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EFFECT OF SOME BIOFERTILIZERS ON THE GROWTH, ESSENTIAL OIL YIELD AND CHEMICAL COMPOSITION OF MARJORAM PLANTS

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ABSTRACT

Two field experiments were conducted at the Experimental Station of Medicinal and Aromatic Plants, Fac. of Agric., Mansoura Univ. during two successive seasons of 2002/2003 and 2003/2004 on marjoram plant, to determine the influence of two rates of each of nitrobein, phosphorein and yeast on the vegetative growth, yield of herbs, essential oil and chemical composition.

The results showed that although all the treatments improved the growth and yield parameters, active dry yeast proved to be the most favorable as significant increases in growth parameters, oil % and oil yield per feddan. The most effective concentration was 4 g/L before each cut.

Hence, this treatment could be recommended for raising marjoram yield, improving oil quality, lowering the productive costs and consequently minimize the pollution of the agriculture environment.

INTRODUCTION

Marjoram (*Majorana hortensis*, Moench) is a perennial herbaceous plant belongs to Family *Lamiaceae*. It is one of the most commercially important medicinal plants. The drug has agreable spicy smell and taste. It is a popular flavouring used a flavour meat dishes and in making salamis and other sorts of sausage. It contains up to 2 percent of an essential oil. The active principles in the drug oil used in pharmaceutical preparation as stimulant and antiseptic material in tooth pastes and drugs of whooping cough, larynx affections and as carminative at the case of gastrointestinal disorder (Frantisek and Valclav, 1975).

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 used for application 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 rhizophere of inoculated plants. Such microbiological processes can change unavailable forms of nutrients into available forms that can be easily assimilated by plants (Alaa EL-Din, 1982 and Subba Rao, 1981). 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.

Phosphorene 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 plants (Ibrahim and Abdel-Aziz, 1977 and Ashour, 1998). *Bacillus megaterium*, was the important group in the solubilization process of insoluble phosphorus in soils (EL-Katkat, 1992). Under the Egyptian soil conditions, 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 cintent (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, Zn and Si) as well as some organic compounds (Nagodawithana, 1991).

Many investigators reported that application of biofertilizers (N_2-fixers, P dissolvers) produced better growth and yield, increased active constituents, reduced the N requirement and enhanced water stress tolerance in many medicinal and aromatic plants such as caraway (Abdou and EL-Sayed, 2002); anise (Soliman, 2002); coriander (Abdel-Khader and Ghaly, 2003) and marjoram (Eid and EL-Ghawwas, 2002; EL-Ghadban *et al.*, 2003 and Kandeel and Sharaf, 2003). While, the role of yeast in promoting and enhancing growth, flowering and chemical composition of different plant species was pointed out by many investigators, Ahmed (1998) and Mansour *et al.*, (1999) on marjoram, Ali (2001) on pot marigold, Badran *et al.*, (2002) on marjoram, EL-Sayed *et al.* (2002) on coriander and EL-Hindi and EL-Boraie (2004) on marigold plant.

The present study was initiated to elucidate the beneficial effect of using three biofertilizers namely nitrobein, phosphorene and active dry yeast on vegetative growth and essential oil content of marjoram plants.

MATERIALS AND METHODS

The field trial was carried out during the two successive seasons of 2002/2003 and 2003/2004 at the Experimental Station of Medicinal and Aromatic Plants, Fac. of Agric., Mansoura Univ. to investigate the beneficial

effect of three biofertilizers on growth , yield of herb, volatile oil and chemical composition of marjoram plant.

Seeds and Soil :

Seeds of marjoram were obtained from Medicinal and Aromatic Research Department of Horticulture Institute, Dokky, Cairo, Egypt. On October 20th, 2002 and 2003 seeds were sown in the nursery beds. The seedlings were transplanted on Feb 10th, 2003 and 2004 into Experimental Farm in plots (2 x 1.8 m) which continued 3 rows, 60 cm apart. The spacing between plants was 25 cm. The physical and chemical properties of the used soil are listed in Table (1).

The field was prepared three weeks before planting and supplemented during preparation with 20 m^3/feddan of 6 months old cattle manure. Regular agricultural practices such as weeding and watering as a basic dressing were carried out whenever necessary as recommended.

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

Particle size distribution %				Chemical analysis				
Coarse Sand	Fine Sand	Silt	Clay	EC mmhos/cm	pH	N ppm	P ppm	K ppm
2.13	21.17	30.4	46.30	1.42	7.90	73.12	15.85	540

Biofertilizers application :

Biofertilizers (nitrobein and phosphorene) were added after mixing with sand each one at two rates, 3 and 6 g/plot 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 six weeks 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, phosphorene 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. Active dry yeast solution was prepared according to EL-Ghamriny *et al.* (1999). Analysis of prepared yeast stock solution was : total protein percent were (5.3), total carbohydrates (4.7), N (1.2), P (0.13), K (0.3), Mg (0.013), Ca (0.02), Na (0.01), micro-elements/ppm, Fe (0.13), Mn (0.07), Zn (0.04), Cu (0.04), B (0.016), Mo (0.0003), IAA (0.5 mg/ml), GA (0.3 mg/ml) and cytokinins. Such analysis was according to Nagodawithana (1991).

The experimental design and treatments :

The experimental design was a complete randomized block with six treatments plus the control. Each treatment was practiced in four replicates.

During the growing season three cuts were taken, on June 15th, on Sept. 1st and on Nov. 15th. The following data were recorded at each cut.

(1) Growth parameters :

Plant growth parameters of marjoram, i.e. plant height, basal branch number per plant as well as fresh and dry weight per plant at each harvest, were recorded in both seasons.

(2) Essential oil extraction and determination :

Essential oil percentage in the dried herb of each treatment was determined by hydro-distillation according to the Egyptian Pharmacopoeia (1984). Qualitative and quantitative determinations of the different main constituents of marjoram oils obtained from the first cut of each treatment had been carried out in parallel with authentic samples of different oil components by Gas Liquid Chromatographic (GLC) technique.

The G.L.C. analysis was carried out at the Central Laboratory of Cairo Univ. The relative retention time (RT) of each peak was compared with the reference authentic sample to identify the oil components. The quantitative estimation for each component was based on the peak area measurement by triangulation (Guenther and Joseph, 1978).

(3) Statistical analysis :

A randomized complete block design with four replicates was utilized according to Steel and Torrie (1980). Data were subjected to the statistical analysis according to the analysis of variance procedure (ANOVA). The treatment means were compared using the least significant difference (LSD) test at the 0.05 level, as described by Gomez and Gomez (1984).

RESULTS AND DISCUSSIONS

1- Vegetative growth parameters :

The data in (Table 2) reveal that, in most cases, the studied vegetative growth characters i.e. plant height (cm), average number of branches / plant as well as both herb fresh and dry weights (g/plant) of marjoram plants were significantly increased, in both seasons, due to the use of the two rates of each of nitrobein, phosphorene 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, phosphorene and concentrations of active dry yeast caused a generally gradual and steady increase in vegetative growth characters up to the high application rate (6 g/plot) for nitrobein and phosphorene 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 phosphorene at 3 to 6 g/plot in all cases and both seasons. Corresponding data (Table 2) show an increase in fresh and dry weights of herb (g/plant) in all cuts due to yeast treatment in comparison with nitrobein or phosphorene treatments and the control especially when active dry yeast was used at the rate of 4 g/plant which produced 163.39, 282.50 and 219.39 (g/plant) of herb fresh weights in the three cuts, respectively, in the 1st season and 163.94, 283.11 and 213.55 in the 2nd season respectively. While, data were 41.62, 71.94 and 55.46 (g/plant) for herb dry weight for three cuts in the 1st season, respectively. Herb dry weight averaged 41.29, 71.46 and 53.91 for three cuts in the second season, respectively (Table 2). This clearly indicates that the highest level of

active dry yeast effectively promoted the growth of marjoram plants. The superior effect of yeast solution on vegetative growth of marjoram 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.

(Nagodawithana, 1991) attributed the favourable effect of yeast to its high content of vitamins especially Vit. B which 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 in roots, and thus, nutrients uptake is stimulated (Buchuala and Schmid, 1979). The present findings are in harmony with those reported by Hafez (1990), Eid and EL-Ghawwas (2002) and Kandeel and Sharaf (2003) on marjoram plant concerning N-fixing bacteria substances.

2- Herb contents of essential oil and its constituents :

2:1- Essential oil content :

The effect of different treatments of biofertilizers on the essential oil percentage (ml/100 g F.W. herb) and oil yield/plant in the shoots of marjoram plants in (Table 3) show that essential oil percentage and oil yield of marjoram in dry herb was significantly increased due to the application of the three biofertilizers (nitrobein, phosphorene 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 oil percentage and oil yield were concerned than the other treatments (nitrobein and phosphorene) in the two seasons. Data presented in (Table 3) show that the highest oil percentages were (1.45 %, 1.65 % and 1.55) for the three cuts in the first season, respectively, when 4 g/L yeast was applied. The same trend was observed in the second season. Similarly, the highest oil yield in herb dry weight was recorded as the same treatment was utilized.

The higher essential oil content and oil yield 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 oil percentage and oil yield of the dry weight of marjoram. In addition, this stimulatory effect on oil content and oil yield could be attributed to the active contents of yeast solution which reflect on metabolic process and enhancing oil synthesis. The present results are in harmony with those reported by EL-Ghadban *et al.* (2002) on marjoram, Kandeel *et al.* (2002) on basil and EL-Hindi and EL-Boraie (2004) on marigold plant.

2:2- Essential oil constituents :

The gas liquid chromatography determination of the distilled oil obtained from herb of marjoram plant is shown in Table (4) and Fig. (1) it indicated that the highest contents of α-terpinol (the main component),

Table (2) : Effect of biofertilization with nitrobein, phosphorene and active dry yeast on growth parameters of marjoram plants in 2002/2003 and 2003/2004 seasons.

Growth parameters	Plant height (cm)			No. of branches / plant			Herb fresh weight (g/plant)			Herb dry weight (g/plant)		
	First season											
Cuts / Biofertilization treatments	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Control	22.22	23.66	23.38	14.22	22.66	26.88	94.75	125.96	125.66	24.10	31.96	31.67
Nitrobein at 3 g/plot	32.83	35.38	32.33	20.33	27.33	32.44	125.78	221.66	171.11	31.89	55.88	43.18
Nitrobein at 6 g/plot	33.21	36.72	33.27	22.11	28.33	33.77	134.33	235.50	182.00	34.30	59.73	45.81
Phosphorene at 3 g/plot	33.16	37.50	35.61	19.55	29.22	34.99	116.22	207.32	161.05	29.57	52.40	40.44
Phosphorene at 6 g/plot	32.94	38.11	36.77	21.55	29.88	36.44	122.00	215.61	165.55	32.14	54.34	41.79
Active dry yeast at 2 g/L	38.94	43.39	40.72	26.55	34.78	42.55	150.77	261.99	202.39	38.44	65.28	51.49
Active dry yeast at 4 g/L	41.54	45.66	43.92	27.99	37.33	44.22	163.39	282.50	219.39	41.62	71.94	55.46
L.S.D at 5 %	1.38	1.23	1.55	1.48	1.44	1.53	1.67	2.19	1.77	1.48	1.19	0.89
	Second season											
Control	24.21	24.44	23.67	14.55	22.00	26.77	95.38	126.38	124.77	23.98	31.85	31.51
Nitrobein at 3 g/plot	32.33	34.83	33.33	22.44	27.11	33.88	128.16	224.61	171.39	29.39	57.35	43.32
Nitrobein at 6 g/plot	33.83	36.83	34.94	24.00	27.66	34.66	134.94	235.00	182.38	34.02	59.07	46.04
Phosphorene at 3 g/plot	34.15	38.11	35.67	22.11	27.89	35.38	121.16	210.72	162.39	30.62	53.33	41.34
Phosphorene at 6 g/plot	37.11	38.88	36.05	23.55	29.00	36.66	124.11	218.11	163.50	31.30	55.21	41.27
Active dry yeast at 2 g/L	39.89	44.94	40.55	27.11	37.66	43.83	153.33	264.55	198.83	38.70	67.37	50.19
Active dry yeast at 4 g/L	42.16	47.05	44.55	28.88	39.44	45.50	163.94	283.11	213.55	41.29	71.46	53.91
L.S.D at 5 %	3.72	1.19	1.18	0.96	1.11	1.48	1.18	1.72	2.11	3.54	1.43	0.64

Table (3) : Effect of biofertilization with nitrobein, phosphorene and active dry yeast on the oil percentage, oil yield (ml/plant) and oil yield (liter/fed.) of dried herb of marjoram plants in 2002/2003 and 2003/2004 seasons.

Growth parameters	Oil content (%)			Oil yield (ml/plant)			Oil yield (liter/fed.)		
	First season								
Cuts / Biofertilization treatments	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Control	1.15	1.25	1.18	0.277	0.399	0.374	7.39	10.64	9.97
Nitrobein at 3 g/plot	1.17	1.40	1.30	0.373	0.782	0.561	9.95	20.85	14.96
Nitrobein at 6 g/plot	1.22	1.42	1.41	0.418	0.848	0.646	11.15	22.61	17.22
Phosphorene at 3 g/plot	1.35	1.45	1.52	0.399	0.759	0.615	10.64	20.24	16.40
Phosphorene 6 g/plot	1.37	1.53	1.49	0.440	0.831	0.623	11.73	22.16	16.61
Active dry yeast at 2 g/L	1.42	1.62	1.51	0.546	1.058	0.777	14.56	28.21	20.72
Active dry yeast at 4 g/L	1.45	1.65	1.55	0.603	1.187	0.860	16.08	31.65	22.93
L.S.D at 5 %	0.02	0.03	0.03	0.03	0.06	0.04	0.31	0.38	0.46
	Second season								
Control	0.96	1.17	1.10	0.230	0.373	0.347	6.13	9.95	9.25
Nitrobein at 3 g/plot	1.09	1.30	1.19	0.320	0.746	0.516	8.53	19.89	13.76
Nitrobein at 6 g/plot	1.12	1.33	1.30	0.381	0.786	0.599	10.16	20.96	15.97
Phosphorene at 3 g/plot	1.20	1.42	1.42	0.367	0.757	0.587	9.79	20.19	15.65
Phosphorene 6 g/plot	1.27	1.45	1.36	0.398	0.801	0.561	10.61	21.36	14.96
Active dry yeast at 2 g/L	1.33	1.57	1.45	0.515	1.058	0.728	13.73	28.21	19.41
Active dry yeast at 4 g/L	1.37	1.50	1.49	0.566	1.072	0.803	15.09	28.58	21.41
L.S.D at 5 %	0.04	0.02	0.04	0.06	0.05	0.03	0.26	0.19	0.21

Table (4) : Effect of biofertilization with nitrobein, phosphorene and active dry yeast on the percentages of identified constituents of marjoram plants in 2003/2004 season.

Biofertilization treatments	Essential oil components (%)													
	α-Pinene	B-Pinene	Eugenol	Limonene	Cineole	α-Terpineol	Linalool	Borneol	Linalyl acetate	Me-charvicol	Carvacrol	Geranyl acetate	Known	Unknown
Control	0.74	5.39	3.25	1.67	8.25	43.05	14.84	6.06	4.98	0.96	1.46	0.59	91.24	8.76
Nitrobein at 3 g/plot	0.42	2.03	1.93	1.86	9.57	41.20	23.17	7.16	3.33	1.21	1.11	1.27	94.26	5.74
Nitrobein at 6 g/plot	0.40	1.68	1.16	2.02	11.46	38.28	27.24	8.09	3.76	1.30	1.06	1.68	98.13	1.87
Phosphorene at 3 g/plot	0.57	2.93	2.16	2.01	10.05	44.01	20.19	7.32	3.14	1.11	1.03	1.02	94.54	4.46
Phosphorene at 6 g/plot	0.64	3.56	4.05	2.16	9.28	40.31	22.05	6.49	3.45	1.16	0.98	0.23	94.36	5.64
Active dry yeast at 2 g/L	0.68	2.42	3.14	1.83	8.11	48.60	19.15	5.13	3.22	0.91	1.13	1.23	95.55	4.45
Active dry yeast at 4 g/L	0.75	2.75	3.73	1.21	6.69	52.15	17.18	3.38	3.79	0.18	1.49	1.41	94.71	5.29

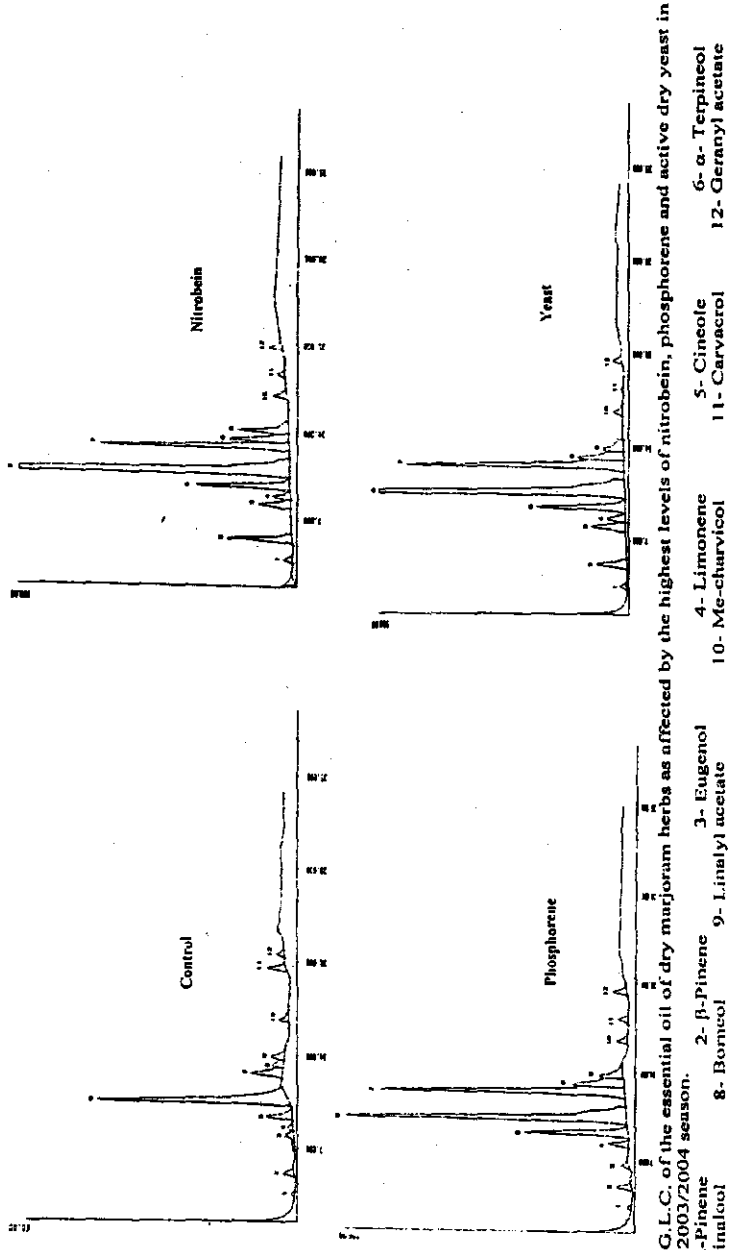

Fig (1): G.L.C. of the essential oil of dry marjoram herbs as affected by the highest levels of nitrobein, phosphorene and active dry yeast in 2003/2004 season.

1- α-Pinene 2- β-Pinene 3- Eugenol 4- Limonene 5- Cineole 6- α- Terpineol
7- Linalool 8- Borneol 9- Linalyl acetate 10- Me-charvicol 11- Carvacrol 12- Geranyl acetate

as well as that of some other important components, i.e. α and B-pinene, Eugenol, Limonene, Cineole, Linalool, Borneol, Linalyl acetate, Me-chavicol, Carvacrol and Geranyl acetate. The data in Table (4) and Fig. (1) showed increases in the content of the main component (α-terpinol) of marjoram oil from all treated plants compared with untreated (control). Plants treated with active dry yeast gave the highest values of the main components. This finding strongly confirm the previous conclusion drawn about essential oil percentage and oil yield (Table 4). These results are accordance with those obtained by both Ahmed (1998) and Mansour *et al.* (1999) on marjoram plants as well as EL-Hindi and EL-Boraie (2004) on marigold plants.

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تأثير بعض الأسمدة الحيوية على النمو والمحصول والتركيب الكيماوى لنباتات البردقوش

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نفذت تجربتان حقليتان على نبات البردقوش فى مزرعة النباتات الطبية والعطرية بكلية الزراعة – جامعة المنصورة خلال موسمى الزراعة ٢٠٠٢/٢٠٠٣ ، ٢٠٠٣/٢٠٠٤ وذلك بهدف دراسة إختبار تأثير ثلاثة أسمدة حيوية هى النتروبين والفوسفورين والخميرة على نمو نباتات البردقوش وإنتاجها من الزيت الطيار وكذا التغير فى محتوى مكونات الزيت .

أوضحت النتائج أن جميع المعاملات السمادية المستخدمة أعطت نموا ومحصولا أفضل بالمقارنة بالنباتات الغير معاملة ، إلا أن النباتات المعاملة بالخميرة الجافة النشطة قبل كل حشة أعطت أعلى زيادة معنوية فى القياسات الخاصة بالنمو ، والنسبة المئوية للزيت الطيار وكذا المحصول من الزيت للفدان ، حيث كان التركيز الأكثر فعالية الرش بالخميرة بمعدل ٤ جرام/لتر قبل كل حشة .

لذا توصى الدراسة بإستخدام معاملة الرش بالخميرة النشطة الجافة للحصول على أعلى إنتاجية من محصول العشب الطازج والجاف وكذلك النسبة المئوية للزيت العطرى لنباتات البردقوش ، وهذا يؤدى إلى الحد من تكلفة الإنتاج والتلوث البيئى .