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Influential Cooperation between Zeolite and PGPR on Yield and Antimicrobial Activity of Thyme Essential Oil

R. A. Zaghloul[1], Y. F. Y. Mohamed[2] and Rasha M. El-Meihy[1,3*]

[1]Department of Agricultural Botany, Agricultural Microbiology Branch, Faculty of Agriculture, Benha University, Moshtohor, Toukh, Qalyoubia, 13736, Egypt.
[2]Department of Horticulture, Faculty of Agriculture, Benha University, Moshtohor, Toukh, Qalyoubia, 13736, Egypt.
[3]Microbial Biotechnology and Fermentation Laboratory, Central Research Laboratories, Faculty of Agriculture, Benha University, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To study the interaction effect among the natural substance (zeolite), plant growth promoting rhizobacteria (PGPR) and *Saccharomyces cerevisiae* extract as foliar spraying on vegetative growth characteristics, yield and essential oil properties as well as chemical composition of its hydrodistilled essential oils by GC were studied. Also, microbial enzymes activity in thyme's rhizosphere and the antimicrobial activity of the essential oil extracts was evaluated against some pathogenic microorganisms.
Methodology: During two successive seasons 2013-2014 and 2014-2015, two field split plot experiments were conducted at the Experimental Farm of Horticulture Dep., Fac. Agric. Benha Univ. The present study include two foliar spraying treatments in the main plot (without and with *Saccharomyces cerevisiae* extract) and five treatments in the sub plot (control, full dose of NPK,

**Corresponding author: E-mail: rashaelmehy@fagr.bu.edu.eg;*

half dose of NPK + PGPR, full dose of zeolite, half dose Zeolite + PGPR).PGPR namely *Azotobacter chrococcum* ML1*, Bacillus circulans* ML2 and *Bacillus megaterium* ML3. Vegetative growth characteristics (plant height, branches number, fresh and dry weight), yield and essential oil properties and chemical composition of its hydrodistilled essential oils by GC were studied. Also, microbial enzymes activity (dehydrogenase, phosphatase and nitrogenase) in thyme's rhizosphere and the antimicrobial activity (minimum inhibitory concentration) of the essential oil extracts was evaluated against some pathogenic microorganisms viz., *Salmonella typhorium, Pseudomonas aureogenosa, Staphylococcus aureus, Bacillus cereus and Candida albicans.*

Results: Results indicated that thyme's rhizosphere amended with half dose of zeolite combined with PGPR and *Saccharomyces* extract gave the highest significant values of all estimated enzymes. In addition, plant height, branches number/plant, fresh and dry weights were higher in plants inoculated with PGPR combined with zeolite at half dose and sprayed with *Saccharomyces* extract compared with other treatments. These results are true in two cuts and in both two growing seasons. On the other hand, chemical analysis of thyme grown under different treatments showed that chemical fertilizer treatments gave the highest significant values of macronutrients and carbohydrates content. Regarding the effect of different treatments on essential oil composition of *Thymus vulgaris*, it was clearly that the main components were thymol, β-phyllandrene and myrcene. These components of thyme's oil were able to inhibit all tested pathogens via minimum inhibitory concentration (MIC) test which resulted that the examined Gram positive bacteria were more sensitive rather than either Gram negative or *Candida albicans*.

Keywords: Zeolite; PGPR; antimicrobial activity; MIC; Thyme's essential oil.

1. INTRODUCTION

Thymus vulgaris L. is an aromatic plant and are extensively used (fresh and dried) as a culinary herb. Their essential oils are utilized as flavour ingredients in a wide variety of food. In addition, because of its antiseptic and antimicrobial properties. Most aspects of medicinal use of *Thymus* spp. are related to their essential oil composition, which show various levels of thymol, carvacrol and phenolic derivatives with strong and wide-spectrum antimicrobial activity [1]. Concerning the composition and the biological properties of *Thymus* essential oils several reports have been published. These studies have emphasized the obvious differences among oils extracted from different species or varieties. More than 20 essential oil chemo types were noticed in different species of *Thymus* genus [2].

Recently, one group of minerals has emerged as having considerable potential in a wide variety of agricultural processes. This group of minerals is the zeolite group. Zeolite minerals are crystalline hydrated alumino-silicates of alkaline-earth metals, formed by AlO_4 and SiO tetrahydral. More than forty types of zeolite have many properties which are interest for agricultural purpose [3]; high cation exchange capacity, water holding capacity and adsorption capacity [4]. Roxana [5] confirms that the suitability of using natural zeolite in agriculture had a positive role in plant nutrition and microbial community stability, as evidenced subject experimentation crops. Regarding the microbiological characteristics of the soil, zeolite increased eleven examined soil microbial counts i.e. total microbial, nitrifying bacteria, cellulose decomposing bacteria, phosphatase, microbial biomass C and microbial biomass N [6]. All these unique properties of zeolite materials promise to contribute significantly too many years of agricultural technology.

Plant growth promoting rhizobacteria (PGPR) are beneficial microorganisms able to produce biological substances that promote vital processes inside the plant. These substances contain vitamins, amino acids, sugars, phytohormones (auxins, cytokinins and gibberelins) and antioxidants. They increase plant yield and quality of products. In addition, they increase plants resistance to inappropriate environmental conditions such as drought, salinity and heavy metals toxicity in soil. This may be attributed to changes made to enzymes activity and antioxidants synthesis [7]. Also, PGPR play an important role in nitrogen fixation, phosphorus solubilization and potassium releasing in soil [8].

Yeast extract act as natural safety and rich source of phytohormones, sugar, vitamins, enzymes, amino acids and minerals. Also, yeast has stimulatory effects on cell division and

enlargement synthesis of protein, nucleic acid and chlorophyll formation. The enhancement effect of yeast could be attributed to its stimulating effect on enzymes activity, phytohormones production, improving the uptake of nutrients which increased vegetative growth of plants. It also releases CO_2 which reflected in improving net photosynthesis [9]. Additionally, it has been demonstrated that yeast cell wall ingredients, such as polysaccharide fraction, can act as elicitors in plant signal transduction pathways as a plant defense response, so it is not only active yeast extract that may be used in agricultural applications. Because of their natural origin, these extracts are environment friendly (eco-friendly) products which can be used in agricultural applications for stimulating the plant defense mechanism and improving the nutraceutical quality of some plants [10].

The aim of the current study is to evaluate the interaction effect among the natural substance (zeolite), plant growth promoting rhizobacteria and *Saccharomyces* extract as foliar spraying on microbial enzymes activity in thymus's rhizosphere, vegetative growth characteristics, yield and essential oil properties and to determine the chemical composition of its hydrodistilled essential oils by GC and GC/MS. As well as evaluating the antimicrobial activity of the essential oil extracts against pathogenic microorganisms.

2. MATERIALS AND METHODS

2.1 Particle Size Distribution and Chemical Analyses of Experimental Soil

Experimental soil was subjected to particle size distribution and chemical analyses according to the methods described by Page et al. [11]. Particle size distribution and chemical analyses are presented in Table 1.

2.2 Fertilizers

2.2.1 Chemical fertilizers

Fertilizers which used were as follows: zeolite which contains 1.02% nitrogen; 0.14% phosphorus and 5.35, 3.23, 0.03, 0.07 mg/l magnesium, calcium, zinc, iron, respectively was obtained from Al-Koptan company for import & export, Qalubia Governorate, Egypt and added to soil at a rate of 48.2 kg/fed in three equal doses. Chemical NPK fertilizers were obtained from Res. Center of Fac. Agric., Benha Univ., Egypt. Inorganic nitrogen fertilizer was applied at a rate of 50 kg N/fed as urea. Also, inorganic phosphorus fertilizer was applied at a rate of 25 kg P_2O_5/fed as calcium superphosphate while, potassium fertilizer was applied at a rate of 40 kg K_2O/fed as potassium sulphate in three equal doses.

2.2.2 Plant growth promoting rhizobacteria (PGPR)

Azotobacter chrococcum ML1*, Bacillus circulans* ML2 and *Bacillus megaterium* ML3 and *Saccharomyces cerevisiae* ML4 were obtained from Microbiology branch, Agric. Botany Dept., Fac. Agric., Benha Univ., Egypt.

2.2.2.1 Inocula preparation

A. chrococcum was grown in Ashbey's broth [12] for seven days (1 ml contains about) 10^6 CFU. *B. circulans* was grown on modified nutrient broth [13] for five days (1 ml contains about) 10^8 CFU. *B. megaterium* was grown on Pikovskaya's (PVK) broth [14] for five days (1 ml contains about) 10^8 CFU. The same prepared inocula were added to soil three times throughout the growing season. *S. cerevisiae* extract was prepared using a technique described by [15] and applied three times throughout growing season as foliar spraying at a rate of 3 l/fed.

2.3 Experimental Design

Two successive field experiments were established in 2013-2014 and 2014-2015 seasons at the Experimental Farm of Horticulture Dep., Fac. Agric. Benha Univ. Treatments were arranged in split plot design with three replicates. The experimental plot area was 10.5 m^2.

2.4 Cultivation Process

Shoot segments of thyme (*T. vulgaris* L.) were obtained from Horticulture Dept., Fac. Agric., Benha Univ., Egypt. Shoot segments were planted in plastic pots (8 cm diameter) at 1st December in the two seasons in medium containing clay + sand 1:1 by volume and lefted

Table 1. Particle size distribution and chemical analysis of the experimental soil

Parameters	Unit	Values	Parameters	Unit	Values
A. Particle size distribution			B. Chemical analysis		
Coarse sand	(%)	5.91	Organic matter	(%)	1.52
Fine sand	(%)	24.73	$CaCO_3$	(%)	0.55
Silt	(%)	25.22	Total nitrogen	(%)	0.23
Clay	(%)	44.14	Total phosphorus	(%)	0.12
Textural class	(%)	Clayey loam	Total potassium	(%)	0.27
			pH		8.2

under low plastic tunnels in the greenhouse for three months. Well rooted transplants of thyme were cultivated after they had 2 to 4 branches in each experimental plots at 1st March in the two seasons. After transplanting, soil was directly irrigated to provide a suitable moisture for added inocula. The normal culture practices for growing thyme were followed as recommended in the region.

2.5 Pathogenic Microorganisms

The essential oils from different treatments were tested against five human pathogenic bacteria, including two Gram positive bacteria namely *Staph. aureus* ATCC 20231 and *B. cereus* ATCC 33018, two Gram negative pathogenic bacteria namely *S. typhimurium* ATCC 14028 and *Ps. aeruginosa* ATCC 4182 and *C.albicans*zus5. Pathogenic microorganisms were obtained from Microbial Biotechnology and Fermentation Lab., Central Research Lab., Fac. Agric., Benha Univ., Egypt.

2.6 Determinations

2.6.1 Enzymes in thyme's rhizosphere

Dehydrogenase activity (DHA) was assayed as µg TPF/g dry soil/24 hr. [16]. Phosphatase activity was estimated as µg inorganic phosphorus released/g dry soil [17]. Nitrogenase activity was measured by using the acetylene reduction technique [18] as µl C_2H_4/g dry soil. Enzymes were estimated in thyme's rhizosphere during the two growing seasons periodically at (45, 90, 120, 150 and 240 days) from transplanting.

2.6.2 Growth characteristics

During each experimental season, the plants were cut twice in each harvest. The first and the second cut were done after three and eight months from transplanting, respectively. Plant height, branch number/plant, herbs fresh weight (g/plant) and herbs dry weight (g/plant) were estimated after 90 and 240 days from transplanting.

2.6.3 Plant chemical analysis

Total nitrogen, phosphorus, potassium and carbohydrates were determined in thyme herbs at flowering stage according to the methods described by [19-22], respectively.

2.6.4 Oil yield

Essential oil yield/plant (g) was estimated after 90 and 240 days from transplanting during two growing seasons.

2.6.5 Essential oil properties

The percentage of oil were determined in fresh herb using 100 g samples for each cut. Distillation of volatile oil to extract the essential oils was carried out according to the method described by [23].

2.6.6 Fraction of thyme's essential oil

The gas liquid chromatography analysis was carried out at the medicinal and aromatic plant Lab., Hort. Res. Cen., Nat. Res. Cen., Dokki, Giza, Egypt to determine the components of thyme's essential oil. The obtained chromatogram and report of GC analysis for each sample were analyzed to calculate the percentage of main components of volatile oil.

2.6.7 Determination of minimum inhibitory concentration (MIC)

For the antimicrobial assays, the method described by Santurio et al. [24] was used. The

MIC was defined as the lowest concentration of essential oil at which no growth was evident compared to positive control (broth only with no essential oil).

2.7 Statistical Analysis

The statistical analysis was carried out using the CoStat package program, version 6.311 (cohort software, USA) [25]. The differences between the mean values of various treatments were compared by Duncan's multiple range test [26].

3. RESULTS AND DISCUSSION

3.1 Interaction Effect among Zeolite, PGPR and *Saccharomyces* Extract on Microbial Enzymes Activity in Thyme's Rhizosphere

Data in (Figs. 1, 2 and 3) indicated that rhizosphere of thyme cultivated in soil without any amendments (control) gave the lowest dehydrogenase, phosphatase and nitrogenase activities at the two determination periods. The obtained results also indicated that all enzymes in various treatments were gradually increased from initial time to reach their maximum records at 150 days for DHA and decreased thereafter while, at 200 days for P-ase and N_2-ase. The observed increase in enzymes activity in cultivated soil could be attributed to the beneficial effect of root exudates which increase during the vegetative stage of cultivated plants and the differences in multiplication rate of different soil microorganisms during this stage. Also, results revealed that the highest significant increase of DHA, P-ase and N_2-ase were observed in thyme's rhizosphere amended with zeolite at half dose of nitrogen and inoculated with PGPR combined with *Saccharomyces* extract. This result is likely be due to not only the promotion of PGPR on microbial proliferation but also to the beneficial effect of zeolite and *Saccharomyces*. In this respect, [27] reported that the microbial populations could respond to zeolite amendment in different ways, but further studies should be performed to understand better the zeolite effects on soil microbial activities. Also, [28] emphasized that zeolite application was significantly increased the number of total bacterial and fungal counts.

In addition, yeast application cause beneficial effect on either introduced or native microbial activities in soil as those stated by [29] who reported that spraying of legumes with *Saccharomyces* extract able to enhance all microbial activities in soil. Also data showed that at all determination periods the inoculation of thyme with PGPR combined with chemical NPK or zeolite gave significant increase in all estimated enzymes activity compared with either zeolite or chemical NPK each one singly. This could be attributed to the beneficial effect of rhizobacteria which known to improve solubilization of fixed soil phosphorus and fixing atmospheric nitrogen [30]. On the other hand, relative lower records of enzymes activity were observed in the experimental periods in soil amended with chemical fertilization than that amended with either zeolite or treatments inoculated with PGPR each one solely. This result could be due to the poor of this treatments to zeolite which cause increase of the microbial activities in soil. [3,31] stated that the beneficial effects of zeolites were observed on microbial populations and their activities in soils. Also, they found that an increase in microbial biomass after zeolite amendment. Moreover, [32] stated that natural zeolites have been accepted as promising materials for native and indigenous soil microorganisms due to their high porosity and large surface area. Also, these results are in agreement with those obtained by [33] who demonstrated that zeolite application has positive effect on soil fertility through the prevention of nitrogen loss from soil. Moreover, data in (Figs. 1, 2 and 3) clearly indicated that thyme inoculated with PGPR in combination with either NPK or zeolite at different doses gave significant higher DHA, P-ase and N_2-ase than that treated with either zeolite or NPK each one individually, this was true at all experimental period. Concerning the effect of *Saccharomyces* extract application, it was clearly that foliar spraying with *Saccharomyces* extract gave higher significant values of DHA and N_2-ase than plants without foliar spraying (control). This result is in accordance with those obtained by [10] who emphasized that the beneficial effect of yeast extract may be due to the fact that it is a natural source of most essential compounds such as phytohormones, sugar, vitamins, enzymes, amino acids and minerals which acts as cofactor for most enzymes.

3.2 Interaction Effect among Zeolite, PGPR and *Saccharomyces* Extract on Thyme's Growth Characteristics

Data presented in (Tables 2 and 3) indicated that the growth characteristics i.e. plant height,

number of branches/plant, dry and fresh weight of herb/plant were greatly affected by different investigation treatments in both cuts during two growing seasons. At the two cuts in the first season, the maximum plant height was observed in plants treated with half dose of zeolite combined with PGPR without *Saccharomyces* extract, while the highest records of branches/plant and plant weight (fresh and dry) were observed in plants treated with full dose of chemical NPK. This may be due to the beneficial effect of zeolite as those stated by [34] who found that either water retention or soil water contents to be greater in soils to which zeolite was applied, this effect likely be play an important role in plant growth activation. Also, [35] reported that the addition of zeolite has improved the nutrient status of root zones, especially selective retention of NH_4^+ and K^+ ions which have major role in plant growth improvement. From another view, the effect of this treatment could be attributed to the beneficial effect of PGPR which known as plant growth promotor as those obtained by [36] who reported that medicinal plants are the most plants response to inoculation with PGPR, which is reflected on the growth performance and healing properties. Also, [37] evaluated the inoculation with rhizobacteria on some medicinal plants i.e. *T. vulgaris* for plant growth promotion. They found that all estimated plants growth parameters were significantly response to inoculation and gave high growth characteristics. Medicinal plants support a great diversity of microflora in their rhizosphere including PGPR. The main mechanisms by which PGPR directly contribute to the plant growth are phytohormone production such as auxins, cytokinins and gibberellins, enhancing plant nutrition by solubilization of minerals such as phosphorus and iron, production of siderophores and enzymes, lowering of ethylene levels and induction of systemic resistance [38]. In addition, data revealed that in the second season the highest values of thyme's growth characteristics were recorded in the treatment of full dose of NPK with *Saccharomyces* extract in both cuts. On the reverse, control plants (without *Saccharomyces*) gave the minimum records of growth characteristics during two growing seasons in both cuts. In this context, [9] stated that the use of yeast extract as a natural, safety and rich source of phytohormones in order to improve plant growth and productivity has acquired a great attention nowadays. Also, [10] reported that yeast has stimulatory effects on cell division and enlargement. This enhancement effect of yeast could be attributed to its improving the uptake of nutrients which increased vegetative growth of plants. Regardless control (without *Saccharomyces* extract), the lowest values of growth characteristics were recorded in the treatment of full dose zeolite without *Saccharomyces* extract at both cuts in the first and second seasons. Generally, the second cut in both seasons produced the maximum records of all estimated growth parameters.

3.3 Chemical Analysis of Thyme Plants

Data presented in (Table 4) demonstrated that all combinations between fertilization with or without *Saccharomyces* extract succeeded in increasing the values of N, P, K percentages and total carbohydrates % in leaves of Thyme plants. The best results of these parameters were obtained in the treatment of full dose of NPK with *Saccharomyces* extract as compared to control (without *Saccharomyces*) in the two growing seasons. Moreover, either treatments of full dose of NPK without *Saccharomyces* extract or half dose of zeolite combined with PGPR resulted in highly increments of NPK content as compared to control (without *Saccharomyces*) in the two growing seasons. On the reverse, the lowest values of N, P, K percentages and total carbohydrates % in leaves were recorded by in the treatment of control, especially without *Saccharomyces* extract in both seasons. In this concern, [39] found that the application of biofertilizers increased N, P and K content in leaves of roselle plants. Also, [40] showed that *Thymus vulgaris L.* inoculated by nitrogen fixing bacteria showed a significant increase in total N, P and carbohydrate content. [41] showed that biofertilizers application increased N, P, K and total carbohydrates contents of *Ocimum basilicum, L.* cv. genovese leaves as compared control. Concerning the effect of zeolite application, [4] reported that zeolite have many properties which are interest for agricultural purpose: high exchange capacity, high water holding capacity and high adsorption capacity. In addition, [5] confirms that the suitability of using natural zeolite (clinoptilolite) in agriculture had a positive effect in plant nutrition and microbial community stability, as evidenced subject experimentation crops. Regarding the microbiological characteristics of the soil, zeolite increased soil microbial parameters i.e. total microbial, nitrifying bacteria, cellulose decomposing bacteria counts, phosphatase, microbial biomass C and microbial biomass N [6].

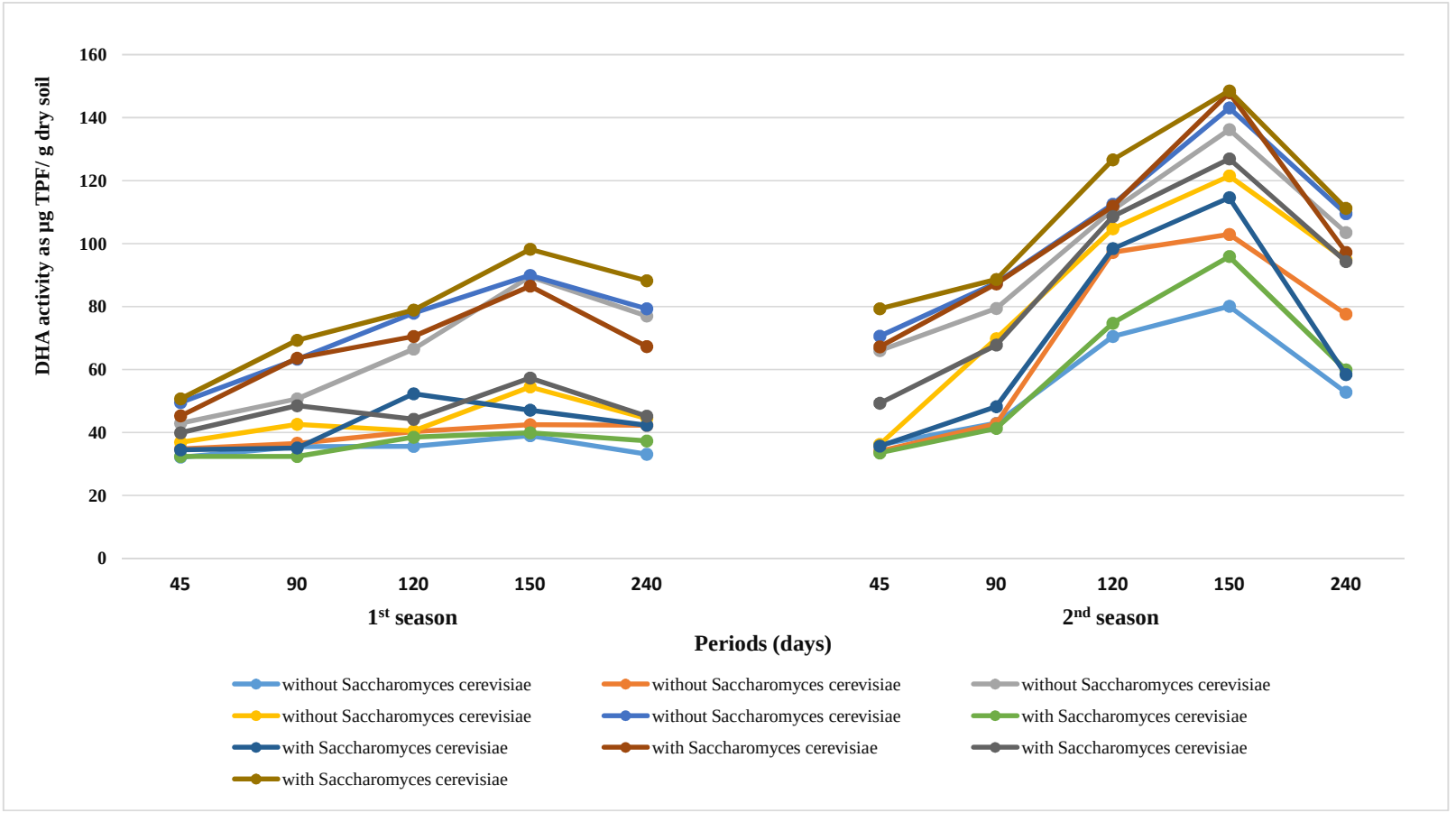

Fig. 1. Periodical changes in DHA in Thyme's rhizosphere

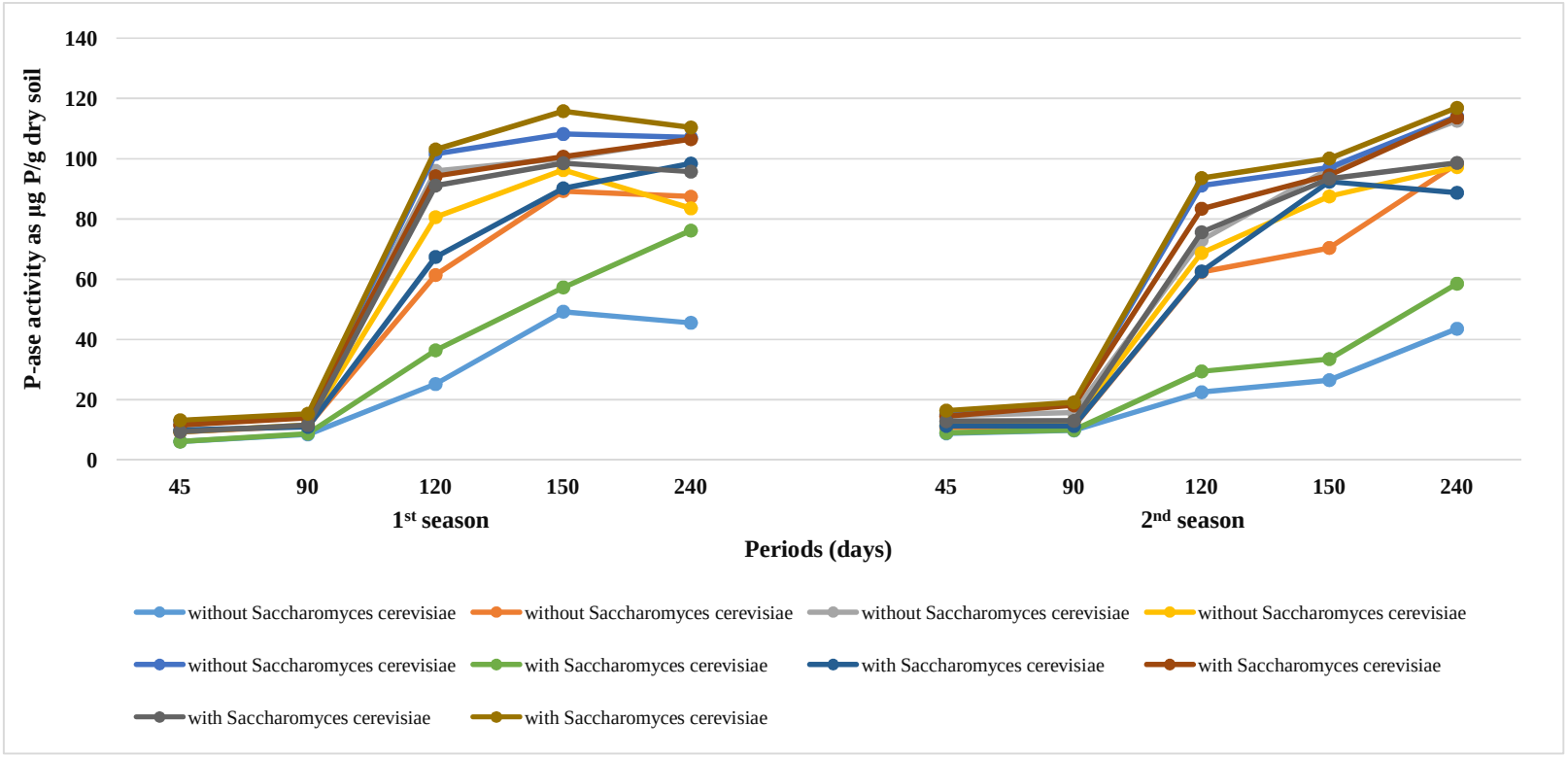

Fig. 2. Periodical changes in P-ase in Thyme's rhizosphere

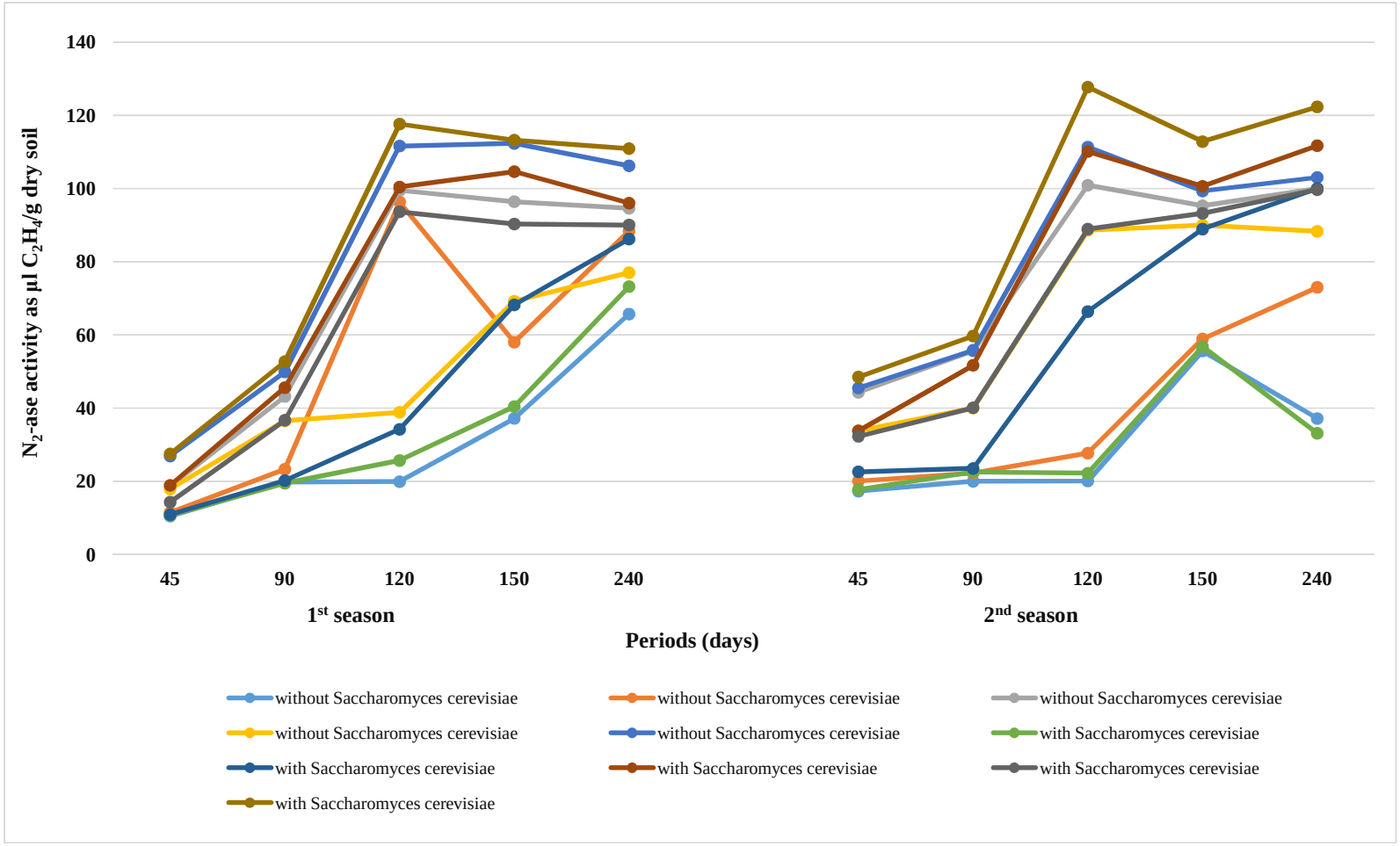

Fig. 3. Periodical changes in N_2 -ase in Thyme's rhizosphere

Table 2. Growth characteristics of thyme plants growing under different treatments during the 1st season

Treatments	Parameters	Plant height (cm)		Branches number/plant		Fresh weight (g/plant)		Dry weight (g/plant)	
		Cuts							
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Without *Saccharomyces cerevisiae*	Control	20.5^{j}	22.2^{i}	30.3^{j}	32.7^{i}	46.0^{j}	48.1^{j}	14.2^{h}	14.8^{g}
	Full dose of NPK	28.5^{f}	33.1^{d}	50.7^{e}	60.0^{b}	105.3^{b}	114.1^{b}	42.4^{b}	47.7^{a}
	½ dose NPK + PGPR	30.5^{e}	31.5^{e}	45.0^{g}	49.7^{e}	96.3^{f}	96.3^{f}	36.0^{d}	40.7^{d}
	Full dose of zeolite	25.1^{h}	26.9^{g}	40.7^{h}	42.7^{g}	88.3^{h}	90.7^{h}	31.7^{f}	36.2^{e}
	½ dose Zeolite + PGPR	35.0^{a}	36.2^{a}	47.3^{f}	54.3^{d}	98.6^{e}	107.3^{d}	39.8^{c}	42.3^{c}
With *Saccharomyces cerevisiae*	Control	22.0^{i}	25.0^{h}	35.7^{i}	34.3^{h}	52.6^{i}	54.4^{i}	17.6^{g}	18.6^{f}
	full dose of NPK	33.4^{c}	34.3^{c}	60.7^{b}	60.3^{b}	110.3^{a}	116.3^{a}	46.6^{a}	48.2^{a}
	½ dose NPK + PGPR	31.5^{d}	33.4^{d}	55.7^{c}	55.7^{c}	101.3^{d}	98.3^{e}	36.1^{d}	41.0^{d}
	Full dose of zeolite	27.4^{g}	28.3^{f}	54.6^{d}	43.7^{f}	92.4^{g}	93.4^{g}	33.7^{e}	36.4^{e}
	½ dose Zeolite + PGPR	34.3^{b}	35.5^{b}	61.4^{a}	61.7^{a}	103.1^{c}	110.7^{c}	39.7^{c}	43.8^{b}

Table 3. Growth characteristics of Thyme plants growing under different treatments during the 2nd season

Treatments	Parameters	Plant height (cm)		Branches number/plant		Fresh weight (g/plant)		Dry weight (g/plant)	
		Cuts							
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Without *Saccharomyces cerevisiae*	Control	22.3^{g}	20.9^{i}	31.7^{g}	30.3^{g}	49.70^{i}	50.6^{i}	15.0^{h}	15.6^{h}
	Full dose of NPK	35.3^{ab}	32.6^{d}	64.7^{a}	61.7^{b}	110.9^{b}	112.6^{b}	47.9^{a}	48.1^{b}
	½ dose NPK + PGPR	29.6^{d}	26.9^{f}	56.0^{c}	58.0^{c}	101.2^{e}	107.9^{d}	40.2^{e}	41.5^{e}
	Full dose of zeolite	26.2^{f}	25.4^{g}	40.7^{e}	43.3^{e}	93.20^{g}	95.1^{g}	35.9^{f}	38.3^{f}
	½ dose Zeolite + PGPR	33.1^{c}	31.3^{e}	60.7^{b}	60.3^{b}	107.1^{d}	106.7^{e}	44.2^{c}	43.2^{d}
With *Saccharomyces cerevisiae*	Control	25.2^{f}	24.2^{h}	35.0^{f}	36.3^{f}	55.20^{h}	58.8^{h}	18.6^{g}	20.0^{g}
	Full dose of NPK	36.3^{a}	34.8^{a}	65.7^{a}	65.7^{a}	112.4^{a}	115.9^{a}	48.2^{a}	49.1^{a}
	½ dose NPK + PGPR	34.9^{b}	34.6^{b}	59.3^{b}	61.7^{b}	107.1^{d}	110.6^{c}	43.8^{c}	45.1^{c}
	Full dose of zeolite	27.7^{e}	33.2^{c}	46.7^{d}	49.7^{d}	97.10^{f}	96.5^{f}	41.3^{d}	44.7^{c}
	½ dose Zeolite + PGPR	35.9^{ab}	34.6^{b}	60.3^{b}	61.0^{b}	108.5^{c}	115.0^{a}	47.0^{b}	48.9^{ab}

Table 4. Chemical analysis of Thyme plants growing under different treatments

Treatments	Parameters	N%		P%		K%		Carbohydrates %	
		Season							
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Without *Saccharomyces cerevisiae*	Control	1.5^{g}	1.3^{h}	0.20^{g}	0.24^{g}	1.0^{j}	1.2^{f}	8.7^{f}	8.7^{g}
	Full dose of NPK	3.8^{b}	3.8^{b}	0.39^{a}	0.42^{b}	2.6^{b}	2.8^{a}	14.2^{ab}	14.^{6b}
	½ dose NPK + PGPR	2.8^{d}	3.2^{d}	0.34^{c}	0.36^{d}	1.5^{g}	1.8^{d}	11.7^{cd}	11.5^{e}
	Full dose of zeolite	2.1^{e}	2.3^{f}	0.27^{e}	0.26^{f}	1.4^{h}	1.5^{e}	10.2^{e}	10.2^{f}
	½ dose Zeolite + PGPR	3.4^{c}	3.5^{c}	0.36^{b}	0.42^{b}	1.8^{f}	2.4^{c}	13.9^{b}	13.9c
With *Saccharomyces cerevisiae*	Control	1.8^{f}	1.5^{g}	0.22^{f}	0.25^{g}	1.3^{i}	1.5^{e}	9.9^{e}	9.9^{f}
	Full dose of NPK	4.1^{a}	4.3^{a}	0.40^{a}	0.54^{a}	2.8^{a}	2.9^{a}	15.0^{a}	15.0^{a}
	½ dose NPK + PGPR	3.4^{c}	3.5^{c}	0.35^{bc}	0.38^{c}	2.4^{d}	2.7^{b}	12.5^{c}	12.5^{d}
	Full dose of zeolite	2.1^{e}	2.8^{e}	0.29^{d}	0.30^{e}	2.1^{e}	1.9^{d}	11.5^{d}	11.7^{e}
	½ dose Zeolite + PGPR	3.7^{b}	3.8^{b}	0.35^{bc}	0.37^{cd}	2.5^{c}	2.5^{b}	14.6^{ab}	14.2^{c}

3.4 Essential Oil %

Data presented in Table 5 indicated that essential oil percentage/plant of thyme was more affected by using all different treatments as compared to control (without *Saccharomyces*) during two growing seasons and in both cuts. Treatment of full dose of NPK combined with *Saccharomyces* extract was the most effective for increasing essential oil yield percentage/ plant, followed by full dose of NPK without *Saccharomyces* extract at the first and second cuts in both growing seasons. On the other hand, the interaction treatment of half dose of zeolite combined with PGPR and *Saccharomyces* extract gave the third value at both cuts, during the first and second seasons. In addition, it was observed that all fertilization treatments with or without *Saccharomyces* scored increases of essential oil percentage/plant in the second cut as compared to the first one in the two growing seasons. Furthermore, the lowest value of oil percentage per plant was produced in untreated plants (without *Saccharomyces*) at both cuts in the two growing seasons. These results are in agreement with those reported by [42] on indian fennel, [43] on *Silybum marianum*, [44] on *Foeniculum vulgare*, [45] on *Origanum syriacum* var. *sinaicum* and [46] *Rosmarinus officinalis*. In addition, [40] showed that *Thymus vulgaris L* .inoculated with a mixture of nitrogen fixing bacteria and compost amendment showed a significant increase in essential oil production. Furthermore, [41] showed that *Ocimum basilicum, L.* cv. genovese which received biofertilizers showed the highest significant increase of essential oil yield.

3.5 Effect of Different Treatments on Essential Oil Composition

Data in Table 6 and (Figs. 4, 5 and 6) showed that the effect of different treatments (control without *Saccharomyces*, full dose of NPK with *Saccharomyces*, and ½ dose zeolite + PGPR with *Saccharomyces*) on the qualitative of the essential oil constituents of essential oils produced from thyme. The volatile oil composition of thyme produced 12 compounds which identified as thymol, β-phyllandrene, myrcene, carvacrol, α-pinene, camphene, P-pinene, sabinene, α-terpinene, terpineolen, P-cymene and Y-terpinene. The main component was the thymol which ranged from 44.39 to 48.84%, β-phyllandrene which ranged from 20.05 to 23.80%, myrcene which ranged from 11.85 to 15.03% and carvacrol which ranged from 1.02 to 1.53%. In-addition, unknown compounds represent 0.54 to 2.85%.

Furthermore, the combination treatments of fertilization and Saccharomyces extract induced an increasable values of thymol from (44.39%) for the control to 46.20, 48.84% for the ½ dose zeolite + PGPR combined with *Saccharomyces* extract and full dose of NPK with *Saccharomyces* extract, respectively. On the other hand, different treatments caused decreases in the percentage of β-phyllandrene from 23.80 in control to 20.09- 20.05%.Similar findings had been obtained by [47] on *T. vulgaris L.* Conclusively, it is preferable from the previous results that treating thyme plants with full dose of NPK with or without *Saccharomyces* extract or with the combined treatment between the half dose of zeolite in combination with PGPR and *Saccharomyces* extract for enhancing thyme growth and oil productivity.

3.6 Interaction Effect among Zeolite, PGPR and *Saccharomyces* Extract on Minimal Inhibition Concentration (MIC) of Thyme's Oil

In vitro, antimicrobial activity of thyme's oil against four pathogenic bacterial strains and one yeast was evaluated in vitro to determine the minimum inhibitory concentration (MIC) which was defined as the lowest concentration of essential oil that completely inhibited the growth of pathogens. The selected microorganisms are representative of the Gram positive and Gram negative and known to cause pathogens in humans. The obtained results for the antibacterial assay are shown in (Table 7). The tested oil samples were more active against Gram negative bacteria than both Gram positive ones and yeast. The obtained results are in agreement with [48] who reported that the Gram positive bacteria were more sensitive to thyme's oil than the Gram negative ones. The mechanisms by which thyme's essential oil can prevent pathogens include different ways and may be due to their hydrophobicity. It could be divided into the lipid bilayer of the cell membrane and rending it more permeable then leading to leakage of vital cell contents [49]. Thyme components such as phenolic and aromatic exert have antimicrobial effects at the cytoplasmic membrane by modifying its structure and function, this loss of the differential permeability of the cytoplasmic membrane is considerably identified as the cause of cell death [2].

Table 5. Essential oil of thyme growing under different treatments

Treatments	Parameters	Essential oil %			
		1st season		2nd season	
		1st cut	2nd cut	1st cut	2nd cut
Without *Saccharomyces cerevisiae*	Control	1.39^{h}	1.10^{i}	1.50^{h}	1.67^{i}
	Full dose of NPK	1.96^{b}	1.96^{b}	2.09^{a}	2.13^{b}
	½ dose NPK + PGPR	1.69^{e}	1.74^{e}	1.71^{d}	1.96^{e}
	Full dose of zeolite	1.60^{f}	1.57^{g}	1.67^{e}	1.94^{f}
	½ dose Zeolite + PGPR	1.74^{de}	1.79^{d}	1.85^{c}	2.03^{d}
With *Saccharomyces cerevisiae*	Control	1.51^{g}	1.30^{h}	1.57^{g}	1.73^{h}
	Full dose of NPK	2.07^{a}	2.19^{a}	2.09^{a}	2.20^{a}
	½ dose NPK + PGPR	1.78^{d}	1.84^{c}	1.90^{b}	2.03^{d}
	Full dose of zeolite	1.57^{fg}	1.65^{f}	1.61^{f}	1.91^{g}
	½ dose Zeolite + PGPR	1.86^{c}	1.85^{c}	1.90^{b}	2.07^{c}

Table 6. Effect of different treatments on essential oil composition (%) of *Thymus vulgaris* L

Peak no.	Component name	Treatments		
		Control without *Saccharomyces cerevisiae*	Full dose of NPK with *Saccharomyces cerevisiae*	½ dose Zeolite + PGPR with *Saccharomyces cerevisiae*
1	α-Pinene	3.19	2.82	3.41
2	Camphene	1.57	1.58	1.29
3	P-Pinene	1.93	1.97	2.31
4	Sabinene	1.77	1.55	2.05
5	Myrcene	11.85	11.89	15.06
6	Thymol	44.39	48.84	46.20
7	α -Terpinene	1.46	1.73	1.91
8	Terpineolen	2.72	3.78	3.43
9	P-Cymene	1.46	1.42	1.09
10	Y-Terpinene	1.75	1.59	1.60
11	β-phyllandrene	23.80	20.05	20.09
12	Carvacrol	1.53	1.04	1.02
*	Unknown	2.58	1.74	0.54
Total		100.00	100.00	100.00

Table 7. Antimicrobial activity of thyme's essential oil (µg/mL) against pathogenic microorganisms

Treatments	Parameters	*S. typhorium*	*Ps. aureogenosa*	*Staph. aureus*	*B. cereus*	*C. albicans*
		MIC (µg/mL)				
Without *Saccharomyces cerevisiae*	Control	5000	5000	2500	2500	5000
	Full dose of NPK	5000	1250	2500	2500	5000
	½ dose NPK + PGPR	1250	625	1250	1250	1250
	Full dose of zeolite	1250	1250	1250	1250	5000
	½ dose Zeolite + PGPR	625	625	312.5	312.5	1250
With *Saccharomyces cerevisiae*	Control	5000	5000	2500	2500	5000
	Full dose of NPK	5000	1250	1250	1250	5000
	½ dose NPK + PGPR	1250	1250	625	625	1250
	Full dose of zeolite	1250	1250	1250	1250	5000
	½ dose Zeolite + PGPR	625	625	312.5	312.5	1250

MIC: Minimum Inhibitory Concentration

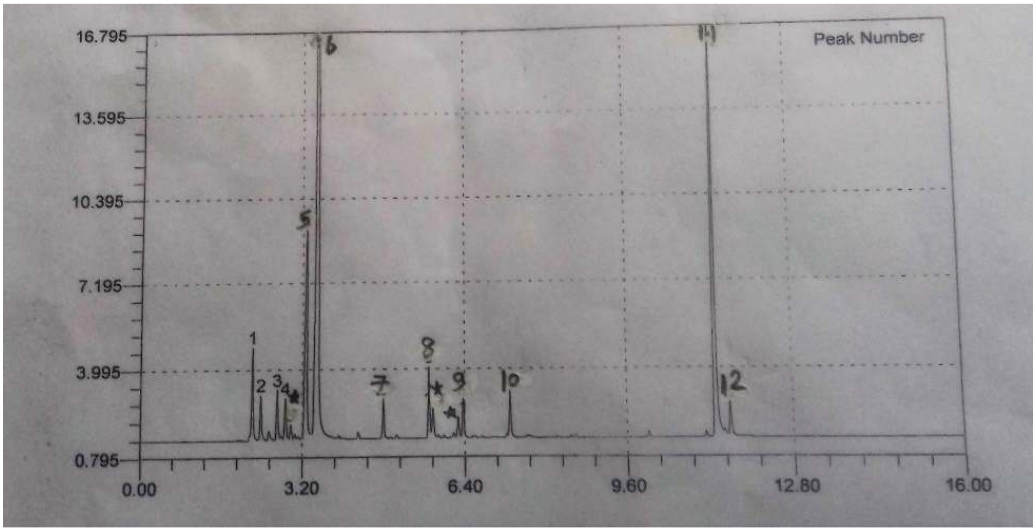

Fig. 4. GLC separation of essential oil components of *T. vulgaris* L. cultivated in soil without any amendments (control)

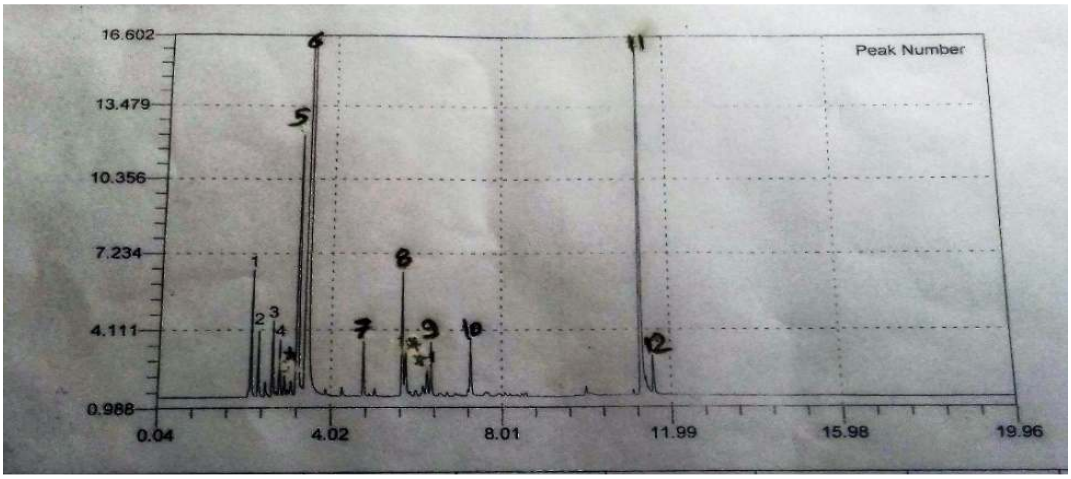

Fig. 5. GLC separation of essential oil composition of *T. vulgaris* L. cultivated in soil amended with full dose of NPK with *Saccharomyces* extract

In addition, [50,51,52] reported that the antimicrobial activity of thyme's essential oil components due particularly to phenols, such as carvacrol and thymol. In reality, even if antimicrobial activity of an essential oil is often mainly attributed to its major components. It is well known that the synergistic or antagonistic effect of one compound in minor percentage of mixture has to be considered [53]. The expected hypotheses for the resistance of Gram negative bacteria, support the adverse effect on the solidity of the bacterial cell membrane [54]. The sensitivity of pathogenic bacteria was related to the morphological structure and chemical composition of their cytoplasmic membrane. Therefore, those bacteria possessing an outer membrane mainly composed from polysaccharides rather impermeable can prevent the inhibitors molecules from passing through it [48]. Given the differences in the cell wall of Gram positive bacteria, it is reasonable that arrival through the cell membrane is more restricted. Although the investigation into the biological activities of chemical mixtures are difficult, it is important to fully understand the mechanism by which these naturally occurring compounds are effective and interact in the treatment of various diseases [55].

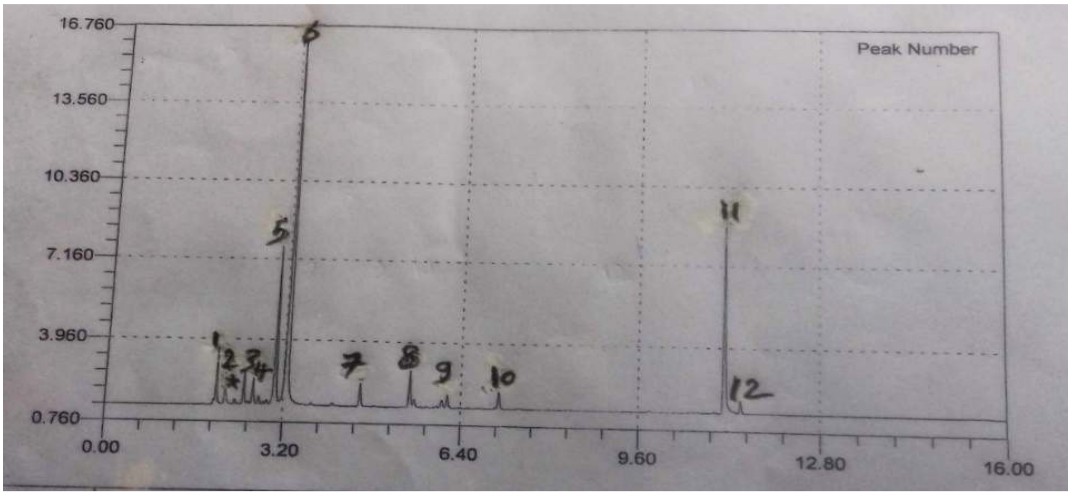

Fig. 6. GLC separation of essential oil composition of *Thymus vulgaris* L. cultivated in soil amended with half dose of zeolite combined with PGPR with *Saccharomyces* extract

4. CONCLUSION

Conclusively, it is preferable from the previous results that treating thyme plants with half dose of zeolite in combination with PGPR and *Saccharomyces* extract gave the highest significant values of dehydrogenase, phosphatase and nitrogenase. In addition, gave higher growth characteristics i.e. plant height, branches number/plant, fresh and dry weights than other treatments. These results are true in two cuts and in both two growing seasons. On the other hand, chemical analysis of thyme grown under different treatments showed that chemical fertilizer treatments gave the highest significant values of macronutrients and carbohydrates content. Concerning the effect of different treatments on essential oil composition of *Thymus vulgaris*, it was clearly that the main components were thymol, β-phyllandrene and myrcene. These components of thyme's oil were able to inhibit all tested pathogens via minimum inhibitory concentration (MIC) test which resulted that the examined Gram positive bacteria were more sensitive rather than either Gram negative or *Candida albicans*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Moghtader M. Antifungal effects of the essential oil from *Thymus vulgaris* L. and comparison with synthetic thymol on *Aspergillus niger.* J Yeast Fungal Res. 2012;3(6):83-88.
2. Coisin Magda, Burzo I, Ştefan M, Rosenhech E, Zamfirache Maria M. Chemical composition and antibacterial activity of essential oils of three *salvia* species, widespread in eastern Romania. Biologie Vegetală. 2012;58(1):51-58.
3. Leggo PJ. The Efficacy of the organo-zeolitic bio-fertilizer. Agrotechnology. 2015;4(1):1-4.
4. Bernardi AC, Oliviera PPA, Monte MB, Polidoro JC, Souza-Barros F. Brazilian sedimentary zeolite use in agriculture. 19th World Congress of Soil Science, Soil Solutions for a Changing World, 1-6 Aug. Brisbane, Australia. 2010;37-40.
5. Roxana V. Researches influence of zeolite on productivity elements and microbiological activity on spring barley, soybeans and maize at ARDS Turda. PhD thesis, Fac. Agric., Univ. Agric. Sci. Vet. Medic., Cluj-Napoca, Romania; 2015.
6. Tállai M, Jakab Anita, Kátai J. Application of bentonite on acidic humic sandy soil. Analele Universităţii din Oradea, Fascicula Protecţia Mediului.2011;XVI:479-84.
7. Sarkhosh MR, Abbaszadeh B, Ardakani MR, Rezaei M. The effect of biological promoters on thyme plant in different harvests. Inter J Biosci. 2015;6(3):109-15.
8. Yadegari M, Farahani GH Nand Mosadeghzad Z. Biofertilizers effects on

quantitative and qualitative yield of Thyme (*Thymus vulgaris*). African J Agric Res. 2012;7(34):4716-23.

9. Hanafy MS, Saadawy FM, Milad SMN, Ali RM. Effect of some natural extracts on growth and chemical constituents of *Schefflera arboricola*plants. J Horti Sci Ornament Plants. 2012;4(1):26-33.
10. Złotek U, Świeca M. Elicitation effect of *Saccharomyces cerevisiae* yeast extract on main health-promoting compounds and antioxidant and anti-inflammatory potential of butter lettuce (*Lactuca sativa* L.). J Sci Food Agric. 2016;96(7):2565-72.
11. Page AL, Miller R, Keeney H. Methods of soil analysis. Part 2, 2nd Ed., Am. Soc. Agronomy, Inc. Mad. Wisconsin, USA; 1982.
12. Abdel-Malek Y, Ishac YZ. Evaluation of methods used in counting *Azotobacter*. J Appl Bacteriol. 1986;31:267-69.
13. Atlas RM. Handbook of Media for Environmental Microbiology. (2ndEd.). CRC press, New York, USA; 2005.
14. Naik PR, Raman G, Narayanan KB, Sokthivel N. Assessment of genetic and functional diversity of phosphate solubilizing fluorescent pseudomonads isolated from rhizospheric soil. BMC Microbiol. 2008;8(230):1-14.
15. Abdel-Rahim EA, Shallan MA, El-Scheik AM. Biochemical studies on production of new thermophilic yeast alkaline proteases applied for the purposes of laundry detergents industry. J Agric Sci Mansoura Univ.1988;21(5):1971-85.
16. Glathe' H, Thalmann A. Uber die microbiello activitat and iher Beziehungenzu Fruchtbrkeitsmerkmalen einiger Acherbodenun terbesonderer Berucksichtigung der dehydrogenase akativitat (TCC. Redukation). Zbl. Bakt. Abt. II. French. 1970;124:1-23.
17. Drobrikova V. Factors influencing the determination of phosphatase in soil. Folia Microbiologica.1961;6:260.
18. Diloworth MJ. The acetylene reduction method for measuring biological nitrogen fixation. Rhizobium News Letters. 1970;15(7):155.
19. Horneck DA, Miller RO. Determination of total nitrogen in plant tissue. In hand book of reference methods for plant analysis (Ed: Kolra, Y.).1998;73.
20. Sandell R. Colorimetric determination of traces of metal 2nd Ed. Inter since. Pub. Inc. New. York; 1950.
21. Horneck DA, Hanson D. Determination of potassium and sodium by flame emission spectrophotometry. In: Karla Y P (ed.). Handbook of Ref Methods for Plant Anal.CRC Press, Washington. 1998;157–64.
22. Herbert D, Phipps PJ, Strange RE. Determination of total carbohydrates. Methods in Microbiol. 1971;5(8):290-344.
23. British Pharmacopeia. Determination of volatile oil in drugs. The Pharmaceutical Press, London, WC L. 1963;213.
24. Santurio DF, Kunz de Jesus FP, Zanette RA, Schlemmer KB, Fraton A, Fries LLM. Antimicrobial activity of the essential oil of thyme and of thymol against *Escherichia coli* strains. Acta Scientiae Veterinariae. 2014;42(1234):1-4.
25. Snedecor GW, Cochran WG. Statistical methods. 8th Ed. Iowa State Univ. Press, Ames Iowa, USA; 1989.
26. Duncan's DB. Multiple range and multiple F. test. Biometrics. 1955;11:11-24.
27. Mühlbachová G, Šimon T. Effects of zeolite amendment on microbial biomass and respiratory activity in heavy metal contaminated soils. Plant Soil Environ. 2003;49(12):536-41.
28. Kátai J, Sándor Z, Jakab A, Tállai M. Effect of bentonite and zeolite on the amount of some microbiological parameters of an acidic humic sandy soil. Analele Universităţii din Oradea, Fascicula: Protecţia Mediului. 2010;XV: 109-14.
29. Javaid A. Foliar application of effective microorganisms as an alternative fertilizer for pea. Agron Sustain Develop. 2006; 26:257-62.
30. Farzana Y, Radziah O, Kamaruza-man S, Saad MS. Effect of PGPR inoculation on growth and yield of sweet potato. J Biol Sci. 2007;7:421-24.
31. Chander K, Joergensen RG. Decomposition of ^{14}C labelled glucose in a Pb-contaminated soil remediated with synthetic zeolite and other amendments. Soil Biol Biochem. 2002;34:643-49.
32. Hrenovic J, Tibljas D, Orhan Y, Buyukgungor H. Immobilization of

Acinetobacter calcoaceticus using natural carriers. Water SA. 2005;31:261-66.

33. Torma S, Vilcek J, Adamisin P, Huttmanova E and Hronec O. Influence of natural zeolite on nitrogen dynamics in soil. Turk J Agric Fores. 2014;38:739-44.
34. Ippolito JA, Tarkalson DD, Lehrsch GA. Zeolite soil application method affects inorganic nitrogen, moisture, and corn growth. Soil Sci. 2011;176(3):136-42.
35. Valadabadi SA, Shiranirad AH, Farahani HA. Ecophysiological influences of zeolite and selenium on water deficit stress tolerance in different rapeseed cultivars. J Ecol Natur Environ. 2010;2(8):154-59.
36. Egamberdieva D, Shrivastava S, Varma A. Plant growth promoting rhizobacteria (PGPR) and medicinal plants. 1st Ed, ISBN: 978-3-319-13400-0 (Print) 978-3-319-13401-7 (Online). Soil Biol. 2015;42: 100-10.
37. Ahmed Eman A, Hassan Enas A, El-Tobgy KMK, Ramadan EM. Evaluation of rhizobacteria of some medicinal plants for plant growth promotion and biological control. Ann Agric Sci. 2014;59(2):273-80.
38. Bhattacharyya Pand Jha D. Plant growth promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol. 2012;28:1327-50.
39. Gendy ASH, Said HAH, Mahmoud Abeer A. Growth, productivity and chemical constituents of roselle (*Hibiscus sabdariffa* L.) plants as influenced by cattle manure and biofertilizers treatments. Aust J of Basic Appl Sci. 2012;6(5):1-12.
40. Barandozi FN, Pourmaleknejad B. Effect of biological, organic and chemical fertilizers on vegetative growth, physiological characteristics and essential oils of (*Thymus vulgaris L*.). Annu Res Rev Biol. 2014;4(11):1847-53.
41. Mohamed SM, Abou El-Ghait Eman M, Ghatas YA, El Shayieb NM, Shahin AA. Effect of some fertilizers on improving growth and oil productivity of basil (*Ocimum basilicum, L*.) cv. Genovese plant. Egypt. J Appl Sci. 2015;30(6):384-99.
42. Abou El-Ghait Eman M, Gomaa AO, Youssef ASM, Atiea EM, Abd-Allah WH. Effect of sowing dates, bio, organic and chemical fertilization treatments on growth and production of Indian fennel under north Sinai conditions. Bull Cairo Univ. 2012;63:52-68.
43. Asghar R, Manijeh K. Different planting date and fertilizing system effects on the seed yield, essential oil and nutrition uptake of milk thistle (*Silybum marianum* (L.) Gaertn). Advan Environ. Biol. 2012;6(5):1789-96.
44. Alireza D. Effect of chemicals and biofertilizers on yield, growth parameters and essential oil contents of fennel (*Foeniculum vulgare* Miller.). J Medic Plants By-Products. 2012;2:101-5.
45. Abdel-Wahab MA. Effect of biofertilizers and chemical fertilization on growth and active constituents of *Origanum syriacum* var. *sinaicum* (boiss.). Plant under Saint Katherine conditions. Res J Agric Biol Sci. 2013;9(5):182-90.
46. El-Khyat LAS. Effect of chemical and bio fertilizer on growth and chemical composition of rosemary plants. M.Sc. Thesis Fac. Agric. Moshtohor, Benha Univ., Egypt. 2013;1-7.
47. Hendawy SF, Ezz El-Din Azza A, Aziz EE, Omer EA. Productivity and oil quality of *Thymus vulgaris* L. under organic fertilization condition. Ozean J Appl Sci. 2010;3(2):203-16.
48. Kamatou GPP. Indigenous Salvia species-an investigation of their pharmacological activities and. phytochemistry. PhD thesis, Uni. Witwatersrand, Johannesburg; 2006.
49. Edris AE. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents, a review. 2007;21:308-23.
50. Valero M, Giner MJ. Effects of antimicrobial components of essential oils on growth of *Bacillus cereus* INRA L2104 in and the sensory qualities of carrot broth. Inter J Food Microbiol. 2006;106:90-94.
51. Pioro-Jabrucka E, Suchorska-Tropilo K, Rzewuska M. Antibacterial activity of the essential oil of common thyme (*Thymus pulegioides* L.) and wild thyme (*Thymus serpyllum* L.). Herbal Pol. 2007;53:297-301.
52. Celikel N, Kavas G. Antimicrobial properties of some essential oils against some pathogenic microorganisms. Czech J Food Sci. 2008;26:174-81.
53. Soković M, Marin PD, Brkić D, van Griensven LJ. Chemical composition and

antibacterial activity of essential oils of ten aromatic plants against human pathogenic bacteria. Food. 2007;1(1):1-7.

54. Delaquis PH, Stanichm K, Girard B, Mazza G. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. Inter J Food Microbiol. 2002;74:101-9.

55. Liu J. Oleanolic acid, ursolic acid: Research perspectives. J Ethnopharmacol. 2005;100:92-105.

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