

EFFECT OF BIO- FERTILIZATION AND SOME PLANT EXTRACTS ON THE GROWTH, YIELD AND CHEMICAL CONSTITUENTS OF BASIL PLANT.

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ABSTRACT

The present work was carried out at the Farm of Medicinal and Aromatic Plants Research Department, El-Kanater El- Khairiya, Horticulture Research Institute, during the two successive seasons 2011 and 2012. The present study aimed to investigate the effect of bio fertilization (active dry yeast with three concentrations 4, 6 and 8 gm/l) and some medicinal plant extracts (garlic at 50% and aloe vera with four concentrations 25, 50, 75 and 100 %) on the growth, yield of herb, oil production and chemical constituents of basil plant *Ocimum basilicum*, L. The results indicated that, most treatments significantly increases plant height, number of branches, dry weight of herb as well as oil yield compared with control. The best treatment of garlic + yeast was garlic 50 % + 8 g. yeast / L, while it was the treatment of aloe 100 % for aloe treatments.

INTRODUCTION

Ocimum basilicum, L. belongs to family Lamiaceae (basil or sweet basil), it concedes the most economic importance and is cultivated and utilized throughout the world, because of the continuous and increase demands for their products from the local and foreign markets. The aromatic leaves are used fresh or dry as a flavoring agent for foods, confectionary products.

Basil is widely used as a vegetable and as aromatic plant and was originated North West India, North East Africa and Middle Asia (Gill and Randhawa, 1992). It grows in subtropical zone from sea level to altitude of 1800 m. This plant includes at least 60 species and numerous varieties.

An important factor affecting the quantity and quality of the harvested basil yield is to find the optimum level of fertilization. The level of naturally fertilization depends on the soil (Hiltunen and Holm, 1999).

Bio-fertilizers are important for medicinal and aromatic plants to produce best product in both quantity and it is also safe for human, animal and the environment.

Bio fertilization of horticulture crops had drawn the attention of research workers and had become in the last decades a positive alternative to chemical fertilizers. Bio fertilizers are reasonably safer to the environment compared to nitrogen chemical fertilizers.

N.R.P. (1977) stated that analysis of dry yeast consisted of dry matter 93%, protein 47%, arginine 2.6%, glycine 2.6%, histidine 1.4%, isoleucine 2.9%, leucine 3.5%, lysine 3.8%, methionine-cysteine 0.6%, phenyl-alanine 3.0%, tyrosine 2.1%, threonine 2.6%, tryptophan 0.5% and vitamin B2.9%

The various positive effects of applying active dry yeast was attributed to its own contents of different nutrients, high percentage of protein, larger amount of vitamin B and natural plant growth regulators such as cytokinins. In soil solution to form low solubility substances called phosphate fixation. This is dominating with high soil PH and greater percentage of calcium carbonate. Soil microorganisms which convert the insoluble form of phosphorus to soluble one play an important role in supplying the plants with available phosphorus (Ahmed *et al.*, 1997).

EIN Sayed (1985) on *Achillia millifolium*, found that, application of 0.2% dry yeast was the effective with respect to the morphological characters, the increase in volatile oil content as result of application of cytokinin as one of active dry yeast components.

In this concern Haridi (1987) working on *Salvia officinalis* concluded that, the positive effect of active dry yeast may be attributed to its components as cytokinine and vit-B which are active in improving the growth and productivity. In addition, Yeast is a natural source of most nutritional elements (Na, Ca, Fe, K, P, S, Zn and Si) (Nagodawithana, 1991).

Refaat and Naguib (1998) investigated the oil contents in *Mentha* plants treated with bio fertilizer (yeast) where they mentioned that, the total oil markedly increased in response to treating the plants with yeast.

Ahmed *et al.*, (1998) studied the effects of spraying active dry yeast at the concentration of 0.0, 0.05, 0.2 and 0.4 % to marjoram plants. The author found that, all concentration from 0.05-0.4%, in most cases significantly increased the plant height, number of branches; the dry weight of plants as well as volatile oil %.

(Heikal (2005) on thyme plants, indicated that, the application of ADY significantly increased plant height, No. of branches/plant, fresh and dry weights.

Seleim (2005) studied the effect of application of active dry yeast on the growth of *Mentha viridis* and *Salvia officinalis* plants, the author found that, application of dry yeast (1.0 or 2.0 gm/l) significantly increased the vegetative growth characters (plant height, fresh as well as dry weight of shoots, leaves and leaf area). Also the same author found that the contents of volatile oil, photosynthetic pigments (chlorophyll a & b) and nutrient of (N, P and K) were increased under the same conditions of treatments. (Abd El-Latif (2006) stated that, spraying *Salvia officinalis* with active dry yeast at 5 g/l significantly increased fresh and dry weights of herb and leaves.

Josias H. Hamman (2008) found that, the Aloe vera juice contain, anthraquinone, enzymes, vitamins, inorganic compounds and essential amino acid Mady (2009) treating *Majorana hortensis* and *Salvia officinalis* by active dry yeast at 4gm/l showed, stimulated it's fresh, dry weights, photosynthetic pigments of chlorophyll (a) and (b) and total soluble carbohydrates content in the first and second cut. Also, the content of total oil, in the present work, was highly significantly increased in the two investigated plants. Azza Ezz EL-Din and Hendawy (2010) showed that, adding active dry yeast at the rate of 6g./L., was the most effective on growth Parameter and oil% on borage plants.

Concerning the effect of different plant extract, (Helmy1992) applied fresh garlic clove extract solution (in ethyl alcohol or tap water) to summer squash cv. Eskandarani plants. He remarked that soil side dressing of garlic extract at 250 mg DW /plant gave the best results in increasing the number of flowers. (Ahmed *et al.*, 2005) confirmed that greater increase in number of pods of pea (cv. Meteor) was obtained with post inoculation treatment with garlic extract at 10 g/8 liters. Mady (2009) treating *Majorana hortensis* and *Salvia officinalis* by garlic extract (*Allium sativum*) with concentration at 50 or 100% showed, stimulated it's fresh, dry weights, photosynthetic pigments of chlorophyll (a) and (b) and total soluble carbohydrates content in the first and second cut Also, the content of total oil, in the present work, was highly significantly increased in the two investigated plants.

(Lindsey *et al.*, 2002). Used aloe plant extract is to improve the germination, vegetative growth and flowering.

DongZhi *et al.*, (2004) concluded that the aqueous leaf extract of Aloe vera could be useful as a natural plant growth regulator. Padmaja *et al.*, (2007) stated that Aloe vera peelings powder at 140 g/pot significantly increased fresh and dry weights of Lady's Finger (*Abelmoschus culentus*) plants. El-Shayeb (2009) declared that all concentration of Aloe vera extract increased fresh and dry weights of flowers of *Oenothera biennis*, the best response resulted from the highest concentration of Aloe. Mady (2009) treating *Majorana hortensis* and *Salvia officinalis* by aloe extract (Aloe vera) with concentration at 50 or 100% showed, stimulated it's fresh, dry weights, photosynthetic pigments of chlorophyll (a) and (b) with respect carotenoides and total soluble carbohydrates content in the first and second cut Also, the content of total oil, in the present work, was highly significantly increased in the two investigated plants.

(The world's healthiest food 2012) showed that, garlic extract contains carbohydrates, manganese, magnesium, selenium, calcium, phosphorus, copper, tryptophan, vit. B1, vit. B6, allicin, and allinase enzyme.

MATERIALS AND METHODS

This experiment was conducted to study the effect of bio fertilization and some medicinal plants extracts on the vegetative growth, herb production, oil production and chemical composition of basil plant (*Ocimum basilicum*, L.).

The field experiment was carried out at the Farm of Medicinal and Aromatic Plants Research Department, El-Kanater El- Khairiya, Horticulture Research Institute, during the two successive seasons 2011 and 2012.

Basil seedlings were planted on 15th May in both seasons in the field experiment on one side of the row, in hills 30 cm apart. The experimental plots were 2.5x 2.0 meters, with 3 rows at distance of 60 cm between rows.

After 21 days from transplanting, seedlings were received three sprays of active dry yeast with three concentrations (4g/l, 6g/l and 8g/l), one spray every 15days.

Garlic extract also used after 21 days from transplanting with one concentration (50%) alternative with active dry yeast, the plant was received three sprays.

As for aloe extract the treatments were every week, the plants received six sprays.

The plant sprayer with the same treatments after every cut, i.e. the plant also treated with active dry yeast alternative with garlic extract four times. The plants also sprayed with aloe extract 8 times with different concentrations.

Active dry yeast was added as bio fertilizer with three concentrations (4, 6 and 8g/l).

Garlic extract was added with one concentration 50%, while aloe vera extract was added with four concentrations 25, 50, 75 and 100%.

The treatments of the experiments were 8 and they were as follows:

- Chemical fertilizer N, P, K (control): The recommended dose of nitrogen was applied at a rate of 500 kg. / fed. using ammonium sulphate (20.5N). Phosphorus was applied at a rate of 300 kg/fed. using calcium super phosphate (15.5%P₂O₅ P). Potassium was applied at a rate of 75 kg / fed. using potassium sulphate (48 % K₂O).

- Garlic extracts with concentration 50% +4g/l active dry yeast.
- Garlic extracts with concentration 50% +6g/l active dry yeast.
- Garlic extracts with concentration 50% +8g/l active dry yeast.
- Aloe Vera extract with concentrations at 25%, 50%, 75% and 100%.

The three cuts of basil plants were done on 10th Jul., 15th Sept. and 15th Nov. in first season while they were 5th Jul., 12th Sept. and 12th Nov. in the second one.

During every cut these data were recorded:

- | | |
|--------------------------------|------------------------------|
| 1 -Plant height | 2 - Number of branch |
| 3 -Fresh and dry herb / plant | 4 -Fresh and dry herb / fed. |
| 5 -Oil %and oil yield | 6 -Pigments in leaves |
| 7 -Total carbohydrates in herb | 8 - GC/MAS analysis. |

All the agricultural practices were done in the field experiment.

The layout of the experimental:

The layout of this experiment was designed as complete randomized blocks with 3 replicates as described by **Snedecor and Cochran (1968)** using the L.S.D. test at 0.05.

Preparation of the plant extract:

1-Garlic plant extract:

According to El- Desouky *et al.* (1998) the fresh mature cloves were blended in the presence of distilled water 1 kg cloves / L, then frozen and thawed two times then filtered. The filter was used for preparation of different garlic extract concentration. The garlic plant extracts were used as a foliar spray at half strength by adding distilled water.

2-Aloe plant extract:

It was prepared as described by Wilfred *et al.*, (1990) where the plant tissues were crushed using a porcelain mortar and pestle, aloe extract presence of distilled. Water at equal rate (1/1by volume), then filtered. The

obtained extract was used for foliar spray at 25%, 50%, 75%, and 100% by adding distilled water.

The oil percentage was determined in the fresh herb according to the method described by the British pharmacopoeia (1963).

Oil samples taken and were analyzed using GC/MS Shimadzu-QP5000 GC 17A series, column DB5-ms 30m x 0.250mm x 0.1 µm film thickness to determine their main constituents as described by EL Tobgy (1999).

The analysis conditions were as follows GC/MS:

Oven temp.: 70oC, injector temp.: 50oC, interface temp.: 250oC, temperature programming: 70oC for 2 minutes, rate: 3o/min to 170oC for 1 minute 8o/min to 210oC/15 minutes, injection type: split, split ratio: 1: 24, carrier gas: helium 99.999%, carrier flow rate: 1.9 ml/min, detector gain: 1.50 kV, mass range: 40: 350 M/Z, run time: 56.33 min.

The estimation content of Chlorophyll a, b, and carotenoides content were determined in fresh leaf samples (mg/g fresh matter) according to Saric *et al.*, (1967), while the total carbohydrates content in the dried herb using the method described by Herbert *et al.*, (1971).

The physical and chemical properties of the soil of the experimental area were shown in Table (1).

RESULTS AND DISCUSSION

Effect of active dry yeast and some medicinal plant extracts (garlic and aloe)

1. Vegetative growth:

Data in Table (2) and Fig. (1, 2) showed the response of plant height and number of branches of *Ocimum basilicum*, L plant to different treatments during three cuts in two seasons.

It is obvious that, the growth of basil plant was enhanced due to different treatments under study i.e. Plant height and number of branches were significantly increased compared with control. These increases were accompanied with increasing the concentrations of yeast. the highest values were obtained when the plant treated with active dry yeast 8 g/l + garlic 50% there were, (93.21cm and 96.31 cm) for plant height in the second cut of the first and second seasons respectively, while, they were recorded (16.17 and 17.20) for the number of branches in the third cut for the two seasons respectively.

As for aloe extract, the data indicates that, all the treatments significantly increased the growth characters compared with untreated plant, and by increasing the concentrations of aloe extract, plant height and number of branches significantly increased up to aloe extract 100 %, and the highest value of the growth was obtained when the plant treated with aloe extract 100%.

These results were observed during three cuts in two seasons. These results were in agreement with those obtained by Ahmed *et. al.*, (1998) on rassel plants and Seleim(2005) on mint and sage plants.

In this concern the explanation could be that, the dry yeast is capable of production of creation growth promoting substances such as hormones and amino acids that may enhance plant growth (Armanious, 1987), that may enhance plant growth. However, the fermentation process that occurred in the presence of dry yeast produces CO₂ in high quantity, a factor that may increase photosynthesis and consequently plant growth, the high content of dry yeast from vit.B5 and minerals might play a considerable role in orientation and translocation of metabolites from leaves into the productive organs (Mohamed *et al.*, 1999).

2. Herb production:

It was mentioned from data in Table (3) and Fig. (3, 4) that the different concentrations of active dry yeast significantly increased fresh and dry weights/ plant of basil plant compared with control, and when the concentrations of yeast treatments increased the fresh and dry weights were significantly increased, and the highest values were obtained from the treatment of highest concentration of yeast recorded 362.48 g. /plant and 367.19g./plant of fresh weight for the second cut in the first and second seasons respectively. While, they were 73.41g./plant and 96.31g./plant for dry weight /plant for the same cut in the two seasons respectively.

Regarding aloe extract, the data in the same table showed that, treating the plant with different concentrations of aloe extract, increased fresh and dry weights/ plant. These increases were statistically significant, and by increasing the concentrations of aloe, these parameters were significantly increased up to the treatment of aloe extract 100%, this treatment produced the highest value.

These results were showed in three cuts during the two seasons. These results were in harmony with those obtained by Heikal(2005) on thyme and Mady(2009) on marjoram and sage, also Josias H. Hamman (2008) found that, the Aloe vera juice contain, anthraquinone, enzymes, vitamins, inorganic compounds and essential amino acid.

Oil production:

1- Oil %

Results presented in Table (4) and Fig. (5) showed the response of oil production to bio- fertilization and some medicinal and aromatic plants extracts.

It is obvious that, oil % of basil plants significantly increased due to different treatments in both seasons during three cuts compared to control. The data showed that, increasing the concentrations of active dry yeast + garlic 50% extract significantly increase oil percentage up to active dry yeast 8g/l. + garlic 50%.

Regarding the different of aloe extract the data in the same table showed that, the aloe extract caused significant increase of oil percentage due to increasing the concentrations of aloe extract and the highest value was obtained when the plants treated with 100% aloe extract. These results were observed during two seasons in three cuts. These results were in agreement with these obtained by Refaat and Naguib (1998)_on peppermint.

t3

F1-2-3

F4-5-6

T4

2- Oil yield

Data in Table (4) and Fig. (6) indicated that, oil yield significantly increased due

to treating basil plants compared to untreated plants. Increasing the concentrations of aloe extract significantly increased oil yield and the highest values were obtained when the plants treated with active dry yeast 4g/l + garlic extract and aloe extract 100%.

Chemical constituents:

GC/MS analysis of the essential oil

Data in Table (5) and Fig.(7) showed GC/MS analysis of *Ocimum basilicum*, L. oil, in the third cut in some treatments, the data cleared that, the chemical composition of the oil are α - Pinene, β - Pinene, 1, 8 Cineol, Linalool, Camphor, α -Terpino, Geranyl acetate, Methyl chavicol, Eugenol and β -caryophyllene. The data also indicated that, the treatments of yeast 8g. /l + garlic 50%, aloe 25% and 100% increased the values of chemical composition of basil oil compared to control and the Linalool considered the main compound of basil oil in different treatments they were 0.43 %, 02.43 %, 02.43 % and 04.79 % respectively.

Table (5): Effect of bio-fertilization and some medicinal plant extracts on the essential oil composition (%) of *Ocimum basilicum*, L plant in the second season (2012) for the third cut.

NO.	Compound %	Treatments			
		Control	Y8g/l.+Gar.50%	Aloe 25%	Aloe 100%
1	α - Pinene	0.17	0.47	0.04	0.04
2	β - Pinene	0.44	1.12	1.21	1.21
3	1, 8 Cineol	10.81	12.00	12.74	13.87
4	Linalool	00.43	02.43	02.43	04.79
5	Camphor	0.31	0.40	0.03	0.00
6	α -Terpinol	7.73	13.84	10.31	17.41
7	Geranyl acetate	0.33	0.40	0.46	0.46
8	Methyl chavicol	3.14	3.01	3.06	3.96
9	Eugenol	0.46	0.97	1.71	1.97
10	β - caryophyllene	3.07	0.96	0.96	0.96

Chemical composition:

1-Total carbohydrates

The results recorded in Table (6) indicated that, total carbohydrates significantly increased by increasing the different concentrations of extracts compared to control the date also showed that, the highest values of total carbohydrates were obtained when the plant treated with active garlic 50% extracts + dry yeast and aloe extract 100% .

These results were observed during the two seasons. Similar trend was observed by Naguib (2002) work on lemon grass plants treated with active dry yeast. He revealed that, the increases of carbohydrates contents were resulted to the increases in the photosynthetic rat.

F7

2- Pigments

It can be noticed from data that in Table (6) the content of pigments (chlor. a, chlor. b and carotenoides) significantly increase by treating basil plants with different treatments compared with control and the highest values were obtained when the plants treated with active dry yeast 4g/l + garlic 50% extract for yeast treatments and aloe 100% for aloe treatments.

The same trend was obtained during the two seasons. Similar results were observed by Selim (2005) on mint and sage and Reda *et al.*, (1999) reported that, foliar application of bio-fertilizer of dry yeast significantly stimulated the photosynthetic pigments (ch. A, b and carotenoides) of *Hyoscyamus muticus* plants.

Conclusion

It was observed from this study that using bio-fertilization and medicinal plant extracts enhanced the growth, oil and chemical composition of basil plant that found in yeast, garlic and aloe may be due to the different compounds, amino acid, enzymes and vitamins.

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تأثير التسميد الحيوي وبعض المستخلصات النباتية على النمو والمحصول والمواد الفعالة في نبات الريحان

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اجريت هذه الدراسة خلال الموسمين المتتاليين ٢٠١١ / ٢٠١٢ في مزرعة قسم بحوث النباتات الطبية بالقناطر الخيرية لدراسة تأثير الرش بالخميرة النشطة الجافة بتركيزات ٤، ٦ و ٨ جم/ل مع مستخلص الثوم ٥٠% ، كذلك مستخلص الصبار بتركيزات ٢٥، ٥٠، ٧٥ و ١٠٠% علي النمو والمواد الفعالة في نبات الريحان .
أوضحت الدراسة النتائج التالية :
أ- ادى الرش بالخميره الجافه النشطه ٨جم/ل ومستخلص الثوم ٥٠% بالنسبة لمعاملات الخميره ومستخلص الصبار بتركيز ١٠٠% الى زيادة النمو الخضري ومحصول الزيت وبعض المركبات زياده معنويه.
ب- ادى الرش بالخميره النشطه الجافه ٨جم/ل ومستخلص الثوم ٥٠% بالنسبة لمعاملات الخميره ومستخلص الصبار بتركيز ١٠٠% الى زيادة معنويه فى الوزن الطازج والجاف للعشب فى نبات الريحان.
ج- ادى الرش بالخميره ٨جم/ل ومستخلص الثوم ٥٠% بالنسبة لمعاملات الخميره ومستخلص الصبار بتركيز ١٠٠% الى زيادة معنويه فى نسبة الزيت العطرى ومحصول الزيت.

د- ادى الرش بالخميره ٨جم/ل ومستخلص الثوم ٥٠% ومستخلص الصبار بتركيز ١٠٠% الى زيادة الكربوهيدرات الكليه والصبغات.

ي- اما بالنسبة الى تحليل ال GC/MS اظهرت النتائج أن زيت نبات الريحان يتكون من المركبات التاليه:

α - Pinene, β - Pinene, 1, 8 Cineol, Linalool, Camphor, α -Terpino, Geranyl acetate, Methyl chavicol, Eugenol and β -caryophyllene. وان

المكون الرئيسي للزيت هو مركب ال Linalool , لذا نوصى : في حالة استخدام الخميرة الرش بتركيز ٨جم /لتر بالتبادل مع مستخلص ثوم ٥٠% ، اما في حالة الرش بمستخلص الصبار فيكون بتركيز ١٠٠%.

قام بتحكيم البحث

كلية الزراعة – جامعة المنصورة

أ.د / حكمت يحيى مسعود

مركز البحوث الزراعية

أ.د / احمد عاطف رضوان فهمى

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

1-Physical properties of the experimental soil.															
Percentages														Texture	
				Sand	Silt	Clay	Gravel								
				29.50	9.25	58.90	2.11				<u>clay</u>				
2-Chemical properties of the experimental soil.															
SO ₄ ⁻ (meq/L)	CL ⁻ (meq/L)	HCO ₃ (meq/L)	Co ₃ (meq/L)	k ⁺ (meq/L)	Na ⁺ (meq/L)	Mg ⁺⁺ (meq/L)	Ca ⁺⁺ (meq/L)	E.C(ds/m)	PH	CaCo ₃ %	O.M %	N %	Total (ppm)		
													K	P	N
16.12	12.31	6.42	-	1.56	10.00	5.56	17.22	3.21	7.31	2.91	1.81	0.210	652	28.62	2.13

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