ENHANCING GROWTH, PRODUCTIVITY AND ESSENTIAL OIL PERCENTAGE OF THYMUS VULGARIS L. PLANT USING SEAWEEDS EXTRACT, CHITOSAN AND POTASSIUM SILICATE IN SANDY SOIL

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ABSTRACT: The present study was carried out at the Experimental Farm, Horticulture Department, Faculty of Agriculture, Suez Canal University, Egypt during two successive seasons of 2017/2018 and 2018/2019 to examine the effect of foliar application of seaweeds extract at 2, 4 and 6 ml/l, chitosan at 2, 4 and 6 ml/l and potassium silicate at 6, 9 and 12 ml/l as well as the interaction between seaweeds extract and chitosan on growth, biochemical and essential oil characteristics of thyme plants grown in a sandy soil. These compounds were applied as foliar spray three times after 15 days from planting and at 15 days interval. The results showed that foliar applications of seaweeds extract at 6 ml/l, chitosan at 6 ml/l and potassium silicate at 12 ml/l significantly affected all studied parameters compared with control. The results indicated that the different applied treatments increased the measured growth characteristics i.e. plant height, number of branches/plant, fresh and dry weights as well parallel increase of photosynthetic pigments. Also, constituents of N, P, K, total carbohydrates, percentage of essential oil and the GLC of essential oil of plant were existed in the two assigned seasons. In addition, the most effective combined treatments were seaweed at 6 ml/l + chitosan at 4 ml/l in this respect.

Key words: Medicinal and aromatic plants, thyme, Thymus vulgaris L., seaweed extract, chitosan, potassium silicate, essential oil.

INTRODUCTION

Thyme (Thymus vulgaris L.), locally known “zaatar”, a member of the family Lamiaceae (Labiatae) is widely used in Egypt and Morocco folk medicine for its expectorant, antitussive, antibroncholytic antispasmodic, anthelmintic, carminative and diuretic properties. The aromatic and medicinal properties of the genus Thymus have made it one of the most popular plants all over the world. Numerous studies were conducted on medicinal plants to increase their productivity using different methods. Chitosan is a natural biopolymer modified from chitin, which can be transformed into chitosan by extracting the acetyl group and turn it into amino. Many investigators reported that using chitosan as foliar spray increased vegetative growth, yield and quality of different crops including Castro et al. (2016) on coriander; Mahdavi (2013) on isabgol (Plantago ovata Forsk) and Kim et al. (2005) on basil. Furthermore, chitosan has been recognized as a product to enhance crop production due to its bioactivities: biodegradability, growth stimulation and seed germination, increasing nutrient uptake, reducing oxidative stress, increasing
chlorophyll content, photosynthetic and chloroplast enlargement in the leaves, antifungal, antiviral and antibacterial properties (Hadrami et al., 2010 and Hadwiger, 2013). Seaweeds extract plays role as an activator of cell division, and gives rise to antioxidants levels for protection against adverse environmental conditions (Smirnoff, 1995). Nasiroleslami and Safaridolatabad (2014) reported that foliar application of seaweed increased vegetative growth of dill plants.

On the other hand, potassium is an important nutrient for plant meristematic growth and physiological functions, including regulation of water and gas exchange, protein synthesis, enzyme activation, and photosynthesis and carbohydrates translocation in plants. Potassium has favourable effects on metabolism of nucleic acids, proteins, vitamins, growth substances, energy transfer, phloem transport, cation-anion balance and enabling their ability to resist pests and diseases (Wang et al., 2013). Potassium silicate is a source of highly soluble silicon; it is used in agricultural production system primarily as a silicon fertilizer (Abou-Baker et al., 2011) on bean plant. Foliar spray with potassium silicate increased plant growth, chlorophyll content, N, P and K contents in the leaves and yield and its components (Wand and Galletta, 1998) on strawberries.

Seaweeds are green, brown and red marine macroalgae. Extracts of brown seaweeds are widely used in horticulture crops largely for their plant growth-promoting effects and for their ameliorating effect on crop tolerance to abiotic stresses such as salinity, extreme temperatures, nutrient deficiency and drought. The chemical constituents of seaweed extract include complex polysaccharide, fatty acids, vitamins, phytohormones and mineral nutrients growth promoting hormones (IAA and IBA), cytokinins, trace elements (Fe, Cu, Zn, Co, Mo, Mn, and Ni) and amino acids. (Battacharyyya et al., 2015).

Therefore, this study aimed to investigate the effect of seaweed extract, chitosan and potassium silicate on the growth, herb, essential oil yield and constituents as well as chemical compositions of thyme (Thymus vulgaris L.) plant.

MATERIALS AND METHODS

Experimental design:

The stem cuttings of thyme (Thymus vulgaris L.) were obtained from Experimental Farm of Medicinal & Aromatic Plants Department, Horticultural Research Institute, Agriculture Research Center, Egypt. The plant materials are planted in the sandy field on December 26th during the two successive seasons of 2017/2018 and 018/2019 at Experimental Farm, Faculty of Agriculture, Suez Canal University. The physical and chemical properties of the used water and experimental soil are shown in Tables (a and b). The plants were treated by spray extracts of seaweeds (2, 4 and 6 ml/l water), chitosan (2, 4 and 6 ml/l water) and potassium silicate (6, 9 and 12 ml/l water) as well as combinations between seaweeds and chitosan (2:2, 2:4, and 2:6 ml/l, 4:2, 4:4, and 4:6 ml/l, and 6:2, 6:4, and 6:6 ml/l). The plants were sprayed with the tested treatments three times with 15 days interval between spraying and the next, the first spray was done 15 days after planting. Seaweed extract coined as Oligo-X was used in this study. It is a mixture of Ascophyllum spp., Laminaria spp., Sargassum spp. and Fucus spp. It was obtained from Union for Agricultural Development Company, Egypt; the chemical constituents are shown in Table (c).

Morphological attributes:

Data of the morphological traits were recorded in each cutting separately for estimating the variability in effect of the different treatments on plant growth. These data included plant height (cm), number of branches/plant, fresh and dry weights of plant (g/plant).
The first cut was done on April 1st and the second cut was done on 15th June of both seasons. The plants were about 5 cm above the ground leaving a lateral branch to renew the plant growth.

**Chemical analysis:**

- The contents of chlorophylls (a, b) and carotenoids were determined in the fresh leaves as described by Mazumdar and Majumder (2003). Total chlorophylls and carotenoids were determined (mg/g f.w.) according to Wettestein (1957).
- Contents of leaf minerals were estimated according to Piper (1947).
- Percentage of nitrogen was determined by using the micro-Kjeldhle method as described by Jackson (1967).
- Phosphorus percentage was calorimetrically determined according to Murphy and Reilly (1962).
- Potassium percentage was determined using the atomic absorption spectrophotometer (3300) according to Wilde et al. (1985).
- Total carbohydrates % of the dry weight were determined according to Herbert et al. (1971).
- Essential oil percentage (EO) was determined according to British Pharmacopoeia (2000).
- The essential oil content was analyzed using gas liquid chromatography (GLC) to determine the main constituents of the essential oil according to Hoffman (1967). GLC was carried out as described by Guenther and Joseph (1978).

**Statistical analysis:**

The experimental design was complete randomized blocks design included 19 treatments + control treatment with three replicates. The replicate area was 1.5×1.5 m.

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**Table a. Chemical and physical properties of the experimental soil.**

| EC dS m⁻¹ | CO₃⁻ | HCO₃⁻ | CL⁻ | SO₄²⁻ | Ca ++ | Mg ++ | Na⁺ | K⁺ |
|------------|------|-------|-----|-------|-------|-------|-----|----|
| 0.58       | --   | 2.12  | 2.54| 1.67  | 4.60  | 0.83  | 0.82| 0.08|

| pH | N | K | P | Cu ++ | Fe ++ | Mn ++ | Zn ++ |
|----|---|---|---|-------|-------|-------|-------|
| 8  | 53.0 | 55.8 | 0.90 | 0.90  | 2.20  | 1.54  | 0.59  |

**Table b. Chemical and physical properties of used water.**

| EC dS m⁻¹ | CO₃⁻ | HCO₃⁻ | CL⁻ | SO₄²⁻ | Ca ++ | Mg ++ | Na⁺ | K⁺ |
|------------|------|-------|-----|-------|-------|-------|-----|----|
| 0.40       | --   | 1.42  | 0.85| 2.00  | 2.82  | 1.08  | 0.26| 0.11|

| pH | NH₄⁺ | NO₃⁻ | B | CU | Fe | Mn | P | Zn |
|----|------|------|---|----|----|----|---|----|
| 7.10 | 1.05 | 1.75 | 0.30 | 0.20 | 0.20 | 0.011 | 1.50 | 0.30 |

**Table c. Chemical constituents of Oligo-X seaweed extract.**

| Components | Oligo-saccharides | Alginic acid | Zeitin | Mannitol | K₂O | Zinc (Zn) | Iron (Fe) | Manganese (Mn) | Growth regulators | Cytokinin | Indole acetic acid (IAA) | Betanin |
|------------|-------------------|-------------|--------|----------|-----|-----------|---------|---------------|----------------|------------|--------------------------|---------|
| %          | 3                 | 5           | 0.003  | 0.001    | 4.71| 0.13      | 0.12    | 0.10          | 0.001       | 0.0002     | 0.02                    |         |

The content of chlorophylls (a, b) and carotenoids were determined in the fresh leaves using the method described by Mazumdar and Majumder (2003). Total chlorophylls and carotenoids were determined using atomic absorption spectrophotometry (3300) according to Wilde et al. (1985).

Total carbohydrates % of the dry weight were determined according to Herbert et al. (1971).

Essential oil percentage (EO) was determined according to British Pharmacopoeia (2000).

The essential oil content was analyzed using gas liquid chromatography (GLC) to determine the main constituents of the essential oil according to Hoffman (1967). GLC was carried out as described by Guenther and Joseph (1978).
Each replicate was included 16 plant; the distance between plants was 35 cm. Data were subjected to analysis of variance (ANOVA) by the general linear models (LMS) procedure using (CoStat) statistical analysis system. Mean comparisons were performed using the least significant differences (L.S.D) method at significance level of 5% according to Gomez and Gomez (1984).

RESULTS AND DISCUSSIONS

Vegetative growth and herb yield:

Effect of seaweed extract, chitosan and potassium silicate:

Thyme plants foliar sprayed with seaweed extract at different levels increased the vegetative growth characteristics compared to control. Plant height (cm), number of branches/plant, herb fresh and dry weights (g/plant) were increased with increasing sprayed foliar concentration of seaweed extract (4 and 6 ml/l) and potassium silicate up to12 ml/l as well as chitosan up to (4 and 6 ml/l) in both seasons and for the two cuts compared with control plants, as presented in Table (1), nevertheless the control treatment gave the lowest value in the two experimental seasons.

With respect to vegetative fresh weight per plant, results shown in Table (1) clear that seaweed extract at 6 ml/l as a foliar spray showed the highest values; on the other hand, control plants recorded the lowest values in the two tested seasons. Such results are in the same line with those of Norrie and Keathley (2006), Hussein et al (2011), and Delucia and Vecchietti (2012). This increment in the vegetative fresh weight/plant may be due to the positive effect of seaweed extract on increasing most vegetative growth characters i.e., plant height, number of shoots and flower stalk length that seaweed extract contains many growth regulators, macro and micro nutrients.

| Treatments  | Plant height (cm) | No. of branches/plant | F.W. (g)/plant | D.W. (g)/plant |
|-------------|-------------------|-----------------------|----------------|----------------|
|             | 1st cut | 2nd cut | 1st cut | 2nd cut | 1st cut | 2nd cut | 1st cut | 2nd cut |
| Control     | 7.33    | 11.3    | 24.3   | 25.6    | 9.86    | 15.81   | 5.10    | 7.26    |
| Sw 2 ml/l   | 12.3    | 14.7    | 44.3   | 51.3    | 21.26   | 25.89   | 7.83    | 10.63   |
| Sw 4 ml/l   | 14.3    | 15.4    | 57.0   | 62.3    | 27.71   | 29.46   | 8.66    | 12.30   |
| Sw 6 ml/l   | 17.0    | 19.6    | 86.3   | 85.3    | 34.80   | 35.57   | 13.83   | 15.30   |
| Ch 2 ml/l   | 11.6    | 13.5    | 35.3   | 48.6    | 13.64   | 25.56   | 7.20    | 9.56    |
| Ch 4 ml/l   | 13.0    | 14.3    | 53.3   | 53.3    | 24.55   | 27.31   | 9.56    | 11.53   |
| Ch 6 ml/l   | 14.0    | 16.8    | 74.3   | 68.3    | 30.39   | 33.22   | 11.10   | 13.33   |
| Ksi 6 ml/l  | 12.0    | 13.6    | 40.6   | 47.0    | 18.87   | 23.50   | 6.86    | 7.63    |
| Ksi 9 ml/l  | 13.3    | 14.3    | 53.3   | 61.0    | 30.39   | 28.98   | 10.16   | 13.13   |
| Ksi 12 ml/l | 15.2    | 18.5    | 85.3   | 80.6    | 32.99   | 34.54   | 13.13   | 14.23   |
| L.S.D. at 5%| 2.33    | 2.53    | 9.94   | 12.92   | 6.67    | 3.19    | 2.44    | 1.76    |

| Treatments  | Plant height (cm) | No. of branches/plant | F.W. (g)/plant | D.W. (g)/plant |
|-------------|-------------------|-----------------------|----------------|----------------|
|             | 1st cut | 2nd cut | 1st cut | 2nd cut | 1st cut | 2nd cut | 1st cut | 2nd cut |
| Control     | 12.3    | 14.6    | 31.3   | 46.3    | 28.41   | 34.41   | 12.46   | 13.7    |
| Sw 2 ml/l   | 20.3    | 20.3    | 80.3   | 98.6    | 35.16   | 43.19   | 18.66   | 21.33   |
| Sw 4 ml/l   | 21.3    | 23.6    | 107.0  | 120.3   | 44.65   | 56.73   | 21.96   | 25.23   |
| Sw 6 ml/l   | 23.6    | 26.0    | 121.6  | 131.6   | 57.43   | 63.14   | 22.66   | 34.23   |
| Ch 2 ml/l   | 18.3    | 17.3    | 63.30  | 65.30   | 32.69   | 38.76   | 15.10   | 15.00   |
| Ch 4 ml/l   | 20.0    | 22.6    | 91.30  | 99.00   | 37.71   | 52.58   | 19.70   | 19.96   |
| Ch 6 ml/l   | 21.8    | 23.6    | 95.30  | 118.3   | 41.25   | 62.12   | 20.73   | 30.96   |
| Ksi 6 ml/l  | 19.0    | 20.3    | 75.30  | 80.6    | 36.36   | 44.46   | 14.33   | 19.66   |
| Ksi 9 ml/l  | 21.0    | 22.3    | 97.30  | 110.0   | 38.29   | 53.37   | 19.13   | 20.33   |
| Ksi 12 ml/l | 22.3    | 24.0    | 119.6  | 129.3   | 55.43   | 63.71   | 21.96   | 31.66   |
| L.S.D. at 5%| 2.42    | 2.21    | 11.06  | 9.51    | 7.42    | 7.23    | 3.84    | 3.59    |

Sw: seaweed extract, Ch: chitosan, Ksi: potassium silicate
as reported by Shaaban (2011) on wheat, and Ordog et al (2004) and Jensen (2004).

**Effect of combination between seaweed extract and chitosan:**

Foliar spraying of chitosan and seaweeds extract significantly affected all growth characters [i.e. plant height (cm), number of branches/plant, herb fresh and dry weights (g/plant) in the two cuts of both seasons of the study (Table 2). However, previous studies proved that seaweeds and/or chitosan can, directly or indirectly, influence the physiological activities of the plants (Kamal and Ghanem, 2011). Moreover, Kim et al. (2005) reported that chitosan has been shown to stimulate growth of sweet basil plants and Saif Eldeen et al. (2014) on globe artichoke plants attained similar result.

**Essential oil production:**

1. **Essential oil percentage:**

   a. Effect of seaweed extract, chitosan and potassium silicate:

   Data presented in Table (3) showed a significant difference in the two seasons, among potassium silicate, seaweed extract and chitosan treatments. The highest values of essential oil % were due to seaweed extract treatment, while potassium silicate and chitosan gave intermediate values. On the other hand, the least values of essential oil % were given by control treatment. It was observed that applying each of seaweed extract, potassium silicate or chitosan at the high concentration gave better results than those obtained from the low concentration. The highest values of the essential oil percentage ranged between 0.36-0.37 and 0.38-0.40% compared to the control 0.23,

Table 2. Effect of combination treatments between seaweed extract and chitosan on vegetative growth of *Thymus vulgaris* L. for the two cuts during 2017/2018 and 2018/2019 seasons.

| Treatments                  | Plant height (cm) | No. of branches/plant | F. W. (g)/plant | D. W. (g)/plant |
|-----------------------------|-------------------|-----------------------|----------------|----------------|
|                             | 1st cut 2nd cut   | 1st cut 2nd cut       | 1st cut 2nd cut| 1st cut 2nd cut|
| **First season (2017/2018)**|                   |                       |                |                |
| Control                     | 7.33 11.3         | 24.3 25.6             | 9.86 15.81     | 5.10 7.26      |
| Sw 2 ml/l + Ch 2 ml/l       | 14.6 15.3         | 69.3 80.0             | 35.18 35.50    | 12.56 14.40    |
| Sw 2 ml/l + Ch 4 ml/l       | 14.0 16.1         | 114.0 116.0           | 36.06 36.08    | 15.36 15.20    |
| Sw 2 ml/l + Ch 6 ml/l       | 16.3 17.0         | 152.3 167.3           | 38.14 39.48    | 13.33 15.33    |
| Sw 4 ml/l + Ch 2 ml/l       | 17.6 18.3         | 176.3 188.3           | 42.26 39.48    | 14.31 16.00    |
| Sw 4 ml/l + Ch 4 ml/l       | 18.3 19.0         | 192.0 196.0           | 49.12 43.19    | 15.41 19.00    |
| Sw 4 ml/l + Ch 6 ml/l       | 19.0 20.1         | 194.0 199.6           | 50.09 47.97    | 16.36 19.00    |
| Sw 6 ml/l + Ch 2 ml/l       | 16.6 17.6         | 188.6 191.0           | 43.13 46.82    | 14.73 17.13    |
| Sw 6 ml/l + Ch 4 ml/l       | 21.3 24.6         | 209.0 215.0           | 52.14 53.14    | 19.46 21.36    |
| Sw 6 ml/l + Ch 6 ml/l       | 20.0 21.6         | 206.0 214.0           | 51.38 52.14    | 18.40 20.00    |
| L.S.D. at 5%                 | 2.62 2.77         | 8.94 8.68             | 5.83 6.09      | 1.85 2.23      |

| **Second season (2018/2019)**|                   |                       |                |                |
| Control                     | 12.3 14.6         | 31.3 46.3             | 28.41 34.41    | 12.46 13.7     |
| Sw 2 ml/l + Ch 2 ml/l       | 21.3 24.3         | 147.0 165.6           | 48.18 68.00    | 24.16 33.83    |
| Sw 2 ml/l + Ch 4 ml/l       | 23.5 24.3         | 175.6 175.3           | 49.47 75.62    | 23.76 36.03    |
| Sw 2 ml/l + Ch 6 ml/l       | 23.3 25.6         | 181.6 184.6           | 60.52 85.96    | 25.43 38.43    |
| Sw 4 ml/l + Ch 2 ml/l       | 24.0 26.3         | 183.0 197.3           | 66.84 91.15    | 25.66 49.76    |
| Sw 4 ml/l + Ch 4 ml/l       | 25.0 27.6         | 207.0 217.3           | 69.96 108.0    | 30.63 73.66    |
| Sw 4 ml/l + Ch 6 ml/l       | 26.0 31.6         | 212.0 222.6           | 76.58 114.0    | 32.33 75.50    |
| Sw 6 ml/l + Ch 2 ml/l       | 24.0 26.6         | 187.3 211.6           | 68.01 97.00    | 28.33 55.66    |
| Sw 6 ml/l + Ch 4 ml/l       | 27.0 32.0         | 247.6 258.0           | 80.21 135.4    | 39.00 78.66    |
| Sw 6 ml/l + Ch 6 ml/l       | 26.3 31.0         | 219.3 225.0           | 73.65 121.9    | 34.50 76.50    |
| L.S.D. at 5%                 | 2.89 2.53         | 19.15 18.10           | 13.64 5.89     | 2.68 5.28      |

Sw: seaweed extract, Ch: chitosan
0.24-0.22 and 0.28% in the first and second cuts in both seasons of study, respectively.

Similar results concerning seaweed extract fertilization were pointed out by Gharib et al. (2014) on rosemary; Salama and Yousef (2015) on Ocimum sanctum L.; Atteya and Amer (2018) on Hibiscus sabdariffa; El-leithy et al. (2019) on Plectuanthus amboinicus. Also, potassium silicate effect was found by Ezz El-Din et al. (2010) on caraway; Khalid (2014) and Shabana et al. (2016) on Salvia officinalis; and concerning chitosan was found by Helaly et al. (2018) on rosemary (Rosmarinus officinalis L.).

b. Effect of combination between seaweed extract and chitosan:

Data presented in Table (4) showed that spraying combination between seaweeds 6 ml/l + chitosan 4 ml/l treatments significantly produced the highest values of essential oil percentage. oil percentage recorded 0.52, 0.55% for the first and second cuts in the first season, respectively and 0.55, 0.58% for the first and second cuts for the second season, orderly. While, the lowest essential oil percentage were recorded (0.23, 0.22) and (0.24, 0.28) resulted from the control treatment at the first cut and second one during both seasons of study successfully.

2. Essential oil components:

The essential oil ingredient (%) of Thymus Vulgaris L. plant as affected by the combination between foliar application of seaweed extract and chitosan in the first cut at the second season were shown in Table (5) and Figure (1). The components were α–pinene, camphene, α–terpinene, p-cymene, limonene, Y-terpinene, terpineolene, borneol, thymol and carvacrol. The spraying combination between seaweeds at 6 ml/l + chitosan at 4 ml/l treatment raised oil contents from thymol, p-cymene and α–terpinene to the highest values of 24.47, 19.07 and 17.47%, respectively. The least total component resulted from untreated plants (83.54%) and thymol, p-cymene and α–Terpinene recorded 10.84, 12.46 and 18.31, orderly. These results were in harmony with those of Kim et al. (2005) on Ocimum basilicum L and Bistgani et al. (2016) on Thymus daenensis.

Chemical constituents:

1. Photosynthetic pigments content:

a. Effect of seaweed extract, chitosan and potassium silicate:

Data illustrated in Table (6) showed that all concentrations of seaweeds extract, potassium silicate and chitosan significantly increased the photosynthetic pigments of chlorophyll a, b, total carotenoids and total
Table 4. Effect of combination treatments between seaweed extract and chitosan on essential oil percentage of *Thymus vulgaris* L. for two cuts during 2017/2018 and 2018/2019 seasons.

| Treatments | 1<sup>st</sup> cut | 2<sup>nd</sup> cut | 1<sup>st</sup> cut | 2<sup>nd</sup> cut |
|------------|------------------|------------------|------------------|------------------|
| Control    | 0.23             | 0.24             | 0.22             | 0.28             |
| Sw 2 ml/l + Ch 2 ml/l | 0.30             | 0.31             | 0.33             | 0.35             |
| Sw 2 ml/l + Ch 4 ml/l | 0.34             | 0.36             | 0.32             | 0.38             |
| Sw 2 ml/l + Ch 6 ml/l | 0.35             | 0.35             | 0.37             | 0.39             |
| Sw 4 ml/l + Ch 2 ml/l | 0.39             | 0.40             | 0.41             | 0.46             |
| Sw 4 ml/l + Ch 4 ml/l | 0.47             | 0.49             | 0.46             | 0.53             |
| Sw 4 ml/l + Ch 6 ml/l | 0.48             | 0.48             | 0.50             | 0.54             |
| Sw 6 ml/l + Ch 2 ml/l | 0.45             | 0.46             | 0.49             | 0.51             |
| Sw 6 ml/l + Ch 4 ml/l | 0.52             | 0.55             | 0.55             | 0.58             |
| Sw 6 ml/l + Ch 6 ml/l | 0.50             | 0.53             | 0.54             | 0.56             |
| L.S.D. 5%  | 0.271            | 0.292            | 0.324            | 0.280            |

Sw: Seaweed, Ch: Chitosan

Table 5. Essential oil components (%) of *Thymus vulgaris* L. plant as affected by the combination between foliar extract seaweed and chitosan in the 2<sup>nd</sup> cut at the second season (2018/2019).

| Components (%) | Sw 2 ml/l | Sw 4 ml/l | Sw 6 ml/l |
|----------------|-----------|-----------|-----------|
|                | CON Ch 2 ml/l Ch 4 ml/l Ch 6 ml/l | CON Ch 2 ml/l Ch 4 ml/l Ch 6 ml/l | CON Ch 2 ml/l Ch 4 ml/l Ch 6 ml/l |
| α-pinnae       | 5.27  4.93  4.85 | 3.32 | 4.37  4.39 | 4.39  4.04 | 4.39  4.50  4.42 |
| Camphene       | 13.10 8.21 9.78 | 12.06 | 9.52 13.20 | 9.93 10.34 | 9.32 4.06 | 8.51 |
| α-terpinene    | 18.31 17.47 15.54 | 18.75 11.78 15.98 | 16.08 14.31 12.07 | 16.08 17.74 16.68 |
| p-Cymene       | 12.46 19.63 17.27 | 22.96 14.93 | 2.77 17.64 | 17.27 16.73 | 17.64 19.07 18.08 |
| Limonene       | 2.02 2.80 2.19 | 1.75 1.91 | 7.02 1.97 | 2.19 2.47 | 1.97 2.26 1.98 |
| γ-Terpinene    | 2.87 6.55 7.24 | 2.98 5.98 | 4.26 6.90 | 7.32 6.97 | 6.90 7.99 7.41 |
| Terpineolene   | 11.53 9.10 7.68 | 13.58 8.07 | 6.39 9.49 | 10.17 9.30 | 9.49 9.83 9.31 |
| Borneol        | 6.39 6.71 6.74 | 6.56 6.83 | 10.79 6.73 | 7.24 7.97 | 6.73 7.57 6.93 |
| Thymol         | 10.84 15.69 22.26 | 11.11 18.24 | 21.93 21.94 | 21.08 22.71 | 21.94 24.47 23.76 |
| Carvacrol      | 0.75 1.40 1.39 | 0.77 5.71 | 2.63 1.43 | 1.95 2.25 | 1.43 1.53 1.45 |
| Total components | 83.54 92.49 94.94 | 95.46 86.29 | 89.34 96.50 | 96.26 90.43 | 96.50 99.42 98.53 |

CON: control, Sw: seaweed, Ch: chitosan
Fig. 1. Essential oil components (%) of *Thymus vulgaris* L. plant as affected by the combination between foliar extract seaweed (Sw) and chitosan (Ch) in the 2nd cut at the second season (2018/2019).
chlorophyll in fresh leaves compared with control. The highest values of photosynthetic pigments were resulted from the application of seaweeds extract at 6 ml/l, potassium silicate at 12 ml/l and chitosan at 6 ml/l, orderly.

b. Effect of combination treatments between seaweed extract and chitosan:

Data presented in Table (7) showed Chlorophyll a, b, carotenoids and the total content of chlorophyll in fresh leaves of Thymus vulgaris, L. plant as affected by combination between seaweeds 6 m/l + chitosan 4 m/l treatments were with order 1.924-1.954, 0.607-0.711, 1.922-1.943 and 2.531-2.665 in the first season and its two cuts and 2.032-2.307, 0.772-0.938, 1.949-1.943 and 2.804-3.245 in the second one compared with control (1.118-1.125, 0.225-0.288, 1.112-1.133, 1.343-1.413) and (1.258-1.364, 0.371-0.406, 1.343-1.348, 1.629-1.770), respectively.

2. N, P, K and total carbohydrates in the leaves:

a. Effect of seaweed extract, chitosan and potassium silicate:

Data presented in Table (8) indicated that the thyme plants sprayed with foliar seaweed extract at different levels increased the chemical components compared to control. Nitrogen, P and K percent contents and total carbohydrates were increased by increasing the concentration of seaweed extract (6 ml/l), potassium silicate up to (12 ml/l) as well as chitosan (6 ml/l), orderly, nevertheless the control treatment gave the lowest values in the two seasons of study.

Table 6. Effect of seaweed extract, chitosan and potassium silicate on photosynthetic pigment contents of Thymus vulgaris L. for two cuts during 2017/2018 and 2018/2019 seasons.

| Treatments | Chl. a (mg/g F.W.) | Chl. b (mg/g F.W.) | Carot. (mg/g F.W.) | Total chl (mg/g F.W.) |
|------------|--------------------|--------------------|--------------------|----------------------|
|            | 1st cut | 2nd cut | 1st cut | 2nd cut | 1st cut | 2nd cut | 1st cut | 2nd cut |
| Control    | 1.118    | 1.125    | 0.225    | 0.288    | 1.112    | 1.133    | 1.343    | 1.413    |
| Sw 2 ml/l  | 1.155    | 1.224    | 0.319    | 0.324    | 1.285    | 1.318    | 1.474    | 1.548    |
| Sw 4 ml/l  | 1.273    | 1.351    | 0.410    | 0.471    | 1.348    | 1.392    | 1.683    | 1.822    |
| Sw 6 ml/l  | 1.325    | 1.498    | 0.478    | 0.493    | 1.537    | 1.692    | 1.803    | 1.991    |
| Ch 2 ml/l  | 1.125    | 1.148    | 0.341    | 0.347    | 1.144    | 1.204    | 1.446    | 1.494    |
| Ch 4 ml/l  | 1.231    | 1.338    | 0.364    | 0.428    | 1.335    | 1.344    | 1.595    | 1.766    |
| Ch 6 ml/l  | 1.288    | 1.417    | 0.441    | 0.471    | 1.447    | 1.469    | 1.729    | 1.888    |
| Ksi 6 ml/l | 1.138    | 1.204    | 0.334    | 0.304    | 1.193    | 1.212    | 1.472    | 1.508    |
| Ksi 9 ml/l | 1.257    | 1.349    | 0.370    | 0.396    | 1.263    | 1.358    | 1.627    | 1.745    |
| Ksi 12 ml/l| 1.291    | 1.436    | 0.456    | 0.483    | 1.463    | 1.675    | 1.747    | 1.919    |
| L.S.D. at 5%| 0.205    | 0.371    | 0.253    | 0.205    | 0.423    | 0.549    | 0.460    | 0.574    |

First season (2017/2018)

| Treatments | Chl. a (mg/g F.W.) | Chl. b (mg/g F.W.) | Carot. (mg/g F.W.) | Total chl (mg/g F.W.) |
|------------|--------------------|--------------------|--------------------|----------------------|
|            | 1st cut | 2nd cut | 1st cut | 2nd cut | 1st cut | 2nd cut | 1st cut | 2nd cut |
| Control    | 1.258    | 1.364    | 0.371    | 0.406    | 1.343    | 1.348    | 1.629    | 1.770    |
| Sw 2 ml/l  | 1.371    | 1.391    | 0.348    | 0.349    | 1.584    | 1.614    | 1.719    | 1.740    |
| Sw 4 ml/l  | 1.588    | 1.588    | 0.452    | 0.479    | 1.666    | 1.686    | 1.910    | 2.059    |
| Sw 6 ml/l  | 1.596    | 1.610    | 0.584    | 0.617    | 1.748    | 1.721    | 2.180    | 2.227    |
| Ch 2 ml/l  | 1.393    | 1.357    | 0.392    | 0.416    | 1.448    | 1.461    | 1.775    | 1.773    |
| Ch 4 ml/l  | 1.427    | 1.431    | 0.431    | 0.458    | 1.601    | 1.582    | 1.873    | 1.889    |
| Ch 6 ml/l  | 1.502    | 1.528    | 0.526    | 0.541    | 1.638    | 1.625    | 2.059    | 2.069    |
| Ksi 6 ml/l | 1.314    | 1.415    | 0.382    | 0.389    | 1.537    | 1.583    | 1.706    | 1.804    |
| Ksi 9 ml/l | 1.349    | 1.499    | 0.446    | 0.496    | 1.644    | 1.654    | 1.780    | 1.995    |
| Ksi 12 ml/l| 1.547    | 1.545    | 0.557    | 0.593    | 1.729    | 1.707    | 2.073    | 2.138    |
| L.S.D. at 5%| 0.338    | 0.246    | 0.213    | 0.211    | 0.405    | 0.373    | 0.551    | 0.457    |

Second season (2018/2019)

Sw: seaweed extract, Ch: chitosan, Ksi: potassium silicate

chlorophyll in fresh leaves compared with control. The highest values of photosynthetic pigments were resulted from the application of seaweeds extract at 6 ml/l, potassium silicate at 12 ml/l and chitosan at 6 ml/l, orderly.

Table 6. Effect of seaweed extract, chitosan and potassium silicate on photosynthetic pigment contents of Thymus vulgaris L. for two cuts during 2017/2018 and 2018/2019 seasons.
Table 7. Effect of combination treatments between seaweed extract and chitosan on photosynthetic pigment contents of *Thymus vulgaris* L. for two cuts during 2017/2018 and 2018/2019 seasons.

| Treatments                  | Chl. a (mg/g F.W.) | Chl. b (mg/g F.W.) | Carot. (mg/g F.W.) | Total Chl. (mg/g F.W.) |
|-----------------------------|--------------------|--------------------|--------------------|------------------------|
|                             | 1st cut            | 2nd cut            | 1st cut            | 2nd cut                | 1st cut            | 2nd cut                | 1st cut            | 2nd cut                |
| **First season (2017/2018)**|                    |                    |                    |                        |                      |                        |                      |                        |
| Control                     | 1.118              | 1.125              | 0.225              | 0.288                  | 1.112                | 1.133                  | 1.343                | 1.413                  |
| Sw 2 ml/l + Ch 2 ml/l       | 1.219              | 1.505              | 0.451              | 0.433                  | 1.421                | 1.692                  | 1.671                | 1.938                  |
| Sw 2 ml/l + Ch 4 ml/l       | 1.299              | 1.592              | 0.476              | 0.501                  | 1.548                | 1.657                  | 1.775                | 2.093                  |
| Sw 2 ml/l + Ch 6 ml/l       | 1.353              | 1.609              | 0.481              | 0.506                  | 1.697                | 1.698                  | 1.834                | 2.110                  |
| Sw 4 ml/l + Ch 2 ml/l       | 1.387              | 1.673              | 0.459              | 0.519                  | 1.658                | 1.702                  | 1.846                | 2.192                  |
| Sw 4 ml/l + Ch 4 ml/l       | 1.748              | 1.718              | 0.524              | 0.582                  | 1.727                | 1.751                  | 2.272                | 2.300                  |
| Sw 4 ml/l + Ch 6 ml/l       | 1.851              | 1.783              | 0.583              | 0.617                  | 1.751                | 1.784                  | 2.434                | 2.400                  |
| Sw 6 ml/l + Ch 2 ml/l       | 1.553              | 1.771              | 0.493              | 0.528                  | 1.683                | 1.715                  | 2.046                | 2.299                  |
| Sw 6 ml/l + Ch 4 ml/l       | 1.924              | 1.954              | 0.607              | 0.711                  | 1.922                | 1.943                  | 2.531                | 2.665                  |
| Sw 6 ml/l + Ch 6 ml/l       | 1.864              | 1.898              | 0.637              | 0.630                  | 1.867                | 1.905                  | 2.501                | 2.528                  |
| L.S.D. at 5%                | 0.702              | 0.825              | 0.382              | 0.423                  | 0.810                | 0.832                  | 0.538                | 0.525                  |
| **Second season (2018/2019)**|                    |                    |                    |                        |                      |                        |                      |                        |
| Control                     | 1.258              | 1.364              | 0.371              | 0.406                  | 1.343                | 1.348                  | 1.629                | 1.770                  |
| Sw 2 ml/l + Ch 2 ml/l       | 1.621              | 1.566              | 0.516              | 0.415                  | 1.643                | 1.664                  | 2.137                | 1.981                  |
| Sw 2 ml/l + Ch 4 ml/l       | 1.677              | 1.685              | 0.537              | 0.492                  | 1.731                | 1.684                  | 2.214                | 2.177                  |
| Sw 2 ml/l + Ch 6 ml/l       | 1.723              | 1.746              | 0.584              | 0.598                  | 1.824                | 1.774                  | 2.307                | 2.344                  |
| Sw 4 ml/l + Ch 2 ml/l       | 1.792              | 1.852              | 0.609              | 0.593                  | 1.814                | 1.758                  | 2.401                | 2.445                  |
| Sw 4 ml/l + Ch 4 ml/l       | 1.835              | 1.929              | 0.690              | 0.721                  | 1.869                | 1.811                  | 2.525                | 2.650                  |
| Sw 4 ml/l + Ch 6 ml/l       | 1.983              | 1.932              | 0.739              | 0.753                  | 1.918                | 1.856                  | 2.722                | 2.685                  |
| Sw 6 ml/l + Ch 2 ml/l       | 1.801              | 1.871              | 0.665              | 0.699                  | 1.838                | 1.799                  | 2.466                | 2.570                  |
| Sw 6 ml/l + Ch 4 ml/l       | 2.032              | 2.307              | 0.772              | 0.938                  | 1.949                | 1.943                  | 2.804                | 3.245                  |
| Sw 6 ml/l + Ch 6 ml/l       | 2.055              | 1.975              | 0.743              | 0.924                  | 1.936                | 1.876                  | 2.798                | 2.899                  |
| L.S.D. at 5%                | 0.774              | 0.943              | 0.372              | 0.532                  | 0.533                | 0.595                  | 0.661                | 0.574                  |

Sw: seaweed extract, Ch: chitosan

b. Effect of combination treatments between seaweed extract and chitosan:

Data presented in Table (9) illustrated that leaves contents of N, P, K % were greatly affected by all treatments compared with the control, in the two cuts of the two seasons of study. Combination between seaweeds 6 ml/l + chitosan 4 ml/l treatments recorded the following values for N, P, K and total carbohydrates in the first and second cuts of the first season (3.23-3.31, 0.45-0.48, 1.44-1.46, 38.43-40.55), respectively. Also, recorded the following values in the second season (3.25-3.42, 0.43-0.49, 1.45-1.49, 40.81-42.86), respectively. While the control treatment recorded 1.14-1.18, 0.12-0.14, 1.02-1.06, 30.05-32.06 in the two cuts of the first season orderly and 1.21-1.25, 0.17 - 0.15, 1.06 - 1.09, 31.10 - 34.12 in both cuts of the second season. The same trend of increasing N, P and K % due to seaweed extract potassium silicate was also reported by Zewail (2014). While, the efficiency of potassium silicate in promoting N, P and K % was revealed. Moreover, previous studies have shown the involvement of chitosan in increasing N, P and K % such as Saif Eldeen *et al.* (2014) on globe artichoke.
CONCLUSION

From our results it could be concluded that the application of seaweed extract and chitosan on thyme had positive effects separately or combining together and significantly increased the growth, yield and percentage of essential oil. The best result gained from using at the highest concentrations of seaweed extract and chitosan (seaweed extract 6 ml/l water + chitosan 4 ml/l water).

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Table 9. Effect of combination treatments between seaweed extract chitosan on nitrogen, phosphorus, potassium and total carbohydrates (% of D.W.) of *Thymus vulgaris* L. for two cuts during 2017/2018 and 2018/2019 seasons.

| Treatments                | N% 1st cut | P% 1st cut | K% 1st cut | Total carbohydrates (%) 1st cut | N% 2nd cut | P% 2nd cut | K% 2nd cut | Total carbohydrates (%) 2nd cut |
|---------------------------|------------|------------|------------|---------------------------------|------------|------------|------------|---------------------------------|
| First season (2017/2018)  |            |            |            |                                 |            |            |            |                                 |
| Control                   | 1.14       | 1.18       | 0.12       | 1.02                            | 1.06       | 0.14       | 1.04       | 30.05                            | 32.06                   |
| Sw 2 ml/l + Ch 2 ml/l     | 1.89       | 1.95       | 0.24       | 1.25                            | 1.24       | 0.25       | 1.27       | 31.12                            | 33.21                   |
| Sw 2 ml/l + Ch 4 ml/l     | 2.29       | 2.34       | 0.28       | 1.29                            | 1.30       | 0.29       | 1.31       | 34.17                            | 34.64                   |
| Sw 2 ml/l + Ch 6 ml/l     | 2.36       | 2.41       | 0.32       | 1.31                            | 1.32       | 0.34       | 1.33       | 35.21                            | 35.77                   |
| Sw 4 ml/l + Ch 2 ml/l     | 2.59       | 2.66       | 0.34       | 1.34                            | 1.35       | 0.36       | 1.35       | 35.18                            | 36.80                   |
| Sw 4 ml/l + Ch 4 ml/l     | 2.82       | 2.77       | 0.41       | 1.38                            | 1.39       | 0.42       | 1.39       | 36.25                            | 38.78                   |
| Sw 4 ml/l + Ch 6 ml/l     | 2.86       | 2.84       | 0.42       | 1.40                            | 1.42       | 0.43       | 1.42       | 38.29                            | 39.45                   |
| Sw 6 ml/l + Ch 2 ml/l     | 2.75       | 2.79       | 0.39       | 1.36                            | 1.38       | 0.45       | 1.38       | 35.22                            | 36.86                   |
| Sw 6 ml/l + Ch 4 ml/l     | 3.23       | 3.31       | 0.45       | 1.44                            | 1.46       | 0.48       | 1.46       | 38.43                            | 40.55                   |
| Sw 6 ml/l + Ch 6 ml/l     | 3.21       | 3.11       | 0.42       | 1.41                            | 1.44       | 0.47       | 1.44       | 39.31                            | 39.51                   |
| L.S.D. at 5%              | 0.750      | 0.770      | 0.330      | 0.390                           | 0.380      | 0.340      | 0.380      | 1.471                            | 1.553                   |
| Second season (2018/2019) |            |            |            |                                 |            |            |            |                                 |
| Control                   | 1.21       | 1.25       | 0.17       | 1.06                            | 1.09       | 0.15       | 1.06       | 31.10                            | 34.12                   |
| Sw 2 ml/l + Ch 2 ml/l     | 1.93       | 1.98       | 0.27       | 1.26                            | 1.29       | 0.29       | 1.29       | 33.42                            | 34.68                   |
| Sw 2 ml/l + Ch 4 ml/l     | 2.31       | 2.46       | 0.28       | 1.28                            | 1.31       | 0.31       | 1.31       | 35.23                            | 35.37                   |
| Sw 2 ml/l + Ch 6 ml/l     | 2.34       | 2.57       | 0.31       | 1.34                            | 1.37       | 0.36       | 1.37       | 34.61                            | 35.86                   |
| Sw 4 ml/l + Ch 2 ml/l     | 2.61       | 2.71       | 0.37       | 1.36                            | 1.39       | 0.39       | 1.39       | 36.10                            | 37.99                   |
| Sw 4 ml/l + Ch 4 ml/l     | 2.84       | 2.95       | 0.44       | 1.40                            | 1.42       | 0.47       | 1.42       | 37.00                            | 38.82                   |
| Sw 4 ml/l + Ch 6 ml/l     | 2.86       | 2.91       | 0.46       | 1.41                            | 1.46       | 0.49       | 1.46       | 38.60                            | 39.97                   |
| Sw 6 ml/l + Ch 2 ml/l     | 2.80       | 2.88       | 0.48       | 1.38                            | 1.39       | 0.39       | 1.39       | 36.45                            | 37.56                   |
| Sw 6 ml/l + Ch 4 ml/l     | 3.25       | 3.42       | 0.43       | 1.45                            | 1.49       | 0.49       | 1.49       | 40.81                            | 42.86                   |
| Sw 6 ml/l + Ch 6 ml/l     | 3.22       | 2.98       | 0.40       | 1.44                            | 1.47       | 0.48       | 1.47       | 39.21                            | 41.79                   |
| L.S.D. at 5%              | 0.720      | 0.730      | 0.230      | 0.380                           | 0.386      | 0.330      | 0.386      | 1.729                            | 1.852                   |

Sw: seaweed extract, Ch: chitosan

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