this investigation was to evaluate effectiveness of associative nitrogen fixing bacteria *Azospirillum* application with

organic and inorganic nitrogen fertilizers on the growth parameters and marketable cut flowers of *C. argentea*.

## MATERIALS AND METHODS

**The bacteria strain:** Associative nitrogen fixing strain of *Azospirillum brasilense*, initially isolated from the rhizosphere of maize grown in sandy soil and identified in the Agriculture Microbiology Department, National Research Centre were used. The bacterial inoculum was prepared by transferring a loopful of 48 h old culture to 50 ml semisolid N-free medium. After five days of incubation at 28°C, the entire broth was transferred to one-liter capacity Erlenmeyer flask containing 500 ml NFM. The flasks were incubated at 28°C for five days. The standard population of *Azospirillum* was adjusted  $10^7$  CFU per ml of broth medium at the time of inoculation.

**The soil used:** The soil used was a sandy loam containing 0.2% organic matter, 90.76% sand, 9.24% silt with pH 7.8 and EC ( $\text{dS m}^{-1}$ ) 0.38. N, P and K content were 0.2, 0.38 and 0.28% respectively.

**Experimental design:** One way experiment planned in Completely Randomized Block Design with four replicates was conducted in the greenhouse of the National Research Centre, Dokki, Cairo, Egypt during the growth seasons of 2004 and 2005. The soil was unamended or amended with the FYM and fertilized with or without mineral nitrogen fertilizers. Chemical properties of FYM was EC ( $\text{dS m}^{-1}$ ) 1.4, pH 7.9, moisture content 28.9%, N, P and K content were 8.5, 2.63 and 6.7 ppm, respectively. The soil was air dried and passed through 2 mm sieve before being dispensed into plastic pots ( $7 \text{ kg pot}^{-1}$ ). Five seeds of *Celosia argentea* were sown in each pot and thinned to two per pot after emergence. FYM were added at the rate of 500g per pot and mixed with soil before sowing. Inoculated pots received 2 ml of *Azospirillum* liquid inoculum for each seed and re-inoculated after three weeks from first. Uninoculated fertilized with recommended doses of ammonium nitrate (33% N) at rate of  $4 \text{ g pot}^{-1}$  applied in two equal doses after thinning and after two weeks from first. One half of the inoculated treatments were amended with 75% from recommended doses of nitrogen fertilizers. All treatments received recommended doses of phosphorus and potassium. Calcium superphosphate (15%  $\text{P}_2\text{O}_5$ ) was added at the rate of  $1.5 \text{ g pot}^{-1}$  before sowing and potassium sulphate (48%  $\text{K}_2\text{O}$ ) was added at a rate of  $1.0 \text{ g pot}^{-1}$ . After

planting, each pot received 150 ml of tap water and watered twice weekly with 200 ml pot<sup>-1</sup> of tap water. Rhizosphere and plant samples were taken at vegetative and flowering stages for enumeration of *Azospirillum* in the rhizosphere as well as plant growth parameters. Rhizosphere samples were taken arbitrarily from the four replicated pots for enumeration of *Azospirillum* spp. using the MPN technique. The method was followed according to the Cochran [15] and expressed as CFU per gram dry soil.

The following data were recorded at vegetative and flowering stages of *C. argentea* in both seasons:

- Plant height (cm)
- Fresh and dry weight of shoot and roots (g plant<sup>-1</sup>)
- Leaf area (cm<sup>2</sup>)
- Number of inflorescences and their length (cm)
- Fresh and dry weights of inflorescences (g plant<sup>-1</sup>)
- Chemical compositions

Chlorophyll (a, b) and carotenoids were determined in the fresh leaf samples according to Saric *et al.* [16]. Total soluble indoles were determined calorimetrically as described by Selim *et al.* [17]. Total soluble phenols were determined by A.O.A.C. [18]. Total anthocyanins as mg g<sup>-1</sup> dry weight were determined by using the methods of Fuleki and Francis [19] and developed by Du and Francis [20]. Soluble and non-soluble sugars (%) were determined using the methods described by Dubois *et al.* [21]. Dried leaves and Inflorescences were ground to pass through a 0.5-mm sieve for determination of mineral nutrients. Macronutrients (N, P and K) concentration in plants were determined according to Kalra and Maynard [22].

Statistical Analysis Software (SAS Institute Inc., Cary, NC). One-way ANOVA and combined analysis for the two seasons were calculated according to Snedecor and Cochran [23].

## RESULTS AND DISCUSSION

At vegetative stage, the population size of *Azospirillum* spp was increased significantly in the inoculated *Celosia argentea* plants than uninoculated control (Table 1). The highest *Azospirillum* population was recorded in the treatments inoculated with *Azospirillum* and fertilized with 75% mineral nitrogen. FYM is an important source of nutrients usable by microorganisms, as a consequence, FYM amendments generally enhanced the development of the microflora and increase the global activity of the soil [24]. The least *Azospirillum* count was recorded in the uninoculated control with or without any nitrogen fertilizers. At flowering stage, the density of *Azospirillum* in the rhizosphere of *Celosia argentea* decreased compared to vegetative stage although, inoculated plants still recorded the highest density of *Azospirillum* spp. population in the rhizosphere of *Celosia argentea*. Since, N fertilization did not modify the densities of *Azospirillum* compared to control treatment. FYM combination with 75% N plus *Azospirillum* inoculation had the highest *Azospirillum* densities (Table 1). Our finding supports the results of many scientists who found that the population size of introduced *Azospirillum* strains increased in the other rhizosphere plants [25]. As such less reports on the survivability of introduced *Azospirillum* in the *Celosia argentea* or any other flower plant [26].

Data in Table 2 show that, in the absent of FYM, *Azospirillum* inoculation significantly increased the fresh and dry weight of roots and shoots of *Celosia argentea* plants as well as leaf area and plant height when compared to uninoculated control. The above parameters increased significantly when nitrogen application at recommended dose (100%). The highest parameters values were recorded when plants received 75% N with *Azospirillum* inoculation, which significantly superior over the all other combination treatments. The least plant

Table 1: The population of *Azospirillum* spp. in the rhizosphere of *C. argentea* as influenced by organic and inorganic forms of N fertilizers

Treatments	Vegetative stage	Flowering stage
Control	32.5X 10 <sup>3</sup>	20.3x 10 <sup>2</sup>
100%N	46.9 X10 <sup>3</sup>	33.7x10 <sup>2</sup>
Azosp.	47.5X10 <sup>6</sup>	37.8x10 <sup>6</sup>
75% N + Azosp.	56.0X10 <sup>7</sup>	44.2x10 <sup>6</sup>
FYM	45.5X 10 <sup>3</sup>	38.1x10 <sup>3</sup>
FYM + 100%N	53.0X10 <sup>3</sup>	45.3x10 <sup>3</sup>
FYM + Azosp.	51.5X10 <sup>6</sup>	43.2x10 <sup>6</sup>
FYM + 75% N + Azosp.	56.5X10 <sup>7</sup>	54.7x10 <sup>7</sup>

Table 2: Effect of *Azospirillum* inoculation, organic and/or inorganic nitrogen fertilizers on growth parameters of *C. argentea* plant (average of two seasons)

Treatments	Fresh wt. (g plant <sup>-1</sup> )		Dry wt. (g plant <sup>-1</sup> )		Plant height (cm)	Leaf area (cm <sup>2</sup> )
	Shoot	Root	Shoot	Root		
Control	28.58f	3.1e	6.35e	0.7f	46.3f	11.35e
100% N	41.22e	9.3d	8.74c	7.5d	70.5d	11.92e
Azosp.	39.32c	16.3b	7.25d	4.2e	78.9c	12.58d
75% N + Azosp.	42.38c	13.2c	10.85b	9.2b	80.5b	13.21c
FYM	41.31e	10.2d	7.81d	4.3e	68.3e	11.89e
100% N + FYM	55.52d	13.4c	8.82c	7.6d	76.5d	12.38d
Azosp. + FYM	53.83b	16.0b	11.12ba	8.5cb	82.9a	14.51b
75% N + Azosp. + FYM	57.11a	19.8a	11.75a	10.1a	82.5a	15.36a

Different letters in each column indicate significant differences at  $p < 0.05$

Table 3: Effect of *Azospirillum* inoculation, organic and/or inorganic nitrogen fertilizers on inflorescences parameters of *C. argentea* (average of two seasons)

Treatments	No. of			
	inflorescences (plant)	Length (cm)	Fresh wt. (g)	Dry wt. (g)
Control	5.74	9.8f	8.04g	1.43f
100% N	11.58e	10.3fe	9.83g	1.73f
Azosp.	14.95d	13.5d	14.85e	2.06cd
75% N + Azosp.	16.35bc	16.7c	17.29d	2.59bc
FYM	6.76f	11.4e	12.56f	1.82d
100% N + FYM	17.38b	14.3d	26.35b	4.17a
Azosp. + FYM	15.51cd	18.2b	19.37c	3.08b
75% N + Azosp. + FYM	19.63a	21.3a	28.52a	4.39a

Different letters in each column indicate significant differences at  $p < 0.05$

parameters were recorded in uninoculated control without nitrogen. Application of 75% N plus *Azospirillum* significantly increased the plant vegetative growth parameters over 100% N application. The same results were obtained when the combination treatments include FYM, which produce highest increase over unamended treatments. Bhavanisanker and Vanangamudi [27] found that FYM and neem cake and NPK fertilization were effective for improving various biometric parameters of pepper plants. Improved vegetative growth of *C. argentea* grown in severely degraded sandy soils and amended with poultry manure has also been reported by Obi and Ebo [28]. A few field studies conducted elsewhere also confirmed the above results of plant parameters of other flowering plants which were increased due to *Azospirillum* inoculation [26]. Several mechanisms by which *Azospirillum* affects plant growth are proposed. Hypothetical mechanisms include hormonal influence, increased water and mineral uptake, changes in membrane function, or a combination of small mechanisms affecting the plant in concert [14]. The response of *Celosia argentea* to FYM application may also be attributed to the increasing total organic matter, macro and micronutrients rendered after the application of manure [29]. Similar results have also been reported by Wong *et al.* [30]. The

authors attributed the increase in plant dry weight may be due to the better nutrient status of N, P and K in the soils as well as in the plants also. The increase vegetative growth may be due to increased rhizosphere aggregate stability which might have favored the beneficial microbes which in turn could have contributed to improved biomass [31]. It suggests the 25% N substituted by *Azospirillum* inoculation. Similar conclusions were also made in other plant elsewhere [26, 32]. This investigation shows that *C. argentea* could be produced for commercial use in FYM plus 75% N + *Azospirillum*.

Data in Table 3 show that the marked increase in length, fresh and dry of *C. argentea* inflorescences as well as their numbers were due to the enrichment of soil fertility through FYM addition to the soil [33] and improved growth parameters and yield attributes [34]. *Azospirillum* inoculation with 75% of the chemical N dose registered higher fresh and dry weight of *C. argentea* inflorescences. Inoculation with *Azospirillum* combined with 75% of the inorganic N plus FYM application recorded highest fresh and dry weight of *C. argentea* inflorescences. The ability to produce high quality flowers with an adequate stem length is of utmost importance for any flower grower [35]. The data in Table 3 indicate that the *Azospirillum* inoculation

Table 4: Effect of *Azospirillum* inoculation, organic and/or inorganic nitrogen fertilizers on chlorophyll, total anthocyanins and carotenoids in *C. argentea* plant

Treatments	(mg g <sup>-1</sup> fresh weight)				Anthocyanine (mg g <sup>-1</sup> dry wt.)	
	Chl a	Chl b	Total Chl	Carotenoids	Leaves	Flowers
Control	0.313e	0.115c	0.428e	0.193d	17.9e	19.13e
100% N	0.416d	0.153c	0.569d	0.219c	22.8c	24.50d
Azosp.	0.432bc	0.186c	0.671c	0.241c	24.3b	28.40c
75% N + Azosp.	0.485c	0.185c	0.617c	0.315b	24.8b	29.30bc
FYM	0.437cd	0.228b	0.669c	0.198d	20.0d	19.53e
100% N + FYM	0.518b	0.257b	0.775b	0.245c	25.2a	25.20d
Azosp. + FYM	0.587ba	0.387a	0.874a	0.349b	25.2a	30.10b
75% N + Azosp. + FYM	0.590a	0.289a	0.828a	0.498a	25.3a	33.20a

Different letters in each column indicate significant differences at  $p < 0.05$

increased number, length, fresh and dry weights of inflorescences in combination with both 75% nitrogen fertilizer and FYM when compared with the 100% N plus FYM treatments without inoculation. The increase in inflorescences parameters may be due to promoted root function [36]. *Azospirillum* inoculation increased the inflorescences parameters over inorganic N alone due to atmospheric N fixation [33] and reduction in the N loss [37]. Inoculation with *Azospirillum* and application of 75% inorganic N substantially increased inflorescences parameters over 100% of inorganic N alone. This indicated that inoculation of *Azospirillum* could reduce the bill of inorganic fertilizer N by 25%. Barr [35] suggested that the commercial standard for stem length for cut flowers in general is 41 cm (16 in.). Our results show that marketability based on stem length is promising for *Celosia argentea* grown under FYM plus 75% N + *Azospirillum*. In fact, *Azospirillum* may be used to excessive stem lengths which occurs when plants are produced in the 100% N fertilizer plus FYM and even in 75% N fertilizer plus *Azospirillum*. In this sense, *Azospirillum* functions as a natural alternative to growth regulators and encourages the efficiency use of nitrogen in the rhizosphere of *Celosia argentea*. Devitt and Morris [38] found that plant height for *C. argentea* L. ranged from 49 cm in 100% N to 30.4 cm in 50% N under greenhouse conditions.

Recorded results in Table 4 regarding leaf chlorophyll (a & b), carotenoids and anthocyanine content of *C. argentea* followed the same pattern observed for plant growth responses. Plants inoculated with *Azospirillum* had greater total chlorophyll, carotenoids and anthocyanine content than uninoculated plants (Table 4). Chlorophyll values range from 0.428 to 0.874 mg g<sup>-1</sup> fresh

weight. In soil amended with FYM, greatest leaf chlorophyll, carotenoids and anthocyanine occurred with the 75% N plus *Azospirillum*. Plants fertilized with 100% N had greater leaf chlorophyll and carotenoids than uninoculated or inoculated with *Azospirillum*. Results of chlorophyll analysis and carotenoids coincide with unamended FYM in the different fertility treatments. The data recorded in Table 4 indicate clearly that all treatments increased the content of anthocyanins in both leaves and flowers compared to control. Also, the anthocyanin content increased gradually in plant amended with FYM plus *Azospirillum* + 75% N, which showed the highest values in this respect. Leaf area and chlorophyll content vary according to mineral status (N, P, K) of plants [39]. Increase leaf area and chlorophyll content and carotenoids of inoculated plants were related to improve N uptake.

The effect of inoculation with *Azospirillum* on photosynthetic pigments in inoculated plants is solely recorded as increased of total chlorophyll content of the inoculated plants, whether in higher plants [40, 41] or single-cell plants [42, 43]. Carotenoids act as light-harvesting molecules inside the cell, allowing the efficient utilization of the light spectrum [44]. Besides this, carotenoids protect the pigment-protein complexes and the chloroplast against photo oxidation [45].

Data in Table 5 showed that soluble, non-soluble sugars, indoles and phenols were markedly improved in the leaves and flower of *Celosia argentea* plants grown in soil amended with FYM. The highest values were obtained from leaves and flower of plants amended with FYM plus *Azospirillum* + 75% N compared with the plant amended with 75% N plus *Azospirillum* without FYM. The beneficial effect of compost on sugars and

Table 5: Effect of *Azospirillum* inoculation, organic and/or inorganic nitrogen fertilizers on total soluble, non-soluble sugars, indoles and phenols of *C. argentea* plants

Treatments	Soluble sugars %		Non-soluble %		Total indoles (mg g <sup>-1</sup> f.w)	Total phenols (mg g <sup>-1</sup> f.w)
	Leaves	Inflorescences	Leaves	Inflorescences		
Control	0.311e	0.543f	4.21d	1.89c	13.15c	2.13c
100%N	0.357e	0.741d	5.32c	2.18b	13.18c	2.18c
Azosp.	0.321e	0.893c	6.18b	2.54b	14.15b	3.15b
75%N + Azosp.	0.521bc	0.934c	6.34b	2.58b	14.93b	3.18b
FYM	0.431d	0.753d	6.42b	2.11b	13.18c	2.19c
100%N + FYM	0.493cd	0.694e	7.31a	3.13a	14.15b	2.93bc
Azosp. + FYM	0.596b	1.034b	7.81a	3.74a	15.23a	3.18b
75%N + Azosp. + FYM	0.611a	1.931a	6.31b	3.24a	15.31a	3.94a

Different letters in each column indicate significant differences at p<0.05

Table 6: Effect of *Azospirillum* inoculation, organic and/or inorganic nitrogen fertilizers on NPK concentration in *C. argentea* plant

Treatments	(mg g <sup>-1</sup> dry weight)					
	Leaves			Inflorescences		
	N	P	K	N	P	K
Control	3.74e	0.19e	1.127c	4.48e	0.28e	2.137e
100%N	4.32d	0.25d	1.634c	5.24d	0.37d	2.644d
Azosp.	6.17b	0.30bc	2.52b1	7.28b	0.40bc	3.531b
75%N + Azosp	4.58d	0.27c	2.153b	5.68	0.39cd	3.163c
FYM	4.85d	0.24d	1.635c	5.37d	0.31e	2.635d
100%N + FYM	5.32c	0.28c	2.210b	6.21c	0.36d	3.210bc
Azosp. + FYM	7.71a	0.35a	3.132a	8.85a	0.46a	4.142a
75%N + Azosp. + FYM	5.58c	0.33ab	2.835a	6.82bc	0.42b	3.844ab

Different letters in each column indicate significant differences at p<0.05

carbohydrates were stated by El-Ashry *et al.* [46] on *Peperomia obtusifolia* and Herrera *et al.* [47] on three medicinal plants. Mona [48] found that, on *Rosmarinus officinalis* L., the increments in soluble and non-soluble sugars were associated with the increase in the level of applied compost in soil.

FYM incorporation recorded higher total soluble and non-soluble sugars compared to unamended. These increases parameters might be due to ensured sustainable supply of nutrients and favorable soil condition. The highest loss of total soluble, non-soluble sugars, indoles and phenols were observed in non-amended soil with FYM. Regarding to biofertilizer and inorganic N, *Azospirillum* with 75% of the recommended N resulted in higher total soluble, non-soluble sugars, indoles and phenols. *Azospirillum* inoculation in combination with 75% of the recommended inorganic N dose recorded significantly more total soluble, non-soluble sugars, indoles and phenols.

Both *Azospirillum* inoculation and mineral nitrogen fertilizers affected plant mineral concentration (Table 6). In general, *Azospirillum* inoculated plants had higher N, P and K than uninoculated. With FYM the *Azospirillum* inoculated plants gave the highest mean values of NPK concentration compared with the other treatments included the control. The inoculated *Azospirillum* capable of fixing the atmospheric nitrogen and producing the plant growth hormones [26]. Inoculated organisms increased the N-availability in the rhizosphere soil with concomitant increase in the N concentration by the plant also (Table 6). In the present study, the *Azospirillum* inoculation significantly increased the N, P and K concentration in plant than uninoculated control plants. The interaction between the *Azospirillum* inoculation and nitrogen levels also significant. Where in the treatments receiving *Azospirillum* and 75% N showed the highest N, P and K concentration when compared to the other of the treatments. However, lowest

N, P and K concentration recorded in the uninoculated control with no nitrogen application. The highest nitrogen N, P and K concentration noticed in the plant receiving 75% N plus *Azospirillum* inoculation closely followed by 100% N, both of which statistically compared with each other indicating that application of 75% N plus *Azospirillum* has same effect as that of only 100% N. Similarly increase in N-uptake also reported in the plant species due to *Azospirillum* inoculation [49]. As mentioned before, data in Table 6 indicate that the concentrations of N, P and K were increased in *C. argentea* plants with FYM application.

### CONCLUSIONS

The *Azospirillum* inoculation significantly increased the growth and inflorescences parameters of *C. argentea* over uninoculated control plants. Application of 75% of N plus *Azospirillum* inoculation treatment results highest plant fresh, dry weights and flower characters than 100% N without inoculation, which indicates the possibility of saving 25% nitrogen fertilizer. *Azospirillum* inoculated plants also showed 13% higher dry weight and 23% higher over uninoculated control. On the other hands, the application of organic N fertilizer produced the same positive yield response from flowers as those produced by application of inorganic N fertilizer. This is related to the availability of N in the fertilizers applied [50]. The inorganic fertilizer studied released their N almost instantaneously on addition to the soil. The FYM appeared to behave similarly. Several workers have reported, for a range of vegetable crops, that organic N fertilizers produced yields similar to those produced by inorganic N fertilizers. However, N applied at all rates plus FYM produced yields significantly higher than those unamended.

The difference between N supplied in FYM and other N fertilizers is also reflected in the higher N concentration of plants fertilized with FYM plus 75% N + *Azospirillum* compared to those fertilized with the other N fertilizers. Comparing the two forms of N fertilizers, indicated that significant higher yield was obtained with FYM plus 75% N + *Azospirillum* compared with other treatments, may be attributed to the higher N concentration and lower C:N ratio of FYM used in the present study.

### REFERENCES

1. Anonymous, 2000. Healing power of Nature. Herald of Health, June, 27.

2. Bhakuni, D.S., M.L. Dhar, M.M. Dhar, B.N. Dhawan and B.N. Mehrotra, 1969. Screening of Indian plants for biological activity: Part II. Indian Journal of Experimental Biology, 7: 250-262.
3. Garcia, C., T. Hernandez, A. Roldan, J. Albaladejo and V. Castillo, 2000. Organic amendment and mycorrhizal inoculation as a practice in afforestation of soils with pinus halepensis miller: effect on their microbial activity. Soil Biol. Biochem., 16: 1173-1181.
4. Pascual, J.A., C. Garcia and T. Hernandez, 1999. Comparison of fresh and composted organic waste in their efficacy for the improvement of arid soil quality. Bioresource Technol., 68: 255-264.
5. Papavizas, G.C., R.D. Lumsden, 1982. Improved medium for isolation of *Trichoderma* spp. from soil. Plant Disease, 66: 1019-1020.
6. Ros, M., C. Garcia and T. Hernandez, 2001. The use of organic wastes in the control of erosion in a semiarid Mediterranean soil. Soil Use Manag., 17: 292-293.
7. Steenhoudt, O. and J. Vanderleyden, 2000. *Azospirillum*, a free living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. FEMS Microbiol. Rev., 24: 487-506.
8. Awad M. Nemat, Azza Sh Turky and A. Mazhar, 2004. Effects of bio- and chemical nitrogenous fertilizers on yield of anise (*Pimpinella anisum*) and biological activities of soil irrigated with agriculture drainage water Egypt. J. Soil Sci., In press
9. Okon, Y. and R. Itzigsohn, 1995. The development of *Azospirillum* as a commercial inoculants for improving crop yields. Biotechnol. Adv., 13: 365-374.
10. Umalia-Garcia, M., D.H. Hubbell, M.H. Gaskins and F.B. Gazzo, 1980. Association of *Azospirillum* with grass roots. Appl. Environ. Microbiol., 29: 219-226.
11. Fallik, E., S. Sarig and Y. Okon, 1994. Morphology and physiology of plants roots associated with *Azospirillum*, In Y. Okon (ed.). *Azospirillum*/plant associations CRC Press Boca Raton, FL., pp: 77-86.
12. Okon, Y. and C.A. Labandera-Gonzales, 1994. Agronomic applications of *Azospirillum*. An evolution of 20 years worldwide field inoculation. Soil Biol. and Biochem., 26: 1591-1601.
13. Alagawadi, A.R. and P.U. Krishnaraj, 1988. Field performance of two local rhizobacteria isolates in sorghum. In Association of Microbiologists of India Manglore, pp: 278.

14. Bashan, Y., G. Holguin and L.E. de-Bashan, 2004. *Azospirillum*-plant relationships: physiological, molecular, agricultural and environmental advances (1997-2003). *Can. J. Microbiol.*, 50: 521-577.
15. Cochran, W.G., 1950. Estimation of bacterial density by means of the most probable number. *Biometrics*, 6: 105-106.
16. Saric, M., R. Kastrori, R. Curic, T. Suplna and L. Geric, 1976. Chlorophyll determination Univ-u. Noven Sadu Praktikum is Fiziologizebil. Jaka, Beogard, Haucna, Anjiga, pp: 215.
17. Selim, H., H.A. Fayek and M.A. Sweidan, 1976. Reproduction of Bircher apple cultivar by layering. *Ann. Agric. Sci. Moshtohor, Egypt*, 9: 72-82.
18. A.O.A.C., 1980. Official Methods of Analysis of Association of Official Analytical Chemists. 12th Ed. Washington. D.C.
19. Fuleki, T. and F.J. Francis, 1968. Quantitative methods for anchocyanine. *J. Foot Sci.*, 23: 72-78.
20. Du, C.T. and F.J. Francis, 1973. Anchocyanin of roselly. *Foot Sci.*, 38: 810-812.
21. Dubois, M., K.A. Cilles, I. Humilton, R. Rebers and F. Smith, 1956. Calorimetric method for determination of sugar and related substances. *Anal. Chem.*, 28: 35-59.
22. Kalra, Y.P. and D.G. Maynard, 1991. Methods Manual for Forest Soil. Forestry Canada Northern Forestry Centre.
23. Snedecor, G.W. and W.G. Cochran, 1980. Statistical Methods. 7th Ed. Lowastate College Press, Iowa, USA.
24. Bailey, K.L. and G. Lazarovits, 2003. Suppressing soil-born diseases with residue management and organic amendments. *Soil and Tillage Res.*, 72: 169-180.
25. Hemavathi, M., 1997. Effect of organic manures and biofertilizer on growth and productivity if chrysanthemum (*Chrysanthemum morifolium* Ramat) Cv. Local Yellow. M.Sc. Thesis, Univ. Agric. Sci., Bangalore.
26. Gadagi, Ravi, 1999. Studies on *Azospirillum* isolates of ornamental plants and their effect on *Gaillardia pulchella* var *picta* fouger, Ph.D. Thesis, UAS, Dharwad.
27. Bhavanisanker, K. and K. Vanangamudi, 1999. Integrated nutrient management in gundumalli (*Jasminum sambac* L.). *South Indian Horticulture*, 47: 111-114.
28. Obi, M.E. and P.O. Ebo, 1995. The effects of organic and inorganic amendments in soil physical properties and maize production in a severely degraded sandy soil in southern Nigeria. *Bioresour. Technol.*, 51: 177-123.
29. Awad M. Nemat and Kh S.M. Fawzy, 2004. Assessment of sewage sludge application on microbial diversity, soil properties and quality of wheat plants grown in a sandy soil. *Egypt. J. Soil Sci.*, In press.
30. Wong, J.W.C., K.K. Ma, K.M. Fang and C. Cheung, 1999. Utilization of a manure compost for organic farming in Hong Kong. *Bioresour. Technol.*, 67: 43-46.
31. Caravaca, F., T. Hernandez, C. Garcia and A. Roldan, 2002. Improvement of rhizosphere aggregate stability of afforested semi-arid plant species subjected to mycorrhizal inoculation and compost addition. *Geoderma*, 108: 133-144.
32. Tippannavar, C.M. and A.R. Alagawadi, 1998. Influence of *Azospirillum* inoculation in combination with reduced levels of nitrogen on rabi sorghum. In 39th annual conference, Association of Microbiologist on India, Manglore, December, 5-7, pp: 256.
33. Gopalsamy, G. and P. Vidyasekaran, 1987. Effect of green leaf manure on soil fertility and rice yield. *IRRN.*, 12: 41-45.
34. Thiagarajan, M., 1991. Yield maximization in rice through green manuring, plant duration and time of phosphorus application. M.Sc. (Ag.) Thesis, Tamil Nadu Agric. Univ., Coimbatore.
35. Barr, C., 1992. The kindest cuts of all: how to evaluate new crops. *Greenhouse Manager.*, 11: 82-84.
36. Abdel Salam, M.S. and W. Klingmuller, 1987. Transposon  $Tn^5$  mutagenesis *Azospirillum lipoferum* isolation of indole actic acid mutants. *Molecular Gen.*, 210: 165-170.
37. Murali, K.J. and D. Purushothaman, 1987. Effect of *Azospirillum* inoculation on upland rice. *IRRN.*, 12: 6-34.
38. Devitt, D.A. and R.L. Morris, 1987. Morphological response of flowering annuals to salinity. *J. Am. Soc. Hort. Sci.*, 122: 951-955.
39. Taiz, L. and E. Zeiger, 1998. Plant physiology. 2nd Ed. Sinauer, Sunserland, Mass.
40. Omar, M.N.A., P. Fang and X.M. Jia, 2000. Effect of inoculation with *Azospirillum* brasilense NO40 isolated from Egyptian soils on rice growth in China. *Egypt. J. Agric. Res.*, 78: 1005-1014.



41. Panwar, J.D.S. and O. Singh, 2000. Response of *Azospirillum* and *Bacillus* on growth and yield of wheat under field conditions. Indian J. Plant Physiol., 5: 108-110.
42. de-Bashan, L.E., Y. Bashan, M. Moreno, V.K. Lebsky and J.J. Bustillos, 2002. Increased pigment and lipid content, lipid variety and cell and population size of the microalgae *Chlorella* spp. when co-immobilized in alginate beads with the microalgae-growth promoting bacterium *Azospirillum brasilenses*. Can. J. Microbiol., 48: 514-521.
43. Gonzalez, L.E., Y. Bashan, 2000. Increased growth of the microalga *Chlorella vulgaris* when coimmobilized and cocultured in alginate beads with the plant growth-promoting bacterium *Azospirillum brasilense*. Appl. Environ. Microbiol., 66: 1527-1531.
44. Porra, R.J., E.E. Pfundel, N. Engel, 1997. Metabolism and function of photosynthetic pigments. In: Jeffrey S.W., R.F.C. Mantoura and S.W. Wright (eds). Phytoplankton pigments in oceanography: guidelines to modern methods. Monographs on oceanographic methodology. UNESCO Publishing, Paris, 10: 85-126.
45. Demmig-Adams, B., 1990. Carotenoids and photoprotection in plants: a role for the xanthophylls zeaxanthin. Biochim. Biophys. Acta., 1020: 1-24.
46. El-Ahsry, A.I., M.S. Auda and M.Y. Bakry, 1997. Effect of different growing media on growth and production of some foliage plants. 1-Response of *Pepermia obtusifolia* to different growth media. Egypt. J. Appl. Sci., 12: 146-159.
47. Herrera, E., N. Tremblay, B. Desroches and A. Cosselin, 1997. Optimization of substrate and nutrient solution for organic cultivation of medicinal transplants in multicell flats. J. Herbs, Spices and Medicinal Plants, 4: 69-82.
48. Mona Y. Khalil, 2002. Biochemical studies on *Rosmarinus officinalis* L. plant tolerance to salinity under compost levels. Ann. Agric. Sci. Ain Shams Univ. Cairo, 47: 893-909.
49. James, E.K., 2000. Nitrogen fixation in endophytic and associative symbiosis. Field Crops Research, 65: 197-209.
50. Goh, K.M. and P. Vityakon, 1983. Effects of fertilizers on vegetable production. 1. Yield of spinach and beetroot as affected by rates and forms of nitrogenous fertilizers. NZ J. Agric. Res., 26: 349-356.