BIOFERTILIZERS AS PARTIAL ALTERNATIVE OF CHEMICAL FERTILIZER FOR *Catharanthus roseus* G. Don.

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ABSTARCT

Catharanthus roseus plants were treated with four different rates of nitrogen fertilizer (0, 28, 56, and 84 kg N/fed) and eight biofertilizer treatments (untreated, Azotobacter, Microben, Nitroben, phosphate solubilizing bacteria (PSB), Azotobacter + PSB, Microben + PSB, and Nitroben + PSB) either individually or in combined treatments. The obtained data indicated that nitrogen chemical fertilizer, when used individually, significantly increased the vegetative growth and enhanced the chemical composition of Catharanthus roseus. The medium rate of chemical fertilizer (56 kg N/fed) gave the best results.

The use of biofertilizers alone led to a significant increase in vegetative growth and chemical composition of the tested plants as compared with the control treatment. The best biofertilizer treatment was Azotobacter + PSB. The interaction between chemical fertilizer and biofertilizer was also significant. It should be noted that the use of biofertilizers led to a significant increase in the efficiency of chemical fertilizers. The addition of low rates of chemical fertilizer (28 kg N/fed) in combination with biofertilizer gave the best results. From these results it is advisable from the economic and environmental point of view to use low rate of chemical fertilizer (28 kg N/fed) with selected biofertilizers.

Keywords: Biofertilizer, chemical fertilizer, azotobacter, Catharanthus roseus, growth, and flowering.

INTRODUCTION

Periwinkle (*Catharanthus roseus* G. Don) is one of the most important medicinal plants. It has a certain reputation in folk medicine for the treatment of diabetes and it was found that extract of leaves caused leukopenic actions in rats (Mohamed *et al*., 1987). In addition, six alkaloids from the extract proved active in cancer therapy (Trease and Evans, 1978). Moreover, the alkaloids of *Catharanthus roseus* could be used as a sterile agent for insects (Sukumar and Osmani, 1981). Two of these alkaloids, Resperpine and Ajmalcine, are used in controlling high blood pressure (Dahatonde and Joshi, 1982). Recently, great efforts have been made in Egypt to increase the area cultivated with medicinal plants to develop and enhance the productivity and quality of their yield as natural source of anticancer and hypotensive alkaloids.

Nitrogenous chemical fertilizers are commonly used in production of medicinal plants including *Catharanthus roseus*. However, the intensive use of chemical fertilizers causes serious problems concerning the environmental pollution (Attia, 1990). Some investigators used different types of biofertilizers as alternatives for chemical fertilizers. Abdel-Ati *et al*., (1996) found that inoculation of potato plants with nitrogen fixing bacteria (Azotobacter) and

phosphate solubilizing bacteria significantly increased the yield/fed. Moftah (2000) who discussed the response of soybean plants to the biofertilizer "biogene" obtained similar results.

With the exception of the work of Kandeel *et al*. (2001) on *Foeniculum vulgare* and the work of Nofal *et al*. (2001) on *Ammi visnaga*, there is no available work on the effect of biofertilizers on the growth and yield of medicinal plants. Therefore, the aim of this work is to use nitrogen fixing microorganisms or phosphate solubilizing microorganisms or their mixture as complete or partially alternative of chemical fertilizer for *Catharanthus roseus* to study their effects on the vegetative growth, herb yield, active ingredients, and some chemical compositions of the plants.

MATERIALS AND METHODS

Two field experiments were conducted during the two successive seasons of 1999 and 2000 at the nursery of ornamental plants, Fac. Agric., Minia Univ. The study aimed to investigate the effect of using biofertilizer on growth of *Catharanthus roseus* as compared with chemical fertilizers.

Seeds of *Catharanthus roseus* G. Don were obtained from the Medicinal and Aromatic plant Section of the Agriculture Research Center (ARC), Giza (Egypt). On the 1st of March during both seasons, seeds were sown in 25 cms clay pots. Fifty days later, the healthy uniform seedlings were transplanted into the experimental field (20th of April). The seedlings were transplanted on rows, 60 cm apart and spaced at 25 cm. The clay loamy soil was analyzed (according to Page *et al*., 1982). Physical and chemical analysis of the experimental soil is shown in Table (1).

Table (1): Physical and chemical properties of the experimental soil

Soil constituents		Value
Sand %		16.16
Silt %		39.78
Clay %		44.06
Organic matter %		2.3
CaCO3 %		1.64
E.C. (m mhose/cm)		0.81
pH		7.97
Ex. Ca (mg/100 g soil)		17.90
Total N %		0.11
Available P (ppm)		17.00
Exchangeable K (mg/100 g soil)		2.16
DTPA extractable (ppm)		
	Fe	8.70
	Zn	3.00
	Mn	25.80

Split plot design with three replicates was followed in this experiment where the nitrogen rates assigned as the main plots (A) and the biofertilizers treatments represented the sub-plots (B). The experimental unit was 3 x 2 meter composed of 4 rows including 32 plants.

Nitrogen fertilizer was used at rates of 0, 28, 56 and 84 kg N/fed. (as ammonium nitrate 33.5% N) and were divided into 3 equal doses, at 3 week intervals, starting one month after transplanting. Eight biofertilizer treatments were applied in this experiment by soaking the roots for 20 mins in suspensions contained 28-30 x 10^8 cells/ml of each biofertilizer, just before transplanting. Uninoculated plants (treated with distilled water) were used as control. The used biofertilizers were as follows:

1- Azotobacter chrococcum and Bacillus megaterium (phosphorus solubilizing bacteria) obtained from Dept. Agric. Microbiology, Fac. Agric. Minia Univ.

2- Commercial biofertilizers, Microben (15-20 x 10^7 viable cels /g) and Nitroben (6-10 x 10^7 viable cells/g) obtained from the Agric. Res. Center, Giza, Egypt.

The commercial biofertilizers were used as recommended by producers at (250g/fed.).

Plants received also potassium sulphate (48% K_2O) at a rate of 50 kg/fed. In two equal portions during plant growth stages. Calcium superphosphate (15.5% P_2O_5) at 150 kg/fed. was also added to all experimental units before transplanting. All other cultural practices were performed as usual.

A representative soil sample from rhizosphere of *Catharanthus roseus* G. Don plants was taken just before inoculation (i.e. zero time). After application of bacterial inoculation rhizosphere soil samples were collected from every treatment after 15, 30, 60, 90 and 120 days from planting time.

Densities of Azotobacter and phosphate solubilizing bacteria (PSB) were determined in the collected rhizosphere soil and expressed as number of cells per one-gram oven dried soil.

Number of Azotobacter was determined by dilution frequency method using Hoskins table (1934). The standard plate method used for determining number of phosphate solubilizing bacteria was as described by Abdel-Moniem *et al*. (1988).

At the end of the experiment (October 25th) of each season, data of vegetative growth parameters were recorded for plant height (cm), number of branches/plant, herb fresh and dry weights (g)/plant. Chemical analyses were made to measure reducing sugar contents in the herb, following the method reported by Moor (1974). Total carbohydrates was determined as described by Dubois *et al*. (1956). Total alkaloids (as perivine) in the herb was determined as described by Masoud *et al*. (1968). Alkaloid fractions mainly perivine, vinblastine and ajmalcine were determined following the method described by Mohamed *et al*. (1987) using PERKIN-ELMER LAMBADA 3 SYSTEM U.V./Visible Recording Spectrometer at wave length of 313, 282 and 270 nm for perivine, ajmalcine and vinblastine, respectively. Concentrations of N, P and K in the herb were also determined according to Page *et al*. (1982). All data were statistically analyzed using ANOVA test

method as reported by Snedecor and Cochran (1973). A computer program (PC-STAT) was used to perform the analysis.

RESULTS AND DISCUSSION

Number of microorganisms.

Data in Tables (2&3) show the number of Azotobacter and phosphate solubilizing bacteria in the rhizosphere soil of Catharanthus roseus G. Don. The recorded numbers of Azotobacter and phoshate solubilizing bacteria (PSB) in rhizosphere soil of Catharanthus roseus G.Don just before inoculation (zero time) were 26.4 x 10^4 and 14 x 10^4 cells/g oven dry soil, respectively. Gradual increases in the densities of these microorganisms in the rhizosphere soil of Catharanthus were detected in all treatments with increasing the age of plants up to 60 days after inoculation and then decreased. Such observation may be due to the stimulation effect of plant roots and their exudates (Abdel-Ati *et al*., 1996 and Saad and Hamad, 1998). Moreover, in the inoculated rhizosphere soil the density of any of these mocroorganisms depended on the applied inocula, i.e. the soil inoculated with Azotobacter contained much higher number of Azotobacter than the other treatments. Moreover, when the inocula included PSB, the number of phosphate solubilizing bacteria was increasing by time (up to 60 days) as compared with inocula without PSB. On the other hand the densities of these microorganisms in the rhizosphere soil were increased with increasing nitrogen fertilizer up to 56 kg N/fed (as ammonium nitrate) and then decreased.

Vegetative growth

Plant height and number of branches/plant. Data recorded in Table (4) indicated that the addition of chemical N fertilizer led to significant increases in the plant height and number of branches/plant as compared with untreated plants. However, the rate of 84 kg N/fed did not exhibit significant differences from the 56 kg N/fed in this matter.

On the other side, data indicated that the addition of biofertilizers, either alone or in a mixture with each other, increased significantly the plant height and number of branches/plant as compared with the untreated plants. In this respect, the addition of PSB to the other biofertilizers (Azotobacter, Microben and Nitroben) increased significantly the plant height and number of branches/plant as compared with the biofertilizers alone without PSB. The highest values in this respect were recorded with the mixture of Azotobacter + PSB, then Microben + PSB treatments.

Concerning the effect of the interaction between the chemical fertilizer and biofertilizers on plant height and number of branches/plant, data in the same Table showed that the addition of biofertilizer enhanced the effect of the chemical fertilizers, particularly at the low rates of chemical fertilizers (28 kg N/fed). The effect of such treatment (28 kg N/fed), with the mixture of Azotobacter + PSB, on growth parameters was equal or even higher than that recorded at 56 kg N/fed when added alone without biofertilizers.

Table 2

Table 3

Although data recorded in Table (4) indicated that the highest values of plant height and number of branches/plant were recorded at 84kg N/fed with the mixture of Azotobacter + PSB, there were no significant differences between these values and those obtained with 56kg N/fed when the used with the same mixture of biofertilizer.

Table (4): Effects of chemical fertilizer and biofertilizer on plant height and number of branches of *Catharanthus roseus* G.Don during the two seasons (1999 and 2000).

Bioferttilizer treatments (B)	Nitrogen fertilization (kg N/fed as ammonium nitrate) (A)									
	First season					Second season				
	0	28	56	84	mean	0	28	56	84	mean
	Plant height (cm)									
Untreated	49.5	70.3	7.85	80.1	69.6	47.3	68.4	75.8	78.5	67.5
Azotobacter	62.1	75.2	82.6	83.2	75.8	60.3	73.2	80.5	82.3	74.1
Microbine	59.5	74.8	81.2	82.7	74.6	56.5	71.7	79.6	81.8	72.4
Nitrobine	58.4	73.6	80.3	81.4	73.4	55.4	70.6	77.3	78.6	70.5
PSB	56.3	72.2	79.4	80.2	72.0	54.3	70.3	76.7	77.5	69.7
Azoto + PSB	66.6	79.7	85.1	86.9	79.6	64.7	77.8	84.5	85.6	78.2
Microb + PSB	63.8	77.6	84.8	85.8	77.9	61.5	75.4	82.3	84.7	75.9
Nitrob + PSB	61.7	76.5	82.3	84.2	76.2	59.6	74.5	79.8	83.4	74.3
Mean	59.7	74.9	81.7	83.1		57.5	72.7	79.6	81.6	
LSD (5%)	A: 1.58	B: 2.62		AB: 5.23		A: 2.12	B: 2.97		AB: 5.95	
	Number of branches/plant									
Untreated	4.9	7.3	8.0	8.2	7.1	4.5	6.8	7.5	7.7	6.6
Azotobacter	6.7	7.8	8.3	8.5	7.8	6.4	7.3	7.8	7.9	7.4
Microbine	6.6	7.7	8.2	8.3	7.7	6.3	7.2	7.7	7.8	7.3
Nitrobine	6.5	7.6	8.1	8.3	7.6	6.1	7.1	7.6	7.8	7.3
PSB	6.3	7.5	8.0	8.2	7.5	5.7	6.9	7.5	7.7	6.9
Azoto + PSB	7.0	8.3	8.8	8.9	8.3	6.6	7.6	8.2	8.3	7.7
Microb + PSB	6.9	8.1	8.6	8.7	8.1	6.5	7.5	8.1	8.1	7.6
Nitrob + PSB	6.8	7.9	8.4	8.6	7.9	6.4	7.4	7.9	8.0	7.4
Mean	6.5	7.8	8.3	8.5		6.1	7.2	7.8	7.9	
LSD (5%)	A: 0.22	B: 0.31		AB: 0.61		A: 0.26	B: 0.27		AB: 0.53	

Herb fresh and dry weights. Statistical analysis showed that herb fresh and dry weights were significantly increased by the addition of chemical fertilizers to reach their maximum rates at 84kg N/fed as compared with control plants. However, there were no significant differences between 84 and 56kg N/fed in this matter (Table 5). In this respect, the herb fresh weight was increased at 56kg N/fed by about 43% in the 1st and 2nd seasons. The increase in the dry weight of the herb was about 40 and 42% at the 1st and 2nd season, respectively.

As for the effect of the biofertilizers, it is clear from the data in the same table that all kinds of inocula increased significantly the fresh and dry weights of the herb when added either alone or together in a mixture. Moreover, the mixture of biofertilizers (Azotobacter, Microben or Nitroben) with PSB showed an enhancing effects as compared with the applied single biofertilizers. In this respect, the most increase in the herb fresh and dry weights were obtained with Azotobacter + PSB, followed by Microben + PSB, then Nitroben + PSB. The percent increases in the dry weight under these treatments were about 19.5, 15.5 and 14%, respectively at the first season

and 21, 17 and 14.5% at the second season, respectively, as compared with control plants.

Table (5): Effects of chemical fertilizer and biofertilizer on herb fresh and dry weights of *Catharanthus roseus* G.Don during the two seasons (1999 and 2000).

Bioferttilizer treatments (B)	Nitrogen fertilization (kg N/fed as ammonium nitrate) (A)									
	First season					Second season				
	0	28	56	84	mean	0	28	56	84	mean
	Herb fresh weight (g/plant)									
Untreated	50.2	81.3	98.5	99.4	82.4	48.3	78.5	95.6	95.1	79.6
Azotobacter	75.3	96.5	103.6	104.6	95.0	72.6	94.2	101.9	102.8	92.9
Microbine	72.6	93.5	100.6	101.3	92.0	70.4	90.6	98.7	99.6	89.8
Nitrobine	70.7	91.6	98.8	99.6	90.2	68.6	89.7	95.7	97.1	87.8
PSB	68.4	83.6	99.6	100.5	88.0	66.2	81.4	97.7	99.0	86.1
Azoto + PSB	80.1	100.7	106.3	106.8	98.5	77.8	97.1	104.3	105.6	96.2
Microb + PSB	78.1	96.3	102.7	103.2	95.1	76.1	94.2	100.5	101.3	93.0
Nitrob + PSB	76.9	95.3	101.2	102.3	93.9	73.5	92.4	98.4	99.9	91.0
Mean	71.2	92.4	101.4	102.2		69.2	89.8	98.8	100.2	
LSD (5%)	A: 2.23		B: 3.07		AB: 6.14	A: 2.34		B: 3.17		AB: 6.33
	Herb dry weight (g/plant)									
Untreated	9.59	15.45	18.62	18.99	15.66	9.18	14.84	17.97	18.07	15.02
Azotobacter	14.38	18.34	19.58	19.98	18.07	13.79	17.80	19.16	19.53	17.57
Microbine	13.87	17.77	19.01	19.45	17.53	13.38	17.12	18.56	18.92	17.00
Nitrobine	13.50	17.40	18.67	19.02	17.15	13.03	16.95	17.99	18.45	16.61
PSB	13.06	15.88	18.82	19.20	16.74	12.58	15.38	18.37	18.81	16.29
Azoto + PSB	15.30	19.08	20.09	20.40	18.72	14.78	18.45	19.61	20.06	18.23
Microb + PSB	14.92	18.30	19.41	19.71	18.09	14.46	17.90	18.89	19.25	17.63
Nitrob + PSB	14.69	18.11	19.13	19.53	17.87	13.97	17.56	18.50	18.98	17.25
Mean	13.66	17.54	19.17	19.54		13.15	17.00	18.63	18.88	
LSD (5%)	A: 0.45		B: 0.54		AB: 1.09	A: 0.47		B: 0.61		AB: 1.23

Concerning the effect of the interaction between chemical fertilizers and biofertilizers on the fresh and dry weight of herbs, statistical analysis showed that there was, in general, significant effects on these parameters. Azotobacter + PSB with 28kg N/fed increased the dry weight by about 23%. The corresponding increase at 56kg N/fed with the same biofertilizer treatment was only about 8% as compared with chemical fertilized plants without addition of biofertilizers. A significant observation was that the percent increase in the fresh and dry weights obtained with the addition of biofertilizers to the 28kg N/fed was almost equal to or even higher than that obtained when chemical fertilizers was increased to 56kg N/fed without biofertilizers. In this concern, the increase in dry weight at 56 kg N/fed was about 94% as compared with control plants, while this increase reached about 99% when Azotobacter + PSB was added to 28 kg N/fed rate, as compared with untreated plants. These results indicate that the use of biofertilizer could replace at least 50% of the chemical fertilizer.

Increasing plant growth due to inoculation with microorganisms may be attributed to increasing soil available nitrogen and consequently increase formation metabolites which encourage the plant vegetative growth and enhance the meristematic activity of tissues to produce more branches. Also, N2 fixers synthesize stimulatory compounds such as gibberellins, cytokinines

and IAA that act as growth regulators (Sperenat, 1990). These results are in agreement with those of Abd El-Kader (1992) and Migahed. (1998) on fennel, El-Sawy *et al* (1998) on Ammi visnaga, Ralie *et al*. (1995) on wheat and farag (1998) on rice.

Chemical composition of the plants

Total alkaloids. Data recorded in Table (6) showed that the chemical fertilizers increased significantly the total alkaloids (mg/g dwt) in the herb as compared with control plants. The most increase in total alkaloids was observed at 56kg N/fed. This increase was about 36% in the 1st season and 37% in the 2nd one as compared with control plants.

It is obvious also that the addition of biofertilizers (Azotobacter, Microben, Nitroben and PSB) either alone or in combination with PSB increased the total alkaloid content in the herb as compared with the untreated plants. Moreover, the mixture of biofertilizers with PSB was more effective in increasing total alkaloids than the use of biofertilizers as single treatments. In this concern, the most effective treatment was the mixture of Azotobacter + PSB, followed by microben + PSB, then Nitroben + PSB. The percent increases in total alkaloids under these treatments were about 9, 8 and 7%, respectively, as compared with control plants.

Table (6): Effects of chemical fertilizer and biofertilizer on total alkaloids and alkaloid fractions of *Catharanthus roseus* G. Don during the two seasons (1999 and 2000).

Bioferttilizer treatments (B)	Nitrogen fertilization (kg N/fed as ammonium nitrate) (A)									
	First season					Second season				
	0	28	56	84	mean	0	28	56	84	mean
	Total alkaloids									
Untreated	4.11	4.50	5.77	5.69	5.02	4.09	4.46	5.73	5.66	4.99
Azotobacter	4.30	5.26	5.86	5.76	5.30	4.27	5.22	5.84	5.71	5.26
Microbine	4.25	5.23	5.84	5.74	5.27	4.21	5.20	5.81	5.69	5.23
Nitrobine	4.17	5.17	5.82	5.73	5.22	4.14	5.15	5.79	5.68	5.19
PSB	4.15	5.05	5.80	5.70	5.18	4.11	5.07	5.76	5.64	5.15
Azoto + PSB	4.48	5.70	5.90	5.82	5.48	4.44	5.69	5.89	5.81	5.46
Microb + PSB	4.46	5.68	5.89	5.80	5.46	4.43	5.65	5.86	5.78	5.43
Nitrob + PSB	4.37	5.50	5.88	5.79	5.39	4.34	5.48	5.84	5.76	5.36
Mean	4.29	5.27	5.83	5.75		4.25	5.20	5.82	5.75	
LSD (5%)	A: 0.11		B: 0.13		AB: 0.27	A: 0.12		B: 0.15		AB: 0.31
	Perivine contents (mg/plant)									
Untreated	2.16	3.02	3.87	3.80	3.21	2.02	2.88	3.72	3.66	3.07
Azotobacter	2.89	3.67	3.92	3.85	3.58	2.77	3.61	3.80	3.72	3.47
Microbine	2.86	3.65	3.90	3.83	3.56	2.73	3.58	3.77	3.69	3.44
Nitrobine	2.82	3.63	3.89	3.81	3.54	2.70	3.55	3.75	3.68	3.42
PSB	2.69	3.52	3.88	3.80	3.47	2.57	3.44	3.74	3.67	3.36
Azoto + PSB	3.02	3.85	3.96	3.88	3.68	2.88	3.75	3.85	3.75	3.56
Microb + PSB	2.99	3.81	3.93	3.86	3.65	2.84	3.72	3.81	3.72	3.52
Nitrob + PSB	2.95	3.78	3.91	3.84	3.62	2.79	3.69	3.79	3.70	3.49
Mean	2.80	3.59	3.91	3.83		2.66	3.53	3.78	3.69	
LSD (5%)	A: 0.05		B: 0.07		AB: 0.14	A: 0.05		B: 0.06		AB: 0.12

Table (7): Effects of chemical fertilizer and biofertilizer on total alkaloids and alkaloid fractions of *Catharanthus roseus* G. Don during the two seasons (1999 and 2000).

Bioferttilizer treatments (B)	Nitrogen fertilization (kg N/fed as ammonium nitrate) (A)									
	First season					Second season				
	0	28	56	84	mean	0	28	56	84	mean
	Vinblastine contents (mg/plant)									
Untreated	1.24	1.72	2.25	2.15	1.84	1.15	1.64	2.16	2.03	1.75
Azotobacter	1.58	2.16	2.29	2.27	2.08	1.55	2.07	2.20	2.07	1.97
Microbine	1.55	2.12	2.28	2.25	2.05	1.51	2.04	2.19	2.06	1.95
Nitrobine	1.51	2.09	2.27	2.22	2.02	1.49	2.00	2.18	2.05	1.93
PSB	1.44	2.02	2.26	2.18	1.98	1.42	1.93	2.17	2.04	1.89
Azoto + PSB	1.70	2.26	2.30	2.28	2.14	1.63	2.18	2.29	2.13	2.06
Microb + PSB	1.68	2.33	2.29	2.26	2.12	1.60	2.15	2.27	2.11	2.03
Nitrob + PSB	1.65	2.22	2.27	2.23	2.09	1.58	2.12	2.25	2.09	2.01
Mean	1.54	2.10	2.28	2.23		1.49	2.02	2.21	2.07	
LSD (5%)	A: 0.04	B: 0.05	AB: 0.11			A: 0.04	B: 0.05	AB: 0.11		
	Ajmalicine contents (mg/plant)									
Untreated	1.04	1.43	1.80	1.79	1.52	0.98	1.37	1.72	1.70	1.44
Azotobacter	1.39	1.76	1.84	1.83	1.71	1.28	1.66	1.78	1.73	1.61
Microbine	1.37	1.74	1.83	1.82	1.69	1.26	1.63	1.76	1.72	1.59
Nitrobine	1.36	1.72	1.82	1.81	1.68	1.24	1.60	1.74	1.71	1.57
PSB	1.29	1.64	1.81	1.81	1.64	1.16	1.52	1.73	1.71	1.53
Azoto + PSB	1.42	1.84	1.86	1.86	1.75	1.36	1.74	1.83	1.78	1.68
Microb + PSB	1.41	1.82	1.85	1.85	1.74	1.34	1.72	1.81	1.76	1.66
Nitrob + PSB	1.40	1.80	1.84	1.84	1.73	1.31	1.70	1.80	1.75	1.64
Mean	1.34	1.72	1.83	1.83		1.24	1.62	1.77	1.73	
LSD (5%)	A: 0.02	B: 0.03	AB: 0.07			A: 0.03	B: 0.05	B: 0.11		

Concerning the interaction between biofertilizer and chemical fertilizers, data in the same table showed a significant effect of this interaction on the total alkaloids. The best values were obtained at 56kg N/fed added with either Azotobacter + PSB, Microben + PSB, or Nitroben + PSB, listed from the most effective to the lowest effective fertilizer mixture. However at 28kg N/fed with same biofertilizer mixtures gave nearly close values to those reported at 56kg N/fed. Thus the use of biofertilizer mixtures with low rates of chemical fertilizers is more preferable than the use of medium or high rates of chemical fertilizers.

These results may be due to the fact that nitrogen plays a major role in synthesis of these secondary products throught maximizing enzymatic activity controlling the biosynthesis of energy-rich molecules. The obtained results were in line with those of Menesi (1995) and El-Sawy *et al*. (1998) on *Ammi visnaga*, and Hornok (1980) on dill.

Alkaloid fractions. Data tabulated in Tables (6 and 7) showed that all chemical fertilizer treatments increased the contents of alkaloid fractions namely perivine, vinblastine and ajmalcine as mg/plant, comparing with control plants. In this respect, the highest values of fractions were obtained at the medium rate (56kg N/fed), under which these increments were about 40, 48 and 37% for perivine, vinblastine and ajmalcine, respectively at the 1st season. During the 2nd season, collected data exhibited nearly the same pattern as in the 1st one.

As for the effect of biofertilizers, data in the same tables indicated that all biofertilizer inoculation significantly increased the alkaloid fraction contents as compared with the untreated plants. The best results in this concern were obtained with Azotobacter followed by Microben, then Nitroben and finaly PSB inocula. It is also clear that the application of the biofertilizers in mixture forms with the PSB gave better results than the use of the biofertilizers as single treatments. In this concern, the highest improvement in the content of perivine, vinblastine, and ajmalcine/plant was observed under the treatment of Azotobacter + PSB, followed by Microben + PSB, then Nitroben + PSB inocula.

Concerning the effect of the interaction of both chemical and biofertilizers on the alkaloid fractions, it was obvious that there was significant effect on the content of perivine, vinblastine and ajmalcine, comparing to the untreated plants. In this respect the highest values of alkaloid fractions were obtained by Azotobacter + PSB., then Microben + PSB, and Nitroben + PSB, all with 56kg N/fed. But, it was obvious that the use of the mentioned biofertilizer mixtures with 28kg N/fed exhibited no significant differences as compared with 56kg N/fed. Therefore it is more convenient to use the the biofertilizer inocula with the low rates of chemical fertilizers to improve the alkaloid content of plants. This may be due to the effect of the amount of nitrogen fertilizers, in addition, inoculation with symbiotic nitrogen fixers in the enzymatic systems which are responsible for the biosynthesis of stored foods and this may be reflected on a higher average of total alkaloids per plant. Migahed *et al* (1998) on fennel and El-Demerdash (1994) obtained similar results on maize.

Carbohydrate concentrations. Statistical analyses of the effect of fertilization on reducing sugars and total carbohydrates of the herb (mg/g dwt) showed that all chemical fertilizer treatments increased significantly the sugars and total carbohydrates (Table 8). The highest values of both reducing and total carbohydrates were recorded at 84kg N/fed, followed by 56 kg N/fed. Taking in consideration that there were no significant differences between the effect of 56 and 28kg N/fed.

Data present in the same table showed that all biofertilizer treatments increased the concentration of reducing sugars and total carbohydrates in the herb as compared with untreated plants.

In this concern, the best results were recorded with Azotobacter followed by Microben, then Nitroben and finaly PSB inocula at the rate of 84kg N/fed as chemical fertilizer. It was found also that the use of biofertelizers as mixtures with PSB with 28kg N/fed gave high values of sugars and total carbohydrate concentrations, these values were very close to those obtained with 84 or 56kg N/fed when added without biofertilizers. Therefore it is more convenient to use low rates of chemical fertilizers with the biofertilizer mixture than the use of medium or high rates of chemical fertilizers.

Table (8): Effects of chemical fertilizer and biofertilizer on reducing sugars and total carbohydrates of the herb of *Catharanthus roseus* G. Don during the two seasons (1999 and 2000).

Bioferttilizer treatments (B)	Nitrogen fertilization (kg N/fed as ammonium nitrate) (A)									
	First season					Second season				
	0	28	56	84	mean	0	28	56	84	mean
	Reducing sugars (mg/g dwt)									
Untreated	10.89	11.64	12.47	12.52	11.88	10.42	11.17	11.95	12.05	11.40
Azotobacter	11.47	12.30	12.55	12.56	12.22	11.01	11.90	12.06	12.08	11.76
Microbine	11.35	12.13	12.51	12.54	12.13	10.88	11.72	12.01	12.06	11.67
Nitrobine	11.23	11.92	12.50	12.53	12.05	10.77	11.64	11.99	12.05	11.61
PSB	11.10	11.70	12.49	12.53	11.96	10.64	11.43	11.97	12.06	11.53
Azoto + PSB	11.61	12.51	12.58	12.60	12.33	11.14	11.98	12.10	12.12	11.40
Microb + PSB	11.57	12.40	12.53	12.57	12.27	11.10	11.92	12.06	12.08	11.79
Nitrob + PSB	11.45	12.35	12.52	12.54	12.22	10.98	11.86	12.03	12.07	11.74
Mean	11.33	12.12	12.52	12.55		10.87	11.70	12.02	12.07	
LSD (5%)	A: 0.07		B: 0.09		AB: 0.18	A: 0.07		B: 0.09		AB: 0.18
	Total carbohydrates (mg/g dwt)									
Untreated	40.17	43.70	46.10	46.50	44.12	38.10	42.42	43.82	44.73	42.27
Azotobacter	41.99	46.59	47.13	47.17	45.71	40.75	44.25	45.16	45.19	43.84
Microbine	41.73	46.23	46.90	47.00	45.47	40.54	43.91	44.93	44.94	43.58
Nitrobine	41.52	45.82	46.70	46.80	45.21	40.28	43.73	44.61	44.81	43.36
PSB	41.30	45.61	46.48	46.59	45.00	39.98	43.50	44.38	44.75	43.15
Azoto + PSB	42.95	46.85	48.18	48.25	46.56	41.39	44.78	45.98	46.03	44.55
Microb + PSB	42.80	46.60	48.90	48.10	46.35	41.23	44.55	45.67	45.68	44.28
Nitrob + PSB	42.60	46.40	47.30	47.40	45.93	41.02	44.32	45.34	45.42	44.03
Mean	41.88	45.98	47.09	47.23		40.41	43.93	44.99	45.19	
LSD (5%)	A: 0.52		B: 0.71		AB: 1.42	A: 0.45		B: 0.66		AB: 1.32

The positive effect of these biofertilizers on reducing sugars and total carbohydrates of medicinal plants was mentioned earlier by Nofal *et al*. (2001) and Kandeel *et al*. (2001). This increase might be attributed to the stimulative effect of these biofertilizers on chlorophyll contents, as discussed above, and consequently photosynthates including sugars.

Elemental concentrations. Data present in Table (9) showed that nitrogen % in the herb of chemical fertilized plants was increased significantly as compared with the untreated plants. The highest increase in N% was found at 84kg N/fed. In addition, it was found that the use of biofertilizers either in single form or as mixture with PSB improved the internal N% within the herb. Best results were recorded in the order, Azotobacter, Microben, Nitroben when they used with PSB inocula.

The interaction between chemical and biofertilizers showed significant effects on N% in the plant herb as compared with the untreated plants. The highest increase in N% was recorded at Azotobacter + PSB, followed by Microben + PSB, then Nitroben + PSB at external N fertilizers of 84kg/fed. However, there was no significant differences when 28kg N/fed was used instead of 56kg N/fed. Therefore it is more preferable using low rates of chemical fertilizers (28kg/fed) than the use of medium or high rates (56 or 84kg N/fed). This might be attributed to increase in the capacity of plants to absorb nutrients in case of using nitrogen fixers which may stimulate the enzymatic system responsible for the biosynthesis of organic compounds. In

this concern Sperenat (1990) stated that solubilization of miniral nutrients, synthesis of vitamins, amino acids, auxin and gibberelliens which stimulate plant growth come as a result of inoculation with Azotobacter sp. These results are in harmony with those of Ewada (1990) on wheat, maize and barley.

Table (9): Effects of chemical fertilizer and biofertilizer on concentrations of nitrogen, phosphorus and potassium (%) in the herb of *Catharanthus roseus* G.Don during the two seasons (1999 and 2000).

Bioferttilizer treatments (B)	Nitrogen fertilization (kg N/fed as ammonium nitrate) (A)									
	First season					Second season				
	0	28	56	84	mean	0	28	56	84	mean
	Nitrogen (%)									
Untreated	1.44	1.81	1.93	1.99	1.793	1.36	1.77	1.89	1.94	1.740
Azotobacter	1.66	1.92	2.03	2.08	1.923	1.64	1.88	2.00	2.03	1.887
Microbine	1.62	1.89	1.99	2.07	1.893	1.60	1.85	1.95	2.02	1.858
Nitrobine	1.58	1.87	1.98	2.06	1.873	1.55	1.84	1.93	2.01	1.834
PSB	1.50	1.81	1.92	1.98	1.803	1.48	1.78	1.88	1.94	1.770
Azoto + PSB	1.74	2.04	2.06	2.10	1.985	1.72	2.00	2.05	2.06	1.958
Microb + PSB	1.73	2.02	2.03	2.09	1.968	1.68	1.96	2.00	2.05	1.923
Nitrob + PSB	1.70	2.00	2.02	2.08	1.950	1.67	1.94	1.98	2.04	1.908
Mean	1.621	1.920	1.995	2.056		1.588	1.879	1.960	2.011	
LSD (5%)	A: 0.076	B: 0.081		AB: 0.162		A: 0.082	B: 0.086		AB: 0.171	
	Phosphorus (%)									
Untreated	0.41	0.40	0.39	0.38	0.395	0.39	0.38	0.37	0.36	0.375
Azotobacter	0.45	0.44	0.43	0.42	0.435	0.44	0.43	0.42	0.41	0.425
Microbine	0.43	0.42	0.41	0.40	0.415	0.43	0.41	0.40	0.40	0.410
Nitrobine	0.42	0.41	0.41	0.40	0.410	0.41	0.40	0.39	0.38	0.395
PSB	0.49	0.48	0.47	0.46	0.475	0.48	0.47	0.46	0.45	0.465
Azoto + PSB	0.48	0.47	0.46	0.45	0.465	0.47	0.46	0.45	0.44	0.455
Microb + PSB	0.47	0.46	0.45	0.44	0.455	0.46	0.45	0.44	0.43	0.445
Nitrob + PSB	0.46	0.45	0.44	0.43	0.445	0.45	0.44	0.43	0.43	0.438
Mean	0.451	0.441	0.433	0.423		0.441	0.430	0.420	0.413	
LSD (5%)	A: 0.012	B: 0.020		AB: 0.040		A: 0.013	B: 0.019		AB: 0.038	
	Potassium (%)									
Untreated	3.14	3.12	3.11	3.10	3.118	3.09	3.04	3.02	3.00	3.038
Azotobacter	3.20	3.19	3.17	3.16	3.180	3.16	3.14	3.12	3.11	3.133
Microbine	3.19	3.18	3.16	3.15	3.170	3.14	3.12	3.11	3.09	3.115
Nitrobine	3.18	3.17	3.16	3.14	3.163	3.13	3.09	3.07	3.06	3.088
PSB	3.17	3.16	3.15	3.13	3.153	3.12	3.08	3.06	3.05	3.078
Azoto + PSB	3.21	3.20	3.18	3.17	3.190	3.19	3.17	3.15	3.14	3.163
Microb + PSB	3.20	3.18	3.17	3.16	3.178	3.18	3.16	3.14	3.13	3.153
Nitrob + PSB	3.19	3.17	3.16	3.14	3.165	3.17	3.15	3.13	3.12	3.143
Mean	3.185	3.171	3.158	3.144		3.148	3.119	3.10	3.09	
LSD (5%)	A: 0.021	B: 0.24		AB: 0.049		A: 0.023	B: 0.26		AB: 0.051	

Data in the same Table indicated that the application of chemical fertilizers caused a decrease in the P% within the herb of treated plants, although this decrease was insignificant.

As for the biofertilizer treatments, it was clear that the application of biofertilizers led to an increase in the P% within plant tissues. The best

results were recorded at PSB alone followed by Azotobacter + PSB, Microben + PSB, and finally, Nitroben +PSB treatments.

The interaction effect between the chemical and biofertilizers on P% was significant in both seasons.

Data recorded in Table (9) showed that K% was decreased with the application of chemical fertilizers. The most reduction in K% was recorded at 84kg N/fed.

As for biofertilizer treatments, it was clear that all treatments enhanced the concentration of K in the herb. In this concern, best results were reported at Azotobacter + PSB, followed by Microben + PSB, then Nitroben + PSB.

Concerning the interaction between the chemical and biofertilizers, data in the same table showed significant effect on the K% in the herb of treated plants.

CONCLUSION

From the present study it could be concluded that the use of biofertilizers such as Azotobacter, nitrogen, microbien and PSB with low doses of chemical fertilizer (i.e. 28 kg N/fed.) improved the growth of Catharanthus roseus and prevent or at least minimized the serious pollution of the environment resulting from the excessive use of chemical fertilizers.

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التسميد الحيوي كبديل جزئي للتسميد الكيماوي لنبات الونكا

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أجرى هذا البحث في مشتل الزينة بكلية الزراعة جامعة المنيا خلال موسمين ١٩٩٩، ٢٠٠٠ و ذلك بهدف دراسة استخدام الأسمدة الحيوية كبديل جزئي عن التسميد الكيماوي على نبات الونكا . و لقد استخدم السماد الكيماوي النيتروجيني بأربع معدلات و هي صفر ، ٢٨ ، ٥٦ ، ٨٤ ك جرام وحدة نيتروجين للفدان (على صورة نترات أمونيوم) و استخدم ٨ معاملات من التسميد الحيوي و هي التلقيح بـ :١- بدون تلقيح ٢- الأزوتوباكتر ٣- الميكروبين ٤- النيتروبين ٥- البكتريا المذيبة للفوسفات ٦- الأزوتوباكتر + البكتريا المذيبة للفوسفات ٧- الميكروبين + البكتريا المذيبة للفوسفات ٨- النيتروبين + البكتريا المذيبة للفوسفات ، و ذلك بنقع جذور الشتلات في معلق من البكتريا لمدة ٢٠ دقيقة قبل الزراعة في الأرض المستديمة ، و لقد وزعت المعاملات السابقة في تصميم القطع المنشقة مرة واحدة و يمكن تلخيص أهم النتائج كالأتي:-

أدى استخدام السماد الكيماوي النيتروجيني بدون السماد الحيوي إلى زيادة طول النبات و عدد الفروع و الوزن طازج و الجاف للعشب و محصول القلويدات الكلية في العشب و محتوى العشب من الكربوهيدرات و النتروجين والفوسفور والبوتاسيوم و كانت أفضل المعاملات هي استخدام المعدل المتوسط (٥٦ كيلو جرام نيتروجين للفدان) .

- - أدى استخدام التلقيح بالأسمدة الحيوية بدون استخدام السماد الكيماوي سواء كل سماد حيوي على حدة أو الخليط بين الأسمدة الحيوية معاً إلى زيادة طول النبات و عدد الفروع و وزن العشب الطازج و الجاف و محصول القلويدات في العشب و محتوى العشب من الكربوهيدرات الكلية و النيتروجين و الفوسفور و البوتاسيوم و كان أفضل المعاملات هو التلقيح بالآزوتوباكتر + البكتريا المذيبة للفوسفات يليه الميكروبين + البكتريا المذيبة للفوسفات ثم النيتروبين + البكتريا المذيبة للفوسفات ثم الآزوتوياكتر ثم الميكروبين ثم النيتروبين و كان أقلهم هو البكتريا المذيبة للفوسفات .

- تأثير التفاعل بين السماد الكيماوي و السماد الحيوي : أدى التلقيح بجميع أنواع الأسمدة الحيوية مع السماد الكيماوي و كان التأثير معنوي بصفة عامة و أدى استخدام السماد الحيوي إلى زيادة كفاءة السماد الكيماوي النيتروجيني خصوصاً عند إضافة الأسمدة الحيوية إلى المعدل المنخفض من السماد الكيماوي (٢٨ وحدة نيتروجين للفدان)

التوصية: ينصح باستخدام الأسمدة الحيوية كبديل جزئي للأسمدة الكيماوية في نبات الونكا حيث يمكن استخدام معدل ٢٨ كجم نيتروجين للفدان من السماد الكيماوي مع التلقيح بأي من الأسمدة الحيوية الآتية ، الأزوتوباكتر أو الميكروبين أو النيتروبين أو الخليط بين كل من البكتريا المذيبة للفوسفات مع أي من الأسمدة الحيوية السابقة .

Table (2) Densities (cells/g) of Azotobacter (x10^4) in rhizosphere soil of inoculated and uninoculated Catharanthus roseus G. Don ammended with different doses of nitogen fertilizer at different growth stages. The recorded densities represent the average of two seasons (1999 and 2000)

Applied inocula (B)	Nitrogen Fertilizer (kg N/fed) (A)																							
	0						28						56						84					
	Days after inoculation						Days after inoculation						Days after inoculation						Days after inoculation					
	0	15	30	60	90	120	0	15	30	60	90	120	0	15	30	60	90	120	0	15	30	60	90	120
Untreated	26.4	37.2	48.1	58.2	50.3	47.6	26.4	38.1	50.6	61.3	52.4	48.5	26.4	40.7	53.2	63.4	54.3	50.2	26.4	37.7	49.8	59.6	51.7	47.8
Azotobac.	26.4	78.3	90.4	101	92.3	69.6	26.4	85.3	113	132	102	80.3	26.4	88.5	116	137	104	81.7	26.4	83.2	108	126	96.4	79.8
Microben	26.4	40.1	46.5	85.2	66.8	65.2	26.4	43.8	88.2	97.8	86.6	62.4	26.4	60.2	98.3	105	88.4	58.8	26.4	56.3	38.6	90.8	77.3	66.2
Nitroben	26.4	39.6	45.8	86.2	67.1	64.9	26.4	44.2	86.5	98.1	85.8	61.3	26.4	61.1	97.8	106	87.6	57.9	26.4	55.7	84.1	92.1	76.9	64.8
P.S.B	26.4	38.4	47.6	60.3	51.6	48.2	26.4	40.2	51.3	63.4	54.2	50.1	26.4	43.2	54.6	65.2	53.8	51.6	26.4	39.2	49.9	60.3	52.2	48.6
Azot+PSB	26.4	80.2	93.6	105	95.1	72.8	26.4	88.6	115	135	104	83.3	26.4	91.8	118	140	108	85.7	26.4	86.2	110	128	98.9	82.3
Micr+PSB	26.4	43.6	49.3	82.3	70.6	68.3	26.4	46.2	90.6	99.7	91.2	65.8	26.4	64.2	102	108	90.2	61.3	26.4	58.8	85.8	94.6	80.2	71.1
Nitr+PSB	26.4	44.2	50.2	89.8	69.7	68.1	26.4	45.9	91.2	101	91.7	67.1	26.4	65.1	101	107	91.1	60.8	26.4	58.6	86.4	93.8	81.3	70.3

PSB = phosphate solubilizing bacteria
Azot = Azotobacter
Micr = Microben
Nitr = Nitroben

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Table (3) Densities (cells/g) of phosphate solubilizing bacteria (x10^4) in rhizosphere soil of inoculated and uninoculated *Catharanthus roseus* G. Don ammended with different doses of nitrogen fertilizer at different growth stages. The recorded densities represent the average of two seasons (1999 and 2000)

Applied inocula (B)	Nitrogen Fertilizer (kg N/fed) (A)																							
	0						28						56						84					
	Days after inoculation						Days after inoculation						Days after inoculation						Days after inoculation					
	0	15	30	60	90	120	0	15	30	60	90	120	0	15	30	60	90	120	0	15	30	60	90	120
Untreated	14	18.3	29.6	35.2	27.9	22.2	14	31.4	38.2	51.4	32.7	26.8	14	33.1	40.3	53.6	34.2	27.6	14	29.3	33.6	45.8	30.2	23.5
Azotobac.	14	17.9	30.1	34.7	27.1	23.2	14	30.8	37.6	52.2	31.9	27.1	14	34.2	42.1	54.2	34.3	28.1	14	28.1	32.8	44.2	31.1	22.6
Microben	14	18.1	29.3	33.9	27.2	22.5	14	30.2	35.1	50.6	30.8	25.8	14	32.8	41.2	51.8	33.6	26.8	14	27.6	31.5	44.6	29.6	22.8
Nitroben	14	18.3	28.7	34.1	26.7	22.4	14	31.1	34.8	51.4	31.2	26.1	14	33.0	41.6	52.1	34.1	27.3	14	27.4	32.1	45.2	30.2	23.1
P.S.B	14	30.1	41.2	45.3	40.6	34.2	14	44.6	53.2	60.4	45.2	41.6	14	48.6	55.2	67.6	47.2	43.5	14	39.8	45.2	53.8	42.6	36.4
Azot+PSB	14	28.2	39.8	43.7	38.2	32.7	14	41.2	48.5	59.3	40.3	36.8	14	45.1	51.8	63.6	42.8	37.3	14	36.7	41.2	50.9	39.3	30.8
Micr+PSB	14	25.4	36.2	40.5	35.8	29.6	14	39.6	45.2	55.8	38.6	33.2	14	43.6	49.7	59.8	33.6	35.4	14	33.2	38.6	47.1	36.7	29.6
Nitr+PSB	14	23.7	37.1	41.2	34.9	29.2	14	40.1	44.8	56.1	37.8	34.1	14	42.8	50.1	60.1	34.2	34.8	14	34.1	37.8	47.5	35.7	28.8

PSB = phosphate solubilizing bacteria
Azot = Azotobacter
Micr = Microben
Nitr = Nitroben