J. Agric. Sci. Mansoura Univ., 34(1):387 - 400, 2009

RESPONSE OF *Calendula officinalis*, L. PLANTS TO SOME BIOFERTILIZER TYPES AND RATES.

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ABSTRACT

This study was carried out during two successive seasons of 2005/2006 and 2006/2007 on pot marigold plant (*Calendula officinalis*, L.) at the Experimental Station of Ornamental Plants, Fac. of Agric., Mansoura Univ., aiming to investigate the response of pot marigold plants to three rates of each of strain of nitrogen fixing bacteria "*Azospirillum* sp." under the commercial name of "Nitrobein", phosphate dissolving bacteria "*Bacillus megaterium*" under the commercial name of "Phosphorein" were applied to pot marigold seedlings after one month of sowing. The soil inoculation was repeated twice every month. While, yeast as a foliar spray at the rates 0, 1, 2 and 4 g/L.

Results showed that all treatments of biofertilization improved different vegetative growth characters, promoted flowering parameters and stimulating various chemical constituents compared with control. However, it is evident that the application of active dry yeast treatments as a foliar spray proved to be the most favorable as significant increases in vegetative growth, best quality and quantity of flower production and various chemical constituents such as carotenoids, carbohydrates and oleanolic in the flower as well as chlorophylls contents and nitrogen and phosphorus percentage in the leaves. The data showed that the growth characters and chemical constituents of pot marigold plants were significantly increased when the plants received the three dry yeast applications. The highest concentration of dry yeast (4 g/L) induced significant improvement in the all growth characters and chemical composition.

Hence, these findings clearly indicate that active dry yeast could be used as effective tools instead of chemical fertilization, lowering the productive costs and consequently minimize the pollution of the agriculture environment.

INTRODUCTION

Calendula officinalis, L. (pot marigold) which belongs to family *Asteraceae* is considered as one of the most popular winter annual flowering plants. This herbaceous plant is a native plant of the Mediterranean region (Earle *et al*., 1964), and is grown widely across Europe and North America as an ornamental and medicinal plant. It is grown as an ornamental plant for planting in beds and borders and the plants carry bright yellow and orange colours of flowers, which make them an attractive cut flowers. Besides the flowers are utilized as aromatic drug as an antiseptic and anti-inflammatory activities (Boucaurd-Maiture *et al*., 1988). Moreover, the flower petals are rich in carotenoids, which are very important in the food industries as a natural colored materials.

Biofertilizers are most reliable tools to reduce the rate of chemical fertilizers applied for medicinal and aromatic plants production in all types of soil in Egypt beside decreasing agricultural costs and environmental pollution. Biofertilizers are microbial inoculates to seed or soil to increase soil fertility, with the objective of increasing the number of such microorganisms and to accelerate certain microbial processes in the rhizosphere of inoculated plants. Such microbiological processes can change unavailable forms of nutrients into available forms that can be easily assimilated by plants (Subba Rao, 1981 and Alaa El-Din, 1982). These biofertilizers may be applied as bacteria, fungi or yeast.

Nitrobein is a biofertilizer product has greater amount of symbiotic and non symbiotic bacteria responsible for N-fixation from atmosphere. Numerous investigators reported that biofertilization using different strains of bacteria induced stimulating effect on plant growth and production by fixing atmospheric nitrogen (Awad, 1998). According to Subba Rao (1993), the symbiotic nitrogen fixing bacteria come under the genera *Rhizobium* and the non symbiotic nitrogen fixing bacteria come under the genera *Azotobacter*, *Azospirillum* and *Clostridium*. Moreover, most of the bacteria in the soil are classified as non nitrogen fixing bacteria. Improvement of plant growth due to inoculation with microorganisms may be attributed to increasing soil available nitrogen and consequently increase formation of metabolites which encourage the vegetative plant growth and enhance the meristematic activity of tissues to produce more branches.

Phosphorein is a biofertilizer product containing phosphate dissolving bacteria which plays a fundamental role in correcting the solubility problem in the soil by transforming the insoluble phosphate to soluble forms by secreting organic acids such as formic, acetic, lactic, propionic, fumaric and succinic acids. Those acids lower the pH and bring the dissolution of bond forms of phosphate and vender them available for growing plant (Ibrahim and Abdel-Aziz, 1977). *Bacillus megaterium*, was the important group in the solubilization process of insoluble phosphorus in soils (El-Katkat, 1992). Under the Egyptian soil condition, El-Sheekh (1997) mentioned that using phosphorene with or instead of mineral-P, apparently, increased the available P-concentration in the soil and plants.

Yeast treatments suggested to participate a beneficial role during stress due to its cytokinin content (Barnett *et al*., 1990), improving the formation of lower initiation due to its effect on carbohydrates accumulation (Winkler *et al*., 1962). Also, it was reported about its stimulatory effects on cell division and enlargement, protein, nucleic acid synthesis and chlorophyll formation (Kriag and Haber, 1980; Spencer *et al*., 1983; Fathy and Farid, 1996 and Tartoura, 2002). In addition, yeast is a natural source of many growth substances of crypoprotective agent, i.e., sugars, proteins and amino acids and most of nutritional elements (Na, Ca, Fe, Mg, K, P, S, W and Si) as well as some organic compounds (Nagodawithana, 1991).

Many investigators reported that application of biofertilizers (N_2-fixers and P dissolvers) in increasing vegetative growth traits, enhancing flowers quality and stimulating leaves content of nitrogen and phosphorus, photosynthetic pigments and carbohydrates, as well as, delaying flowering date. Examples are El-Sayed (1991) on *Calendula officinalis,* Attia and Ahmed (1997) on *Chrysamthemum*, Badran *et al*. (2001) on *Tropaeolum* *majus*, Abdou *et al*. (2003) on *Calendula officinalis*, Abdou (2003) on *Chrysanthemum morifolium*, El-Hindi and El-Boraie (2004) on marigold, Heikal (2005) on thyme, Massoud (2006) on *Salvia officinalis*, Swaefy and Milad (2006) on *Euryops pectinatus* and Hamza *et al*. (2007) on *Plantago ovata*. While, the role of yeast in promoting and enhancing growth, flowering and chemical composition of different plant species was pointed out by many investigators, Ali (2001) on pot marigold, El-Sayed *et al*. (2002) on coriander, El-Hindi and El-Boraie (2004) on marigold, Heikal (2005) on thyme and Massoud (2006) on *Salvia officinalis*.

The present study was initiated to elucidate the beneficial effect of using three biofertilizers namely nitrobein, phosphorein and active dry yeast on vegetative growth characters, flowering parameters and chemical constituents.

MATERIALS AND METHODS

Pot experiments were carried out during the two successive seasons of 2005/2006 and 2006/2007 at the Experimental Station of the Faculty of Agriculture, Mansoura University to investigate the beneficial effects of three biofertilizers on growth and chemical composition of marigold plant (*Calendula officinalis*, L).

Soil and seeds :

Before planting, both physical and chemical analysis of the soil under investigation were undertaken according to Jackson (1973) and the data are presented in Table (1).

Table (1): Some physical and chemical characteristics of the experimental soil during 2005/2006 and 2006/2007 seasons.

Physical analysis	Values		Chemical analysis	Values	
	2005/06	2006/07		2005/06	2006/07
Sand (%)	27.9	27.2	**Total N (%)**	0.12	0.10
Silt(%)	31.5	31.9	**Available P (ppm)**	11.2	11.0
Clay (%)	40.6	40.9	**Exchangeable K (ppm)**	304	293
Texture class	Clay-Loam				
CaCO3(%)	3.6	3.4	**pH**	7.9	7.7
OM (%)	2.3	2.4	**EC**	0.9	0.7

Seeds were provided by Hort. Res. Station, Seeds, Dokky, Cairo, Egypt and were sown in the nursery beds on October 5^{th} of both seasons, then transplanted 45 days later in 30 cm diameter earthenware pots filled with ten kg clay loam soil as in Table (1).

Three weeks after transplanting, all plants including the control received 1 g/pot ammonium sulphate (20.6 % N), 2 g/pot potassium sulphate (48 % K_2O) and 2.5 g/pot calcium superphosphate (15.5 % P_2O_5), with the exception of those received phosphorein were not supplied with calcium superphosphate.

Biofertilizers application :

Biofertilizers (nitrobein and phosphorein) were added after mixing with sand each one at three rates, 2.5, 5.0 and 10 g/pot as soil addition of nitrobein and 1.25, 2.5 and 5.0 g/pot divided into three equal portions. The seeds were inoculated with one portion before sowing, whereas, the second portion was added to the plants during transplanting practice and the last one was added after 45 days from transplanting the plants.

Nitrobein (a commercial name in Egypt) is a biofertilizer containing live cells of efficient bacteria strains for N-fixation (*Azotobacter and Azospirillum*). However, phosphorein is used as a biofertilizer containing live cells of efficient bacteria strain *(Bacillus megaterium)* as phosphate solubilizing bacteria (PSB). They were provided by the General Organization for Agriculture Equalization Fund (G.O.A.E.F.), Ministry of Agriculture, Egypt.

Active dry yeast was applied as foliar spraying 3 times starting one month from transplanting with 2 weeks intervals at 3 concentrations 1, 2 and 4 g/L.

Preparation of yeast solution was done according to EL-Ghamriny *et al.* (1999). The chemical composition of the used active baker's dry yeast *(Saaccharomyces cerevisiae)* was recorded in Table (2) according to the results obtained by Nagodawithana (1991).

Table (2) : Chemical composition of Baker's dry yeast.

Major compositions %		Vitamins contents U/g		Approximate composition of minerals			
				mg/g		µg/g	
Protein	47	**Thiamine**	60-100	**Ca**	0.75	**Cr**	2.2
Carbohydrates	33	**Riboflavin**	35-50	**Fe**	0.02	**Cu**	0.1
Minerals	8	**Niacin**	300-500	**K**	21.0	**Li**	0.17
Nucleic acid	8	**Pyridoxine HCl**	28	**Mg**	1.65	**Mn**	0.02
Lipids	4	**Pantothenate**	70	**Na**	0.12	**Mo**	0.4
		Biotin	1.3	**P**	13.5	**Ni**	3.0
		Cholin	4000	**S**	3.9	**Se**	0.1
		Folic acid	5.13	**Si**	0.03	**Sn**	3.0
		Vit. B_{12}	0.001	**Zn**	0.17	**Va**	0.04

The experimental design and treatments :

A complete randomized block design was used with ten treatments being involved such as soil addition of nitrobein and phosphorein at 3 rates. As well as, foliar application of active dry yeast at 3 concentrations, in addition to the control treatment which was sprayed with tap water. The ten treatments were replicated three times, and each replicate contained eight pots.

The following data were studied :

1. Plant height (cm) : it was measured from the surface of the ground to the tallest part of each plant.
2. Branches number per plant.
3. Herb dry weight per plant (g).
4. Flower diameter (cm) and total number.
5. Fresh and dry weights of flowers per plant (as flowers were collected

four times at two-weeks intervals during the flowering period).

6- Total carotenoids in the flowers per plant (mg/g) were estimated according to Fadl and Seri-ELdeen (1978).

7- Chlorophyll A and B in the leaves (mg/g fresh weight) were determined using the method of Moran (1982).

8- Oleanolic acid in flowers/plant (mg/plant) was determined according to EL-Gaingihi *et al*., (1982).

9- Reducing and non-reducing sugars percentage were assessed according to A.O.A.C. (1990).

10- Nitrogen and phosphorus percentage in the leaves were determined following the method described by Page *et al*., (1982).

Statistical analysis :

A randomized complete block design with three replicates was used. Data were subjected to statistical analysis according to Steel and Torrie (1980). The treatment means were compared using the least significant differences (L.S.D) test at the 0.05 % level.

RESULTS AND DISCUSSION

1- Effect of different biofertilizers levels on some vegetative characters :

The data in Table (3) reveal that, in most cases, the studied vegetative growth characters i.e. plant height (cm), average number of branches/plant and herb dry weight/plant (g) of pot marigold plants were significantly increased, in both seasons, due to the use of the three rates of each of nitrobein, phosphorein and active dry yeast in comparison with the untreated plants. However, the high rates of each biofertilizer were much more effective than the low rates since, raising the application rates of nitrobein, phosphorein and concentrations of active dry yeast caused in general gradual and steady increase in vegetative growth characters up to the high application rate (10 g/pot) for nitrobein, (5 g/pot) for phosphorein and (4 g/L) concentration for active dry yeast. It was also, found that spraying active dry yeast at the rate of 2 to 4 g/L was preferable in enhancing vegetative plant growth than using nitrobein and phosphorein at 5 to 10 g/pot for nitrobein and 2.5 to 5 g/pot for phosphorein in all cases and both seasons. Corresponding data Table (3) show an increase in fresh and dry weights of herb (g/plant) due to yeast treatment in comparison with nitrobein or phosphorein treatments and the control especially when active dry yeast was used at the rate of 4 g/pot which produced 328.5 and 335.1 (g/plant) of herb dry weights in the 1st season and in the 2nd season, respectively. This clearly indicates that the highest level of active dry yeast effectively promoted the growth of pot marigold plants. The superior effect of yeast solution on vegetative growth of pot marigold plant under this work conditions could be attributed to its composition as shown previously in analysis of yeast extracted which consists of majority of macro and micro elements. Moreover, it contains a natural growth regulators especially cytokinins which plays an important role and had stimulative effect on cell division, enlargement, protein and nucleic acids synthesis (Kriag and Haber, 1980 and Spencer *et al*., 1983). The yeast also, contains tryptophan which is considered as the precursor of IAA (Moor, 1979). Consequently, it is probable that the contents of yeast solution enhanced vegetative growth of the plant.

Table (3): Effect of nitrobein, phosphorein and yeast on vegetative growth characteristics of *Calendula officinalis*, L. plants during 2005/2006 and 2006/2007 seasons.

Biofertilization treatments		Plant height (cm)		No. of branches/plant		Herb dry weight (g/plant)	
		1st	2nd	1st	2nd	1st	2nd
Control		49.4	52.5	5.80	6.09	289.5	293.3
Nitrobein (g/pot)	**2.5**	53.1	54.9	5.95	6.22	294.2	295.2
	5.0	53.7	55.6	6.13	6.71	308.9	313.5
	10.0	54.3	56.2	6.98	7.33	320.9	322.3
Phosphorein (g/pot)	**1.25**	53.8	55.8	6.12	6.62	305.9	311.3
	2.5	54.9	56.3	6.64	7.22	314.0	319.4
	5.0	56.3	57.3	7.46	7.76	325.4	331.5
Yeast (g/L)	**1.0**	55.0	57.4	6.43	7.35	319.9	325.6
	2.0	56.9	57.9	7.67	8.43	319.5	324.9
	4.0	57.4	60.4	8.69	9.90	328.5	335.1
L.S.D at 5 %		**0.6**	**0.4**	**0.2**	**0.3**	**5.3**	**4.4**

(Nagodawithana, 1991) attributed the favourable effect of yeast to its high content of vitamins especially vit. B is essential for plant growth and development. It plays an active role in polar movement of native auxins from the site of their synthesis towards the site presumed use it roots, and thus, nutrients uptake is stimulated (Buchuala and Schmid, 1979). The present findings are in harmony with those reported by Hassanein *et al*. (2003) on *Calendula officinalis* and EL-Hindi and EL-Boraie (2005) on marjoram plant and Massoud (2006) on *Salvia officinalis*.

2. Effect of different biofertilizers levels on some flowering aspects :

Concerning, the effect of different biofertilizers levels on flowering characters, it could be noticed that the studied flowering growth characters; flower diameter (cm), number as well as fresh and dry weight of total flowers/plant Table (4) were significantly increased, due to the use of biofertilizers (nitrobein, phosphorein and yeast), in both seasons at three rates in comparison with the unfertilized pot (control) marigold plants. Within each fertilizer source, the high, followed by the medium rate were more effective than the low one and gave significantly higher values in the three traits than those of the control plants. However, treating plants with yeast extract had, relatively higher favourable effect as flower parameters were concerned than the other treatments (nitrobein and phosphorein) in the two seasons. Data presented in Table (4) show that the highest flower diameter, number and dry weight of total flowers/plant were (5.49 cm, 92.1 and 417.5 g) in the first season, respectively, when 4 g/L yeast was applied. The same trend was observed in the second season.

Table 4: Effect of nitrobein, phosphorein and yeast on flowering aspects of *Calendula officinalis*, L. plants during 2005/2006 and 2006/2007 seasons.

Biofertilization treatments		Flower diameter (cm)		Flowers No./plant		Flowers weight (g)			
						f.w./plant		d.w./plant	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control		4.04	4.14	74.8	76.0	392.5	395.1	77.3	80.5
Nitrobein (g/pot)	**2.5**	4.16	4.23	76.2	78.0	395.7	407.1	86.3	88.3
	5.0	4.20	4.29	83.9	85.4	396.9	410.0	89.0	92.0
	10.0	4.81	4.71	85.4	87.0	412.5	414.0	91.2	94.4
Phosphorein (g/pot)	**1.25**	4.20	4.38	78.3	83.7	415.1	416.6	88.7	83.3
	2.5	4.30	4.51	84.6	87.7	406.7	408.2	92.3	94.0
	5.0	5.21	4.64	90.3	91.5	415.2	416.5	93.4	94.5
Yeast (g/L)	**1.0**	4.34	4.50	87.8	90.4	417.1	418.4	92.0	92.7
	2.0	4.44	4.65	91.6	93.2	413.2	415.6	93.6	96.1
	4.0	5.49	5.62	92.1	100.0	417.5	420.4	94.7	102.7
L.S.D at 5 %		**0.2**	**0.1**	**1.4**	**2.1**	**1.5**	**1.7**	**2.1**	**1.7**

The stimulatory effect of yeast application on flowering parameters could be attributed to its increasing effect on the synthesis of plant growth promoters specially GA_3, IAA and cytokinins which improve cell division and elongation (Tarrow and Nakase, 1975 and Subba Rao, 1984). The obtained results are in harmony with those found by Ali (2001) on pot marigold in regard to active dry yeast, Kandeel and Sharaf (2003) on marjoram plant concerning N-fixing bacteria substances and Heikal (2005) on thyme.

3. Effect of different biofertilizers level on some chemical composition:

3.1. Leaf pigments:

The effect of different treatments of biofertilizers on photosynthetic pigments, i.e. chlorophyll A and B in the leaves and carotenoids in the flowers of pot marigold plants in Table (5) show that photosynthetic pigments in dry herb were significantly increased due to the application of the three biofertilizers (nitrobein, phosphorein and yeast) at different rates in comparison with the untreated plants, in both seasons. However, treating plants with yeast extract had, relatively, higher favourable effect as chlorophyll A and B as well as carotenoids were concerned than the other treatments (nitrobein and phosphorein) in the two seasons. Data presented in Table (5) show that the highest chlorophyll A and B were (0.325 and 0.13) in the first season, respectively, when 4 g/L yeast was applied. The same trend was observed in the second season. Similarly, the highest carotenoids in flowers dry weight was recorded as the same treatment was utilized.

The obvious increase in chlorophyll contents of the yeast treated leaves (Tables 5) can be interpreted by two functions. At the first, yeast as a source of cytokinins (Ferguson *et al*., 1987) delays the degradation of chlorophyll via obstructing activity of both chlorophyllase and magnesium-dechelatase enzymes (Idso *et al*., 1995). Secondly, this treatment provides the plant with certain macro and micro elements which play a direct or indirect role in the biosynthesis of chlorophylls. Yeast can activate magnesium chelatase through providing Mg and P (Pis needed for ATP production).

The previous results as affected by two biofertilizers of (N_2-fixers, P dissolvers), are in harmony with those found by EL-Sayed (1991) on *Calendula officinalis*, Attia and Ahmed (1997) on chrysanthemum, Badran *et al*. (2001) on *Tropaeolum majus*, Abdou *et al*. (2003) on *Calendula officinalis*, EL-Hindi and EL-Boraie (2005) on marjoram, Massoud (2006) on *Salvia officinalis*, Swaefy and Milad (2006) on Euryops pectinatus and Hamza *et al*. (2007) on *Plantago ovata*. In relation to active dry yeast, the present investigation supported the positive effects of foliar spray of yeast on plants and in harmony with these findings were those reported by (Ali, 2001) on pot marigold, EL-Sayed *et al*., (2002) on coriander, EL-Hindi and EL-Boraie, (2004) on marigold, Heikal (2005) on thyme and Massoud (2006) on *Salvia officinlais*.

3.2. Sugars percentage

Concerning reducing and non-reducing sugar content in the flowers of pot marigold, it was greatly and significantly increased due to the use of the three rates of nitrobein, phosphorein and active dry yeast in the two seasons Table (6).

Table 5: Effect of nitrobein, phosphorein and yeast on photosynthetic pigments content in dry herb of *Calendula officinalis*, L. plants during 2005/2006 and 2006/2007 seasons.

Biofertilization treatments		Carotene in flowers (mg/plant)		Chlorophylls (mg/g f.w.)			
				Chlorophyll A		Chlorophyll B	
		1st	2nd	1st	2nd	1st	2nd
Control		339.6	343.9	0.309	0.312	0.110	0.111
Nitrobein (g/pot)	**2.5**	344.4	346.6	0.313	0.315	0.114	0.115
	5.0	346.4	350.6	0.316	0.318	0.116	0.117
	10.0	351.8	362.2	0.317	0.320	0.119	0.121
Phosphorein (g/pot)	**1.25**	346.8	350.7	0.315	0.317	0.116	0.119
	2.5	347.6	350.6	0.317	0.323	0.120	0.123
	5.0	353.8	356.2	0.319	0.324	0.123	0.125
Yeast (g/L)	**1.0**	348.4	351.3	0.317	0.322	0.118	0.121
	2.0	353.0	355.5	0.323	0.330	0.125	0.127
	4.0	362.0	370.1	0.325	0.333	0.130	0.131
L.S.D at 5 %		**1.3**	**1.4**	**0.001**	**0.002**	**0.001**	**0.001**

It is of interest to note that no significant differences exit between medium and high rate within each biofertilizer. The highest values were resulted from the use of high rate of nitrobein (10 g/pot), phosphorein (5 g/pot) and active dry yeast (4 g/L). However, treating plants with yeast extract had, relatively, higher favourable effect as reducing and non-reducing sugar percentage were concerned than the other treatments (nitrobein and phosphorein) in the two seasons.

The higher reducing and non-reducing sugars percentage might be attributed to the increase in the amount of metabolites synthesized by the plant, which in turn, accelerated different plant growth parameters and dry weight of herbs that resulted due to yeast applications and finally reflected on the reducing and non-reducing sugars percentage of the dry weight of pot marigold.

3.3. Oleanolic acid (mg/plant):

It was evident from the data presented in Table (6) that oleanolic acid content in the flowers of pot marigold, it was greatly and significantly increased due to the use of the three rates of nitrobein, phosphorein and active dry yeast in the two seasons. It is of interest to note that no significant differences exist between medium and high rate within each biofertilizer. Numerically, the increase in oleanolic acid due to the high rate of nitrobein, phosphorein and yeast reached 50.5, 53.0 and 57.8 %, respectively, in the first season and 54.4, 57.5 and 61.8 % respectively, in the second, in comparison with that of the control plants. These results are in agreement with those found by Ali (2001) and Hassanein *et al*., (2003) pointed out that active dry yeast effectively increased oleanolic acid in the flowers of pot marigold plants. Moreover, this favorable effect of yeast tested could be attributed to their important functions in plant physiological processes which lead to activate cell division and elongation and consequently plant growth as previously indicated.

Table 6: Effect of nitrobein, phosphorein and yeast on reducing and non reducing sugars and oleanolic acid in flowers (mg/plant) of *Calendula officinalis*, L. plants during 2005/2006 and 2006/2007 seasons.

Biofertilization treatments		Sugars %				Oleanolic acid in flowers (mg/plant)	
		Reducing		Non-reducing			
		1st	2nd	1st	2nd	1st	2nd
Control		1.17	1.19	9.81	9.91	30.5	34.5
Nitrobein (g/pot)	**2.5**	1.20	1.22	10.1	10.16	48.5	51.4
	5.0	1.23	1.25	10.24	10.28	49.2	52.9
	10.0	1.26	1.29	10.32	10.44	50.5	54.4
Phosphorein (g/pot)	**1.25**	1.22	1.23	10.21	10.30	49.1	55.0
	2.5	1.25	1.26	10.32	10.44	51.2	55.4
	5.0	1.27	1.30	10.39	10.46	53.0	57.5
Yeast (g/L)	**1.0**	1.26	1.28	10.26	10.35	52.4	58.2
	2.0	1.26	1.29	10.43	10.53	55.7	59.3
	4.0	1.31	1.34	10.46	10.64	57.8	61.8
L.S.D at 5 %		**0.01**	**0.02**	**0.04**	**0.03**	**0.2**	**0.2**

3.4. Minerals percentage:

Relevant data in Table (7) show the percentages of nitrogen and phosphorus in the leaves of marigold plants as affected by biofertilizer levels. In regard to the leaves content of nitrogen and phosphorus, both elements were significantly increased due to the use of all rates of the biofertilizers in the two seasons in comparison with those of the untreated plants. However, the highest N percent was obtained from nitrobein, while, the highest P percent was from phosphorein Table (7).

Also, both N and P in the leaf were gradually increased by the gradual increase in the rate of each of nitrobein, phosphorein and active dry yeast. The role of N-fixing bacteria in increasing N and P in the leaf % was stated by Gomaa and Abou-Aly (2001) and Soliman (2002) on anise. While, the effect of phosphate-dissolving bacteria in promoting such elements was reported by Ibrahim (2000), Soliman (2002) and Badran *et al*. (2002) on tomato, fennel, anise and *Nigella*, respectively. Meanwhile, Ali (2001) on pot marigold, Abdou and EL-Sayed (2002) on caraway, EL-Sayed *et al*. (2002) on coriander and Massoud (2006) on *Salvia officinalis* pointed out the role of yeast in increasing both N and P leaf percentage. These findings for the three biofertilizers are in agreement with the present results. In addition, yeast contains different nutrients, amino acids and vitamins which promote the uptake of different nutrient elements through the modification of the pH value of soil solution towards acidity medium (Subba Rao, 1984).

Table 7: Effect of nitrobein, phosphorein and yeast on N and P percentage in leaf petioles of *Calendula officinalis*, L. plants during 2005/2006 and 2006/2007 seasons.

Biofertilization treatments		N %		P %	
		1st	2nd	1st	2nd
Control		1.144	1.152	0.329	0.343
Nitrobein (g/pot)	**2.5**	1.148	1.163	0.368	0.363
	5.0	1.182	1.191	0.373	0.380
	10.0	1.161	1.173	0.380	0.386
Phosphorein (g/pot)	**1.25**	1.241	1.275	0.377	0.380
	2.5	1.319	1.351	0.383	0.391
	5.0	1.327	1.345	0.389	0.409
Yeast (g/L)	**1.0**	1.313	1.379	0.386	0.399
	2.0	1.386	1.426	0.393	0.409
	4.0	1.402	1.479	0.408	0.434
L.S.D at 5 %		**0.02**	**0.02**	**0.01**	**0.01**

In conclusion, it could be observed that the studied vegetative growth characters; plant height, branch number and herb dry weight of pot marigold were significantly increased, in both seasons, due to the use of the three rates of each nitrobein, phosphorein and active dry yeast in comparison with the untreated plants. However, both the medium and the high rates of each biofertilizer were much more effective than the low rate. Also, all flowering parameters including flower diameter, as well as, number, fresh weight and dry weight of total flowers/plant were significantly increased due to the use of biofertilizers (nitrobein, phosphorein and yeast), in both seasons. Although all the applied treatments improved the growth and yield parameters of marigold, active dry yeast at (4.0 g/L) proved to be the best treatment for increasing the fresh and dry yields per plant, as well as chemical composition of plants in both seasons of the study. Thus, it can also be observed from the results recorded in the two seasons that the marigold plants responded to spray with active dry yeast up to the higher application rate (4.0 g/L) and still more reach is needed to know the most effective level of yeast under Dakahlia governorate growing conditions.

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إستجابة نباتات الأقحوان لبعض المعدلات من الأسمدة الحيوية المختلفة

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تم إجراء هذه الدراسة بمحطة البحوث الزراعية بقسم الخضر والزينة – كلية الزراعة – جامعة المنصورة خلال موسمى ٢٠٠٥/٢٠٠٦ ـ ٢٠٠٦/٢٠٠٧ وذلك بهدف دراسة إستجابة نباتات الأقحوان إلى إستخدام مصادر مختلفة من الأسمدة الحيوية و هى النتروبين المثبت للأزوت الجوى والفوسفورين الميسر لعنصر الفوسفور والرش الورقى بالخميرة بمعدلات (صفر ، ١ ، ٢ ، ٤ جم/لتر) على الصفات الخضرية والنباتات الزهرية والمكونات الكيماوية.

أظهرت النتائج أن معاملة نباتات الأقحوان بمستويات مختلفة من كل من الأسمدة الحيوية المختلفة أعطت فروق معنوية على صفات النمو الخضرى والقياسات الزهرية والمكونات الكيماوية والتى تتمثل فى محتوى الكاروتنويدات والسكريات وحامض الأولولنيك فى بتلات الأزهار وكذلك محتوى الكلوروفيلات والنسبة المئوية للنتروجين والفوسفور فى الأوراق مقارنة بالكنترول.

تشير أهم النتائج إلى أنه رغم أن كل المعاملات المستخدمة قد أدت إلى تحسين قياسات النمو الخضرى والزهرى والمكونات الكيماوية لنباتات الأقحوان مقارنة بالكنترول إلا أن معاملة الرش بالخميرة بالجافة النشطة بالمستويات المختلفة كانت أفضل معاملات التسميد الحيوى المستخدمة وخاصة عند إستخدام التركيز العالى منها (٤ جم/لتر) حيث أعطت نمواً خضرياً جيداً وأفضل إنتاج زهرى نوعاً وكماً.

ومن ثم فإن النتائج تشير بوضوح إلى إستخدام معاملة الرش بالخميرة النشطة الجافة كبديل لإستخدام التسميد الكيماوى مما يؤدى إلى الحد من تكلفة الإنتاج ويقلل من تلوث البيئة الزراعية.