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EFFECT OF SPRAYING ACTIVE DRY YEAST ON GROWTH AND STORAGE PERIOD ON THE ESSENTIAL OIL OF MARIGOLD PLANT (*Tagetes minuta,* L.)

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ABSTRACT

The present investigation was carried out during the two successive seasons of 2002 and 2003 on marigold plant (*Tagetes minuta,* L.) at the Experimental Station of Medicinal and Aromatic Plants, Fac. of Agric., Mansoura Univ.

This work aimed to study the possibility of using spraying active dry yeast at the concentrations of 0, 1, 2 and 3 g/L applied three times to improve growth and yield parameters as well as the oil percentage and oil yield per plant. Also, to study the effect of length of the storage period of the dried herbs on essential oil percentage and its constituent.

The results showed that although all the treatments improved the growth and yield parameters, active dry yeast at the rate of 3 g/L proved to be the most favorable as increases in both fresh and dry herb yields per plant, as well as the oil percentage and oil yield per plant were achieved. Regarding storage period, the results also showed that increasing the storage period of the dried herbs resulted in reduction of the essential oil percentage. Gas Liquid Chromatography analysis of oil samples revealed seven identified compounds. Storage period had a clear effect on the chemical composition of the extracted marigold oil, since some of the principle components increased such as limonen and cis-tagetone as the storage period increased, while those of dihydrotagetone and β-pinene decreased over the same period compared with the control.

INTRODUCTION

Medicinal and aromatic plants are natural therapy source to replace the chemical one, and are considered as a good source of national income as potential exportable crops. One of the most important medicinal and aromatic plants is marigold plant (*Tagetes minuta,* L.) which belongs to family Asteraceae. It is grown in the open field an as a back ground for the summer annual flowers and also, as cut flowers beside its uses as aromatic, drug and colour containing plant. Besides, it is very valuable enter crop for controlling plant parasitic nematode as recorded by Basu and Roy (1975). The aerial parts of the plant contain high quality essential oil and is also used for the scenting soaps, perfumary, cosmetic and pharmaceutical industries.

Human health has received a great attention nowadays. It was documented that artificial fertilizers have a pollutant effect in the soil and plants, in turn, on the human health. Owing to that, the scientists are looking forward to substitute the artificial fertilizers (partially) with the natural ones like yeast.

Recently, great attention has been focused on the possibility of using natural and safety substituents, i.e. yeast in order to improve plant growth, flowering, fruit setting and total yield of horticultural plants.

Meanwhile, yeast treatment plays a beneficial role in improving the formation of flower initiation due to its effect on carbohydrates accumulation (Winkler *et al.*, 1962). Also, it was reported that yeast has a stimulatory effect on cell division and enlargement, protein and nucleic acid synthesis and chlorophyll formation (Kraig and Haber, 1980; Spencer *et al.*, 1983; Fathy and Farid, 1996; Fathy *et al.*, 2000 and Eata *et al.*, 2001). Tartoura (2002) reported that spraying pea plants with yeast extract greatly increased the total yield of fresh pods by 32.13 %.

In addition, yeast is a natural source of many growth substances of cryoprotective agent, i.e., sugars, proteins and amino acids and also several vitamins (Shady, 1978) and most of nutritional elements (Na, Ca, Fe, Mg, K, P, S, Zn, Si) as well as some organic compounds (Nagodawithana, 1991).

On the other hand, storage conditions are important in keeping the product in a good marketable quality, since increasing the storage period affected the essential oil content (Fehr, 1980) and Paakkonen *et al.* (1990).

Length of the storage period of plant material affected the percentage and composition of the essential oil and the relative percentage of its constituents (Singh *et al.*, 1994). These changes depend on condition of plant material, method and conditions of storage (i.e. temperature and humidity), and the chemical composition of the essential oil (Paakkonen *et al.*, 1990 and Kotb and Eid, 1996).

Therefore, the aim of the present work was to study the possibility of enhancing growth and yield parameters as well as improving the oil percentage and oil yield per plant by using natural and safety compound such as yeast extract. Moreover, to study the effect of length of the storage period on the oil percentage and composition extracted from dried herb of marigold plant.

MATERIALS AND METHODS

Two field experiments were carried out during the two successive seasons of 2002 and 2003 at the experimental Station of Olericulture and Floriculture Dept., Fac. of Agric., Mansoura Univ. to evaluate the beneficial effect of using active dry yeast solution on growth, oil yield and chemical composition of marigold plant (*Tagetes minutia*, L.).

Soil samples were taken before planting from experimental soil at depth of 30-50 cm to determine the physical and chemical analysis (Table 1).

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

Particle size distribution				Chemical analysis					
Coarse sand %	Fine sand %	Silt %	Clay %	O.M. %	EC mmhos/cm	pH	N ppm	P ppm	K ppm
2.13	21.17	30.4	46.30	1.73	1.42	7.90	73.12	15.85	540

Foliar applications:

Yeast extract: Baker's yeast mixed with sugar at a ratio of 1:1 and left for 3 hours at room temperature (25 ± 1 °C). Then it was frozen for

disruption of yeast tissue and releasing their content. Preparation of yeast solution was done according to El-Ghamriny *et al.* (1999). The chemical composition of the used active baker's dry yeast (*Saccharomyces 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 acids	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:

The experimental design was a complete randomized block. Treatments were three plus the control. Each treatment included four replicates and each replicate contained seven plants.

The plants were treated with three doses of active dry yeast (1, 2 and 3 g/L). Foliar sprays of these doses were done after 30, 60 and 90 days from transplanting.

Planting method:

Seeds of marigold (*Tagetes minuta*, L., Family; Asteraceae) were obtained from Medicinal and Aromatic Research Department of Horticulture Institute, Dokky, Cairo, Egypt, to be used in this study. Farmyard manure (FYM) was added to the whole soil area before cultivation. Seeds were sown in the nursery on February 10th and 15th in 2002 and 2003 seasons, respectively, and transplanting took place in 1st April. All the plants received normal agricultural practices, whenever they were needed.

Storage:

The dried samples (25 g of dried herbs) were placed in paper bags for two storage periods (0, 1 and 2 months). Herbs were dried in perforated paper bags at room temperature until constant weight.

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- Branches length per plant (cm).
4- Fresh and dry weights of herb per plant (g).
5- Fresh and dry weights of leaves per plant (g).
6- Oil percentage (%): The essential oil percentage was determined in samples of dried herbs (25 g each) in both seasons by hydro-distillation using Clevenger apparatus according to methods described by the Egyptian Pharmacopoeia (1984).
7- Oil yield (ml/plant): was calculated in proportion to the herb fresh weight/plant.
8- Oil yield (liter/fad): was calculated by multiplying essential oil (%) in dried samples by the yield of the dried herbs per feddan.

Gas Liquid Chromatography (G.L.C.):

This study was carried out at the Central Laboratory of Cairo Univ. by G.L.C. (Varian VISTA series 6000, FID detector) in order to compare the composition of the essential oil samples resulted from plants of the tested yeast extract treatments with the control samples. The relative retention time (RT) of each peak was compared with the reference authentic sample to identify the unknown samples. The quantitative estimation for each component was based on the peak area measurement by triangulation (Guenther and Joseph, 1978).

Statistical analysis:

A randomized complete block design with four replicates was used 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 DISCUSSION

1- Plant growth parameters:

Data of growth performance of marigold plants as influenced by active dry yeast foliar application are presented in Table (3). It was clear that all level applications, in general, resulted in increasing vegetative growth over the control in the two seasons of the study. In addition, it could be noticed that spraying yeast for three times at 3 g/L per plant was superior than the other applications since all the studied growth parameters were enhanced i.e., plant height (cm), number of branches, branches length, herb fresh and dry weights (g), as well as leaves fresh and dry weights (g) of vegetative growth/plant in relative to those of control plants during 2002 and 2003 seasons.

application rate (3 g/L), at which maximum effectiveness of yeast was reached (Table 3). The superior effect of yeast extract on vegetative growth of marigold plant under this work conditions could be attributed to its composition as shown previously in analysis of yeast extract which consists of majority of macro and micro elements. In addition, 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. The yeast also contains tryptophan which considered as the precursor of IAA (Moor, 1979). Consequently, it is probable that the application of yeast produced more IAA which increased plant growth.

Such findings could be attributed to the high content of vitamins in yeast (Nagodawithana, 1991) 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 toward the site presumed use in roots, and thus nutrients uptake is stimulated (Buchuala and Schimd, 1979).

Therefore, it is well documented in our study that yeast is synergistic in their uptake. Reverselly, the promotional effect of yeast extract treatment on marigold plant growth could be attributed to its auxins, GAS, carbohydrate, protein and minerals content. Thus, it might accelerated cell division and enlargement and all metabolic processes, all known as physiological functions for best growth.

Similar findings about the beneficial effect of yeast were obtained by Winkler (1962), Roberts (1976), Kraig and Haber (1980), Spencer *et al.* (1983), Kurtz *et al.* (1986), Weiderrecht *et al.* (1988), Fathy and Farid (1996), Abd El-Kafie *et al.* (2001) on chrysanthemum, Eid (2001) on coriander and Dawa *et al.* (2003) on pea.

2. Herb contents of essential oil and its constituents:

2.1 Essential oil content:

Data presented in Table (4) clearly show that all the applied treatments 1, 2 and 3 g/L of yeast extract enhanced the synthesis and accumulation of essential oil percentage and oil yield in fresh and dry herbs of marigold plants in both seasons. Also, yeast extract levels caused an increase in oil percentage and oil yield with increasing levels when compared with the untreated plants. The maximum values were recorded at 3 g/L of yeast extract in two seasons. The increases in oil percentage and oil yield obtained from two treatments (2 and 3 g/L) were significant when compared with the control. However, the differences between these two treatments were not significant.

The higher essential oil content and oil yield might be attributed to 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 (Table 3) and finally reflected on the oil percentage and oil yield of the fresh and dry weights of marigold.

The high level of yeast had more efficiency than the lower level. Such results coincided with those of Ahmed *et al.* (1997) and Eid (2001) on coriander. These results might be due to the function of the chemical fractions composed inside the oil may be affected by spraying with active dry

yeast. The stimulatory effect on oil content and oil yield 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 trend of the oil yield is identical to that of the herb yield. Thus, the data clearly indicated that the difference in oil yield was a result of the effect of each yeast treatment on the yield of the dried herbs rather than its effect on oil percentage. These results agreed with those of Shalaby *et al.* (1993) who showed that all increases in the oil yields of *Melissa officinalis* were a result of increased crop yield rather than increased essential oil concentrations.

Concerning the storage period, increasing storage period of marigold dried herbs significantly decreased the essential oil percentage and oil yield (Table 4) in both seasons. These results are in agreement with those obtained by Fehr (1980) who mentioned that storage period caused significant decrease in the oil of anise, caraway and fennel fruits by 1, 2.8 and 0.5% when stored for a month, respectively. Similar results were obtained by Abd El-Kader *et al.* (2004) who reported that increasing the storage period of the dried flowers (*Achillea millefolium*) significantly decreased the essential oil percentage by an average of 0.06% every two months. On the contrary, Shalaby *et al.* (1988) reported that the essential oil content of the stored samples of *Mentha arvensis* did not change throughout the storage period. However, the losses of essential oil from air dried plant material during storage depends on condition of material, method and conditions of storage, length of storage period and the chemical composition of the oil (Fehr, 1980; Kotb and Eid, 1996).

2.2. Essential oil constituents:

Separation of oil samples by GLC revealed seven components and β-pinene, limonene, dihdrotagetone and cis-tagetone were the major oil constituents in marigold oil sample from plants of the control treatment, while the others components were found in small quantities. Comparing such values with those under yeast treatment, it could be observed from the data presented in Table (5) and illustrated in Fig (1) that some oil constituents were increased as yeast levels increased in the fresh and dried herbs of marigold, while the others were decreased or disappeared. The highest percentage of dihydrotagetone was in oil sample from plants under active dry yeast treatment, which ranged from 24-78% to 63.91%. On the contrary, active dry yeast treatments decreased the other components as limonene, which was reduced from 24.88 to 8.20% with increasing yeast levels. Also, both β-pinene and cis-tagetone were decreased in stressed herbs oil sample.

As regarding storage period, data presented in Table (5) and Fig (2) showed the effects of storage on marigold essential oil percentage. The relative concentrations of each constituent changed with the length of the storage period. Increasing the storage period resulted in an increase in the percentages of limonene and cis-tagetone, while β-pinene and

dihydrotagetone percentages were decreased as the storage period increased.

These data and those of the previous ones in Table (4) showed that the main effect on the changes of the essential oil percentage was mainly due to the length of the storage period. The mentioned changes during storage might be due to oxidation, disintegration, or transformation of different compounds. Similarly, Singh *et al.* (1994) showed that the essential oil composition during storage changed with length of the storage period, some constituents increased, some decreased, while others remained constant. These findings are in agreement with those obtained by Abd El-Kader *et al.* (2004) on *Achillea millefolium*.

Conclusions

Considering the forecited results, it could be concluded that, using foliar applications of new promising, like yeast (safety material) in low concentration and cost with higher efficiency to counteract the internal nutritional ionic case. Although all the applied treatments improved the growth and yield parameters of marigold, active dry yeast at 3 g/l proved to be the best treatment for increasing the fresh and dry yields per plant, as well as the oil percentage and oil yield per plant. 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 (3 g/l) and still more research is needed to know the most effective level of yeast under Dakahlia governorate growing conditions. As the storage period, the relative concentrations of each constituents changed with the length of the storage period. Increasing the storage period resulted in an increase in the percentages of limonene and cis-tagetone, but a decrease of dihydrotagetone and β-pinene compared with control.

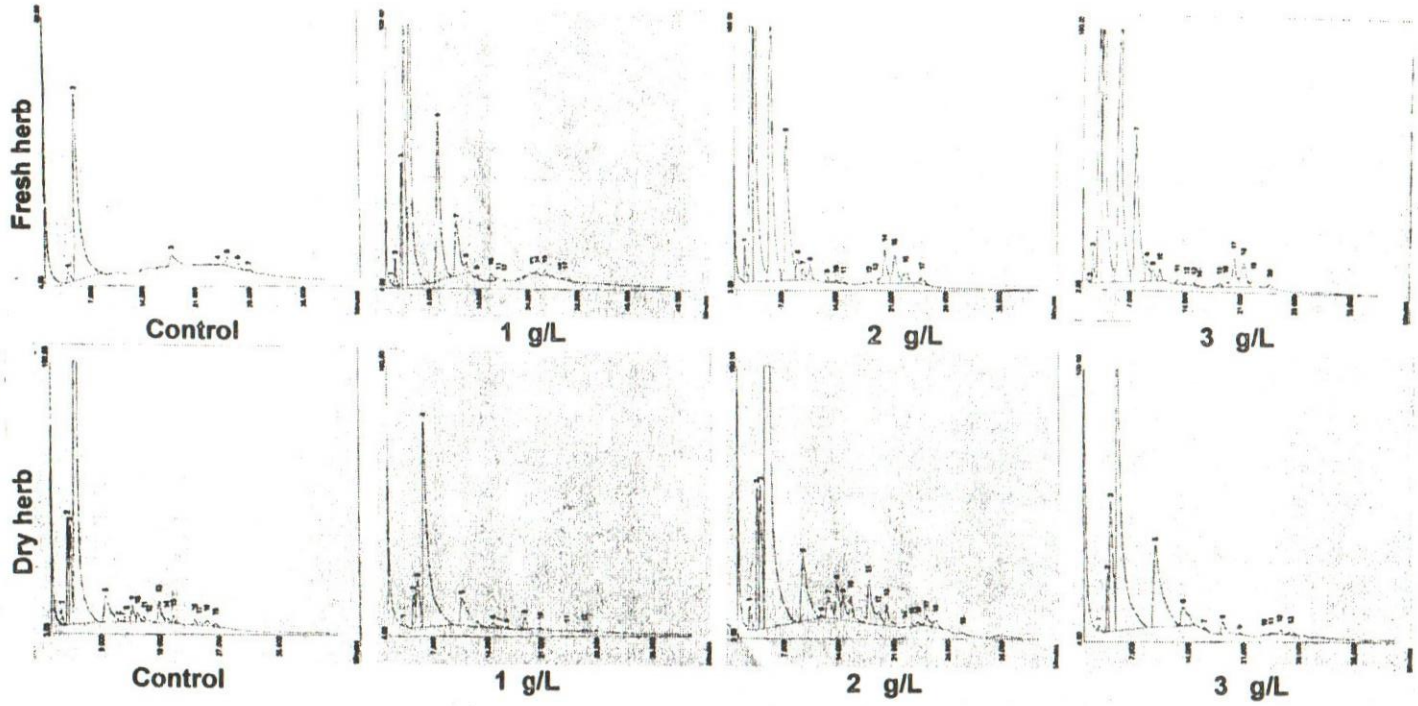

Fig (1): G.L.C. of the essential oil of fresh and dry marigold herbs (*Tagetes minuta L.*) as affected by different yeast concentrations.

1- α–Pinene 2- β–Pinene 3- Limonene 4- Dihydrotagetone 5- Cis-tagetone 6- β–Ocimene 7- Carvon

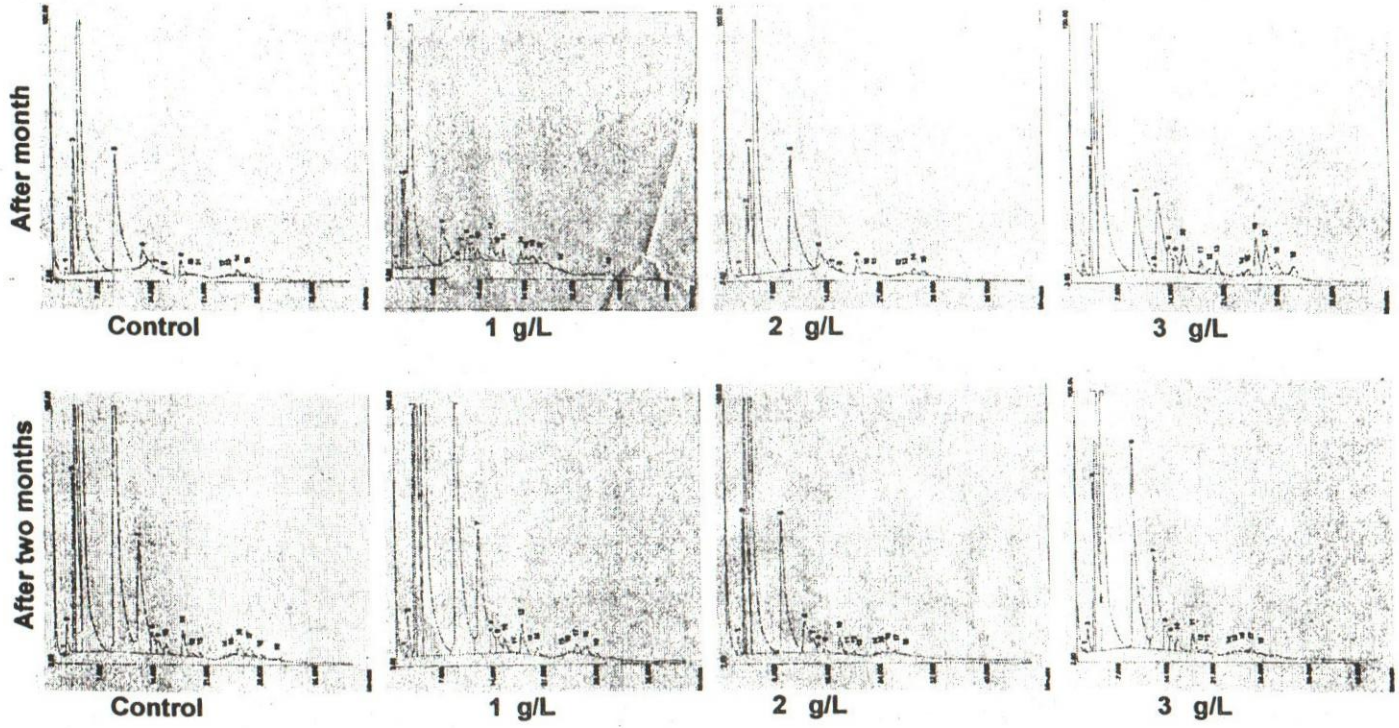

Fig (2): G.L.C. of the essential oil at different storage period of marigold plants (*Tagetes minuta L.*).

1- α–Pinene 2- β–Pinene 3- Limonene 4- Dihydrotagetone 5- Cis-tagetone 6- β–Ocimene 7- Carvon

Table (3): Effect of spraying active dry yeast on vegetative growth of marigold plant (*Tagetes minuta*, L.) during 2002 and 2003 seasons.

Treatments (g/l)	Vegetative growth characteristics						
	Plant height (cm)	No. of branches /plant	Av. of branch length (cm)	Fresh weight (g/plant)		Dry weight (g/plant)	
				Herb	Leaves	Herb	Leaves
2002 season							
0	184.8	14.20	29.35	291.5	131.10	96.3	26.56
1	224.5	20.90	35.85	396.0	177.19	130.6	35.55
2	235.7	19.05	34.35	412.3	200.14	144.8	39.05
3	241.4	20.25	40.60	462.1	215.95	151.6	42.94
LSD at 5%	10.55	3.46	5.67	0.96	0.37	0.54	0.07
2003 season							
0	190.4	17.55	29.90	315.6	142.44	103.2	28.28
1	223.5	22.23	34.95	453.2	210.03	139.9	41.69
2	230.4	21.90	31.20	486.7	227.66	159.2	44.55
3	236.6	24.05	37.15	498.3	230.45	164.2	45.36
LSD at 5%	9.49	3.55	4.84	0.43	0.25	0.18	0.05

Table(4):Effect of Spraing active Dry Yeast and Storage Period on The Oil Percentage,Oil Yield (ml/plant) AND OIL Yield (litre/fed) in Fresh and Dry Herb Of Marigold Plant (Tagetes Minuta,L.) During 2002 and 2003 Seasons.

Treatments (g/l)	Oil content (%)				Oil yield (ml/plant)				Oil yield (liter/fed)			
	Fresh herb	Dry herb at harvest	First month of dry herb	Second month of dry herb	Fresh herb	Dry herb at harvest	First month of dry herb	Second month of dry herb	Fresh herb	Dry herb at harvest	First month of dry herb	Second month of dry herb
2002 season												
0	0.33	1.33	1.33	1.27	0.42	0.34	0.35	0.33	6.77	5.50	5.62	5.23
1	0.38	1.53	1.57	1.37	0.70	0.54	0.55	0.49	11.15	8.57	8.77	7.90
2	0.47	1.60	1.67	1.50	0.90	0.60	0.62	0.56	14.35	9.55	9.98	8.97
3	0.45	1.73	1.70	1.43	1.00	0.77	0.76	0.64	16.07	12.29	12.09	10.18
LSD at 5%	0.08	0.17	0.21	NS	0.14	0.07	0.12	0.13	2.22	1.17	1.88	2.05
2003 season												
0	0.33	1.30	1.27	1.17	0.44	0.36	0.35	0.32	7.10	5.76	5.6	5.15
1	0.43	1.50	1.47	1.27	0.77	0.64	0.63	0.54	12.3	10.24	10.02	8.62
2	0.47	1.53	1.60	1.47	1.04	0.71	0.74	0.68	15.04	11.38	11.84	10.86
3	0.47	1.77	1.63	1.47	1.01	0.78	0.73	0.65	16.12	12.57	11.62	10.40
LSD at 5%	NS	0.73	0.45	0.61	0.46	0.35	0.28	0.21	7.39	5.74	4.53	3.50

Table (5): Effect of spraying active dry yeast on the percentages of identified constituents of marigold essential oil samples in 2003 season.

Treatments (g/l)		Essential oil components (%)							
		α-pinene	β-pinene	Limonene	Dihydrotagetone	Cis-tagetone	β-ocimene	Carvon	Unknown %
Herb fresh	0	--	--	3.29	85.71	--	--	--	11
	1	1.45	15.95	22.47	26.81	20.94	1.02	0.84	10.52
	2	1.44	19.99	24.70	24.78	15.56	1.93	0.96	10.64
	3	1.41	19.10	24.88	27.44	14.84	1.77	0.70	9.86
Dry herb at harvest	0	1.06	7.37	9.11	62.88	6.60	2.27	2.14	8.57
	1	1.51	6.24	8.20	63.91	8.61	2.33	2.75	6.45
	2	1.34	6.44	9.07	53.94	9.58	2.18	2.99	14.46
	3	1.05	5.82	14.92	50.95	10.76	4.43	2.33	9.74
First month of dry herb	0	1.06	5.42	12.65	50.48	15.57	1.61	1.73	11.48
	1	1.35	6.51	10.06	56.24	14.20	1.72	2.47	7.95
	2	1.12	6.26	14.70	46.39	15.70	1.93	2.29	11.61
	3	0.66	4.40	16.88	38.25	21.53	2.55	1.48	14.25
Second month of dry herb	0	0.56	4.51	15.25	28.67	29.62	1.25	1.61	18.53
	1	0.71	5.19	15.99	28.96	27.32	1.55	1.69	18.59
	2	0.90	6.08	20.32	38.84	21.81	1.68	1.58	8.79
	3	0.42	3.83	19.67	32.17	22.28	2.07	1.33	18.18

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تأثير الرش بالخميرة الجافة النشطة على النمو وفترة التخزين على الزيت العطرى لنبات القطيفة

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أجرى هذا البحث فى مزرعة النباتات الطبية والعطرية بكلية الزراعة جامعة المنصورة خلال موسمى الزراعة ٢٠٠٢ ، ٢٠٠٣ على نبات القطيفة. بهدف دراسة تحسين النمو والمحصول وكذلك النسبة المئوية للزيت العطرى ومحصول الزيت/نبات عن طريق الرش بالخميرة بمعدلات صفر ،١، ٢ ، ٣ جرام/لتر، كما استهدفت الدراسة أيضا تأثير فترة التخزين على نسبة الزيت العطرى فى العشب الجاف.

وتشير أهم النتائج إلى أنه رغم أن كل المعاملات المستخدمة قد أدت إلى تحسين قياسات النمو والمحصول لنبات القطيفة مقارنة بالكنترول إلا أن معاملة الرش بالخميرة بمعدل ٣ جرام/لتر كانت أفضل المعاملات بالنسبة لزيادة محصول العشب الطازج والجاف وكذلك النسبة المئوية للزيت العطرى.

ولقد أوضحت النتائج أيضا أن زيادة فترة تخزين العشب الجاف أدت إلى نقص فى نسبة الزيت العطرى. كما أظهر التحليل الكروماتوجرافى لعينات الزيت سبعة مركبات حيث أدت زيادة طول فترة التخزين إلى زيادة بعض المركبات الرئيسية مثل الليمونين والسيس تاجيتون ولكن أدت إلى انخفاض نسبة مركبات الداى هيدروتاجيتون والبيتابينين مقارنة بالكنترول.

توصى الدراسة باستخدام معاملة الرش بالخميرة النشطة الجافة بمعدل ٣ جرام/لتر على ثلاثة جرعات بعد ٣٠، ٦٠و٩٠ يوما من الزراعة للحصول على أعلى انتاجية من محصول العشب الطازج والجاف وكذلك النسبة المئوية للزيت العطرى لنباتات القطيفة تحت ظروف محافظة الدقهلية.