J. Phytol. Res. **15** (2) : 155-160, 2002

USEFULNESS OF BIOFERTILIZERS IN ECONOMISING NITROGENOUS FERTILIZERS IN *TAGETES ERECTA L.*

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The effect of *Azotobacter* and *Azospirillum* biofertilizers in marigold under different levels of chemical nitrogen was investigated. Both bacterial inoculants responded to all levels of chemical nitrogen with an increase in growth, yield and biochemical attributes as compared to corresponding control. *Azospirillum* inoculation with low-level chemical nitrogen (50%) markedly increased growth, yield and biochemical attributes over control. *Azotobacter* with low level chemical nitrogen (50%) also showed better performance but not comparable to *Azospirillum* with low level chemical nitrogen (50%). However, the performance was better with respect to 50% level of chemical nitrogen, resulting in a nitrogen economy of 50%.

Keywords : Biochemical parameters; Biofertilizers; Growth; Marigold; Urea; Yield.

Introduction

Agriculture is one of the major occupations of mankind all over the world. Men, depend upon agriculture for his food, and clothing. In India nearly 70% of the people are engaged in agricultural practices.

All plants need nitrogen compounds as it forms the major constituent of proteins. The demand for fertilizers is increasing day by day with the increasing in the human population, hence, the synthetic fertilizers are used in large scale by the agriculturists of India. They pollute the land and contribute to biological magnification, hence there is a great need to develop new methods by which other fertilizers could be used to enhance the good grain production.

In recent years, use of microbial inoculants as a source of biofertilizers has become a hope for most of the countries, as far as economical and environmental viewpoints are concerned[1] .Biologically fixed nitrogen is such a source, which can supply an adequate amount of nitrogen to plants and other nutrients to some extent[1] . The free-living bacteria (*Azotobacter*) associate (*Azopirillum*) and symbiotic (*Rhizobium*) bacteria are gaining much popularity and such practices being encouraged to save the chemical fertilizers, national economy and the environment [2] . The various graded levels of nitrogen and bioinoculants have increased growth and yield of marigold[3] . The performance was better with respect to 75% levels to chemical nitrogen, resuting a nitrogen economy of 25%[4]. *Azospirillum* inoculation has been reported to significantly increase the growth, yield, nutrient uptake, dry matter and vitamin C contents in cabbage, Cauliflower, tomato and chilli[5-8] . Similarly inoculation with *Azotobacter* has also exhibited an increase of growth, yield and quality attributes of various vegetables[9] . Moreover, both the inoculants are reported to be economical in saving 50% of the recommended dose of nitrogen fertilizer [7-10] . Orange and yellow flowered local variety marigold is one of the important ornamental plants, belonging to the family Asteraceae. Limited work has been done so far on marigold (*Tagetes erecta L.*), the peritnent information regarding growth and biochemical analysis like chlorophyll content, total sugar, total free amino acids and nitrate reductase activity was not avilable. Therefore the present investigation was carried out to examine the effect of biofertilizers alone as well as in combination of Urea on the assess of growth, yield and quality attributes in one cultivar of marigold.

Material and Methods

This experiment has been conducted during the rabi season at the place adherent to the

glass house field at St. Joseph's College, Tiruchirappalli. The area taken for the experiment was 18 sq. m. Three random soil samples were taken from the field before starting the experiment and are tested in soil testing laboratory at Tiruchirappalli, to find out the soil pH and the amount of nutrients present that soil. The experiment was conducted in three replications. Eight different treatments separately and in combination such as T_0 (control (100% Urea), T_1 (*Azotobacter*), T_2 (*Azospirillum*), T_3 (50% Urea), T_4 (50% Urea with *Azotobacter*), T_5 (100% Urea with *Azotobacter*), T_6 (50% Urea with *Azospirillum)* and T_7 (100% Urea with *Azospirillum*) of the recommended dose. Recommended doze of fertilizer included 45 N: 90 P_2 O: 75 K_2 O Kg/ha and FYM 15 t/ha. Full dose of phosphorus, potash and 50% of the nitrogen was applied as basal dose. While remaining 50% of the nitrogen was applied as top dressing after 20 days of transplanting. Carriers based inoculants, procured from the Stan's company Ltd. Tiruchirappalli, and were used as seedling inoculant (200 gm in 50 ml of rice gruel). The local variety of organge and yellow flowered marigold was cultivated. Observations were undertaken from random sample of 12 to 16 plants/plot from each treatment. Plant height, leaf number, leaf length and number of flower buds of all the 12 to 16 plants was counted and thus the plant height (cm)/plant, leaf number/plant, leaf length (cm)/plant, number of flower buds/plants and also biochemical analysis was worked out, Chlorophyll content (100 mg of fresh leaves) was estimated by Arnon[11] method, total free aminoacids (200 mg of plant material) was estimated following the method of Troll and Cannon[12]. Total sugars (200 mg matured dried leaves) by the method of Dubois et al.[13] and nitrate reductase activity (200 mg of fresh leaves) was estimated using the method of Haugeman and Hucklesby [14], from each treatment. The data weas subjected to 10th, 20th and 30th days of variance in order to test the significance of results.

Results and Discussion

Effect of growth and yield attributes : Biofertilizers had a beneficial effect on growth attributes of marigold. Both *Azospirillum* and *Azotobacter* and 100% urea resulted in a significant increase in plant height/plant over control at 10th days. In 20th and 30th *Azospirillum* with 50% urea and 100% urea increased the plant height/ plant significantly over the control (Table 1). Similar results were also made by Chatto *et. al*[4] .

Azospirillum with 100% urea increased the leaf length/plant significantly over the control of 10th day. Leaf length per plant was significantly enhanced by *Azospirillum* with 50% urea in both the 20th and 30th day (Table 1). This significant increase in growth attributes on 20th and 30th days was due to nitrogenous materials secreted by the microbial inoculation, which in turn might have lead to better root development, better transportation of water, uptake and deposition of nutrients[15]. The response of *Azospirillum* with 50% and 100% urea was enhanced in leaf no./plant over the control at 10th ,20th and 30th day (Table.1). Similar results were obtained in other crops[16,17].

Yield of marigold (flower bud formation) shows a good response of bacterial inoculants. *Azospirillum* and *Azotobacter* with 50% and 100% urea resulted in a significant increase in 55 days after treatment (Table 1). Some other related results were obtained in general, the growth and yield attributes exhibited maximum values in treatments of phosphorus solibilising bacteria and seedling treatments in combination with 75% and 100% nitrogen application[3,18,19].

Effect of biofertilizers on biochemical attributes : Biochemical parameters of marigold showed a good response of bacterial inoculants, *Azospirillum* and *Azotobacter* with 50% and 100% urea

Table 1. Effect of *Azotobacter, Azospirillum* and urea on growth and yield (no. of flower buds) attributes of *Tagetes erecta*.

S. No.	Parameters	Treatments Mean ± SD							
		T0	T1	T2	T3	T4	T5	T6	T7
1.	**Plant height (cm)**								
	(a) 10th day	21.0 ± 4.6	20.0 ± 2.6	22.0 ± 1.0	19.5 ± 1.57	21.3 ± 2.0	22.6 ± 1.87	22.5 ± 2.36	22.7 ± 3.45
	(b) 20th day	55.0 ± 3.0	56.5 ±1.5	57.4 ± 1.12	53.1 ± 2.66	65.0 ± 3.0	62.5 ± 2.29	70.7 ± 2.04	68.5 ± 3.27
	(c) 30th day	63.3 ± 4.6	65.5 ± 1.6	73.0 ± 1.0	61.8 ± 2.36	73.8 ± 3.78	71.9 ± 3.26	79.3 ± 3.60	73.7 ± 2.04
2.	**Leaf Length (cm)**								
	(a) 10th day	4.2 ± 0.2	4.2 ± 0.2	4.3 ± 0.34	4.0 ± 0.2	4.3 ± 0.2	4.3 ± 0.3	4.4 ± 0.36	4.6 ± 0.85
	(b) 20th day	4.9 ± 0.1	5.3 ± 0.36	5.6 ± 0.2	4.7 ± 0.3	6.00 ± 0.3	6.4 ± 0.2	6.7 ± 0.3	6.5 ± 0.4
	(c) 30th day	5.9 ± 0.4	6.1 ± 0.1	6.5 ± 0.26	5.5 ± 0.36	7.2 ± 0.4	7.1 ± 0.3	7.4 ± 0.52	7.3 ± 0.52
3.	**Leaf Numbers**								
	(a) 10th day	14 ± 1.0	14 ± 1.0	15 ± 3.0	12 ± 0.52	16 ± 2.0	15 ± 3.0	19 ± 2.0	17 ± 0.87
	(b) 20th day	25 ± 2.6	24 ± 1.0	26 ± 2.0	24 ± 2.0	30 ± 3.0	28 ± 2.08	34 ± 3.0	32 ± 1.27
	(c) 30th day	30 ± 2.0	28 ± 2.0	32 ± 4.0	28 ± 2.64	48 ± 2.0	47 ± 2.64	56 ± 2.12	52 ± 1.77
4.	**No. of Flowers**	9 ± 1.0	11 ± 1.0	13 ± 2.0	5 ± 0.4	15 ± 3.0	14 ± 2.0	17 ± 2.0	16 ± 0.96

T_0 - Control, T_1 - *Azotobacter*, T_2 - *Azospirillum*, T_3 - 50% urea, T_4 - 50% Urea + *Azotobacter*,
T_5 - 100% Urea + *Azotobacter*, T_6 - 50% Urea + *Azospirillum*, T_6 - 100% Urea + *Azospirillum*.

Table 2. Effect of *Azotobacter*, *Azospirillum* and urea on biochemical attributes of *Tagetes erecta*.

S. No.	Parameters	Treatments Mean ± SD							
		T0	T1	T2	T3	T4	T5	T6	T7
1.	**Chl.a (mg/g.F.Wt)**								
	(a) 10th day	3.94 ± 0.29	2.7 ± 0.26	3.5 ± 0.36	3.5 ± 0.3	4.9 ± 0.1	4.7 ± 0.21	5.20 ± 0.3	4.25 ± 0.27
	(b) 20th day	4.78 ± 0.23	5.33 ± 0.32	6.22 ± 0.23	4.06 ± 0.26	7.04 ± 0.44	6.98 ± 0.50	7.75 ± 0.42	7.51 ± 0.18
	(c) 30th day	4.95 ± 0.15	5.71 ± 0.25	6.47 ± 0.45	5.57 ± 0.30	7.75 ± 0.39	7.49 ± 0.47	8.76 ± 0.67	8.38 ± 0.192
2.	**Chl.b (mg/g.F.Wt)**								
	(a) 10th day	4.58 ± 0.40	3.43 ± 0.15	3.89 ± 0.17	3.72 ± 0.47	7.33 ± 0.35	7.09 ± 0.20	8.47 ± 0.30	8.09 ± 0.37
	(b) 20th day	5.72 ± 0.62	4.80 ± 0.2	5.26 ± 0.26	4.58 ± 0.15	8.93 ± 0.12	9.02 ± 0.60	10.8 ± 0.2	10.5 ± 0.75
	(c) 30th day	6.87 ± 0.23	8.03 ± 0.11	8.79 ± 0.47	5.95 ± 0.51	11.8 ± 0.2	11.45 ± 0.52	13.28 ± 0.39	12.4 ± 0.65
3.	**Total Chlorophyll (mg/g.F.Wt)**								
	(a) 10th day	252.7 ± 1.75	171.2 ± 2.53	219.9 ± 1.87	227.8 ± 1.92	319.2 ± 2.90	303.6 ± 2.66	336.3 ± 0.7	480.3 ± 1.85
	(b) 20th day	301.2 ± 2.45	341.1 ± 1.85	397.6 ± 2.81	260.7 ± 2.46	465.0 ± 3.40	395.2 ± 3.30	498.7 ± 1.15	482.5 ± 1.32
	(c) 30th day	318.8 ± 1.61	369.9 ± 2.78	416.7 ± 3.27	299.9 ± 2.85	499.5 ± 3.27	483.3 ± 2.86	565.0 ± 1.04	540.2 ± 0.76

T_0 - Control, T_1 - *Azotobacter*, T_2 - *Azospirillum*, T_3 - 50% urea, T_4 - 50% Urea + *Azotobacter*,
T_5 - 100% Urea + *Azotobacter*, T_6 - 50% Urea + *Azospirillum*, T_6 - 100% Urea + *Azospirillum*.

Table 3. Effect of *Azotobacter, Azospirillum* and urea on biochemical attributes of *Tagetes erecta*.

S. No.	Parameters	Treatments Mean ± SD							
		T0	T1	T2	T3	T4	T5	T6	T7
1.	**Total Sugar (µg/mg.D.Wt)**								
	(a) 10th day	0.60 ± 0.02	0.58 ± 0.03	0.61 ± 0.04	0.55 ± 0.03	0.70 ± 0.04	0.69 ± 0.03	0.76 ± 0.04	0.72 ± 0.02
	(b) 20th day	0.62 ± 0.04	0.63 ± 0.09	0.68 ± 0.03	0.60 ± 0.02	0.77 ± 0.03	0.75 ± 0.05	0.81 ± 0.03	0.79 ± 0.03
	(c) 30th day	0.79 ± 0.09	0.85 ± 0.01	0.98 ± 0.11	0.71 ± 0.02	1.50 ± 0.05	1.47 ± 0.03	1.55 ± 0.05	1.58 ± 0.06
2.	**Total free amino acids(mg/mg.F.Wt)**								
	(a) 10th day	01.8 ± 0.04	01.8 ± 0.04	0.20 ± 0.04	0.16 ± 0.01	0.23 ± 0.02	0.21 ± 0.02	0.31 ± 0.02	0.29 ± 0.01
	(b) 20th day	0.27 ± 0.02	0.28 ± 0.04	0.31 ± 0.03	0.22 ± 0.03	0.41 ± 0.02	0.39 ± 0.03	0.52 ± 0.01	0.49 ± 0.02
	(c) 30th day	0.35 ± 0.05	0.38 ± 0.02	0.41 ± 0.04	0.32 ± 0.03	0.51 ± 0.03	0.49 ± 0.04	0.63 ± 0.04	0.59 ± 0.01
3.	**Nitrate reductase** activity (N.R.A/mgF.Wt)								
	(a) 10th day	0.08 ± 0.01	0.09 ± 0.01	0.11 ± 0.01	0.07 ± 0.02	0.12 ± 0.02	0.13 ± 0.02	0.15 ± 0.04	0.14 ± 0.03
	(b) 20th day	0.17 ± 0.03	0.14 ± 0.02	0.21 ± 0.02	0.13 ± 0.02	0.26 ± 0.04	0.25 ± 0.04	0.33 ± 0.02	0.29 ± 0.01
	(c) 30th day	0.24 ± 0.02	0.27 ± 0.02	0.34 ± 0.03	0.30 ± 0.03	0.41 ± 0.02	0.39 ± 0.03	0.47 ± 0.01	0.44 ± 0.02

T_0 - Control, T_1 - *Azotobacter*, T_2 - *Azospirillum*, T_3 - 50% urea, T_4 - 50% Urea + *Azotobacter*,
T_5 - 100% Urea + *Azotobacter*, T_6 - 50% Urea + *Azospirillum*, T_6 - 100% Urea + *Azospirillum*.

resulted in significant increase in chl a, chl b, and total chlorophyll contents over the control in 10th, 20th and 30th days, whereas *Azospirillum* with 50% urea showed a significant increase over control and other treatments in 20th, 30th days (Table 2). The increase in biochemical attributes like chlorophyll contents could be due to more leaf number and leaf length (more photosynthetic area). This result corroborates with the findings of other workers in different crops[4,7].

The increase in biochemical attributes like total sugar content in *Azospirillum* with 50% and 100% urea and 10th, 20th and 30th day (Table 3) while increase in sugar content could be due to the increased efficiency of nicrobial inoculants to fix atmospheric nitrogen and secrete growth promoting substances which accelerates the physiological processes like synthesis of carbohydrates. This result was found in chilli[5]. Increasing levels of introgen resulted in improvement of biochemical attributes of marigold. In 10th, 20th, and 30th days, higher total free amino acid content was recorded at *Azospirillum* with 50% urea and 100% urea level and was significantly higher than control and other treatment (Table 3).

There was gradual response in biochemical attrubutes of marigold to the increasing levels of 50% urea with *Azospirillum*. Maximum nitrate reductase activity was recorded at 10th, 20th and 30th days, which was significantly higher than the NRA recorded at other levels (Table 3). This result corroborates with the findings of other workers on different crops [16, 20]. Interaction between bacterial inoculants and applied nitrogen with respect to growth, yield and biochemical attributes were significant.

Acknowledgements

We are grateful to Dr. N. Jayabalan, Reader, Department of Plant Biotechnology, Bharathidasan University, Tiruchirappalli for his valueable suggestion. We are thankful to Dr. Muthusamy, Dr. Jeyakumar and Mr. Ganesan for their help in various ways.

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