INFLUENCE OF SOME BIOSTIMULANTS AND CHEMICAL FERTILIZERS ON GROWTH, SEED YIELD, CHEMICAL CONSTITUENTS, OIL PRODUCTIVITY AND FIXED OIL CONTENT OF CHIA (SALVIA HISPANICA L.) PLANT UNDER ASWAN CONDITIONS

Eman M. Abou El-Ghait*; H.M.M. Abd Al Dayem**; Y.F.Y. Mohamed* and Y.I.H. Khalifa*

* Horticulture Dept., Fac. Agric., Benha University, Egypt
** Botany Department, Faculty of Agriculture, Benha University, Egypt

ABSTRACT: This study was carried out at a private farm in AL-Radisiya city, Markaz Edfu, Aswan Governorate, Egypt on the banks of Nile River and Experimental Laboratory of Horticulture Department, Faculty of Agriculture, Benha University, EL-Qaloubia Governorate, Egypt during the two successive seasons of 2018/2019 and 2019/2020 on chia (Salvia hispanica L.) plant. A study was initiated through various biostimulant and chemical fertilizer aiming to evaluate effectiveness of arbuscular mycorrhiza fungi (AMF) or seaweed extract and/or humic acid as well as chemical fertilizer (N.P and K) and the combination of them on vegetative growth, seed yield, chemical constituent's, fixed oil productivity and fixed oil composition of chia (Salvia hispanica L.) plant. Plants sprayed by biostimulants gave the significantly highest mean values for all of studied characteristics mentioned above, particularly T6: the combined of mycorrhiza, humic acid, seaweed and recommended dose of NPK followed by T5: recommended dose of NPK (350:200:100 kg/feddan) in the first and second seasons. Meanwhile, T4: seaweed (1 ml/l) ranked the third values in parameters mentioned before in this concern. Whereas, the richest fixed oil percentage was scored by T6, followed descendingly by T5 in the 1st and 2nd seasons. Chromatography analysis of chia fixed oil revealed the identification of four components. i.e. palmitic acid, oleic acid, linoleic acid and α-linolenic acid. The main component was α-linolenic acid (43.34 to 53.28%). It can be concluded that T6 was the best for improving growth, seed yield, fixed oil productivity, chemical constituents and fixed oil components of Salvia hispanica L. plant.

Key words: Salvia hispanica, chia, fixed oil, humic acid, arbuscular mycorrhiza fungi, seaweed, chemical fertilizer, oil productivity and GLC.

INTRODUCTION

Chia (Salvia hispanica L) is native to Mexico and Guatemala belongs to the family (Lamiaceae) Ixtaina et al. (2008). In pre-Columbian times, chia is one of the basic foods of Central American civilizations (Ayerza and Coates, 2005). Possible to grow under drought conditions recommended cultivation as an alternative to strategic crops (Peiretti and Gai, 2009). As chia is considered a healthy and well-nourished food, it is widely cultivated in Africa (Ayerza and Coates, 2000).

Chia can be up to 1 meter long and have opposite leaves. The color of the seed ranges from black to gray and speckled black to
white, the shape is oval and the size ranges from 1 to 2 mm. Shoots are small flowers (3-4 mm) with small corollas and compact parts that contribute to a high rate of self-pollination. Chia seeds are rich in dietary fibers, protein, oil and mucilage. It contains from 25 to 40% oil with 60-68% of its comprising (omega) ω-3 α-linolenic acid (ALA) and 20% of (omega) ω-6 linoleic acid (Cahill and Provance, 2002; Peiretti and Meineri, 2008; Reyes-Caudillo et al., 2008; Bresson et al., 2009 and Ayerza, 2013).

Chia seed contains many important chemical components, such as carbohydrates (26-41%), protein (15-25%), fats (30-33%), ash (4-5%), minerals, vitamins and dry substance (90-93%). It also contains a high recent of antioxidants (Ixtaina et al., 2008).

Clearly, any reliable source of omega 3 fatty acids that can be found which is safe for consumption would be attractive. Chia contains omega 3 fatty acids and its oil provides the richest plant alpha linolenic fatty acid known (Ayerza and Costa, 2005). Coorey et al. (2012) reported that, S. hispanica can be easily added to commercial products. It is characterized by its nutritional value, as it is one of the largest plant sources of linoleic acid. Chia has recently been revived as a new crop particularly due to its high oil and highest omega-3 fatty acid content among productive oil seeds (Cahill, 2003; Cahill, 2004).

Mycorrhizal fungi (AMF) can act as a biofertilizer, increase plant uptake of immobile phosphate ions from root and enhance plant yield, as well as N, P, K, Mg and some micronutrients leading to stimulating growth (Van der Heijden et al., 2008; Smith et al., 2011 and Veresoglou et al., 2011). Hence, Moghith et al. (2021) declared that plants inoculum of mycorrhizal fungi score the highest significant increases of all vegetative growth and seed yield characteristics as compared of uninoculated plants (control) of (Salvia hispanica L.) plants.

Humic acid is of great importance to plants in terms of its effect on growth in addition to its obvious importance to soil as it improves soil properties and is an important component of organic matter in aquatic systems. It increases the rate of bacterial activity as it enhances the absorption of many nutrients as a chelating agent. Humic acid is a vital stimulant of natural growth. On the other hand, activates microorganisms in the soil and works to increase humic and organic matter, especially in alkaline soils containing little of the organic matter. It is known that humic materials increase the activity of root growth such as auxins, which in turn is reflected in the increased growth of the plant in various stages of growth, and leaf pigments (Berlyn and Russo, 1990).

The other investigators of humic acid on some medicinal plants (Hendawy et al., 2015, Jamali et al., 2015 and Vafaei et al., 2015). Also, Mohamed (2020). In general, it could be recommended that the combinations of seaweed extract and inoculum arbuscular mycorrhizal fungi (AMF) could be achieved the greatest values of all studied traits of Dutch fennel. Also, humic acid at 5.0 ml/l or amino acid (amino bower) at 2 ml/l, combined with inoculum mycorrhizal fungi recorded highly increments in this concern.

Biostimulants are used as safe alternatives to some chemical fertilizers because they contain hormones, such as auxins, gibberellins and cytokinins. (Du Jardin, 2015). It also leads to increased absorption of both macro and micro elements, thus reducing fertilizer requirements (Ertani et al., 2012). Seaweed contains essential minerals and hormones as growth stimulants. It gained importance in the later period as a foliar spray for many crops including various herbs, grains, flowers, medicinal and aromatic plants (Mooney and Van Staden, 1986; Crouch and Van Staden, 1994).

Seaweed extracts are widely used as biostimulants and are widely used in all
countries of the world (Du Jardin, 2015; Crouch and VanStaden, 1994). Biostimulants are expected to reach 894 million Euro in 2022 (Eef et al., 2018).

Some previous studies of marine seaweed extract, i.e., Mohamed et al. (2016) on basil (Ocimum basilicum, L. Ghatas et al. (2021) on Artemisia annua.

Finally, in recent decades the use of chemical fertilizers led to an increase of 33-66% in the field of agriculture (Fageria and Baligar, 2005). On the other side, there was a significant decrease in soil fertility and productivity, due to the excessive misuse of mineral fertilizers (Zargar Shooshtari et al., 2020). In addition to many problems such as soil salinity, heavy metal pollution (Hatamian et al., 2020). In contrast, biostimulants benefit different soil properties, including soil structure, moisture-holding capacity, and microbial activity (Suresh et al., 2004; Shahram and Ordookhani, 2011). In this context, Abou El-Ghait et al. (2021) demonstrated that, T 3 75% R.D. + 25% organic+biotreatment, followed by T 2 100% R.D. of mineral NPK treatment which recorded the greatest values of vegetative, seed yield and chemical components of the S. hispanica plant. It was necessary to estimate the yield of the chia under the conditions of Aswan. For this reason, the aim of this study was to evaluate the influence of using some biostimulants treatments and chemical fertilizers on growth, seed yield, chemical constituents, oil content and fixed oil constituents of Salvia hispanica L. plant.

MATERIALS AND METHODS

The present study involved field experiments conducted at a private farm in AL-Radisiya city, Markaz Edfu, Aswan Governorate, Egypt on the banks of Nile River and Experimental Laboratory of Horticulture Department, Faculty of Agriculture, Benha University, EL-Qaloubia Governorate, Egypt during the two successive seasons of 2018/2019 and 2019/2020 on Chia (Salvia hispanica L.) plant. A study was initiated through various biostimulant and chemical fertilizer aiming to evaluate effectiveness of arbuscular mycorrhiza fungi (AMF) or seaweed extract and/or humic acid as well as chemical fertilizer (N, P, K) and the combination of the abovementioned treatments on vegetative growth, seed yield, chemical constituents, fixed oil productivity and fixed oil composition of (Salvia hispanica L.) plant.

Plant material and procedure:

Seed of chia (1000 seed weight 1.25 g) were obtained from Experimental Farm of Floriculture of Faculty of Agriculture, Benha University, seeds were sown in the field on 20th and 15th October in the first and second seasons respectively, directly in the hills at the rate of 10 seeds/hill at distances of 50 cm between hill (thinned to two plants/hill) and 70 cm between rows (33600 plant/fed) soil samples representing the experiment area was taken at 0–30 cm depth. The soil physical and chemical analysis of this experiment were evaluated according to Jackson (1973) and Black et al. (1965). The obtained results of soil analysis are presented in Table (1).

Calcium superphosphate and compost were added at the time of soil preparation and mixed thoroughly with the soil of the experimental area at the rate of 20 m³/fed of compost and 200 kg/fed of calcium superphosphate (15.5% P₂O₅) for each season.

Experimental procedure:

This study included six treatments:

1. Control (without any addition; T₁)
2. Inoculated with arbuscular mycorrhiza fungi (AMF) treatments (T₂): abuscular mycorrhizal fungi According to Badr El-Din et al. (1999) Arbuscular mycorrhizal fungi obtained from Agricultural Microbiology Department National Research Center. AMF inoculation was done by mixed 20 g of it with 5g of chia seed before cultivation and then after 10
days by infection into the soil in roots area of seedlings from four side at 3.5 g/hill of inoculum material.

3. Humic acid (Humate at 75%, K₂O at 10%) (T₃): Humic King product of Egyptian Spain company Egypt. Treatment of humic acid was applied as soil dressing starting from one month after planting seeds of chia beginning with the rate of 0.5 g/plant until the second month the doses were increased to 1 g/plant and the third doses became 1.5 g/plant in the third month.

4. Seaweed extract (T₄): *Spirulina platensis* algae extract was obtained from Agricultural Microbiology Department National Research Center. Seaweed extract contains plant hormones, especially cytokinins and minerals such as (Fe, Zn, Cu, Mn and Mo) enzymes, vitamins,, amino acids. Treatments of seaweed extract was applied as foliar sprays repeated 10 times starting from 30 days after planting (1 ml/l) at 10 days' intervals until the end of the treatment. Also seaweed extract applied as soil dressing beside each plant immediately after irrigation to avoid any leaching effect at the same concentration (1 ml/l). The plants were irrigated every 15 days until the end of treatment.

5. Chemical fertilizer N, P and K (T₅): chemical fertilizer N, P and K as recommended dose 350 kg/fed of ammonium sulfate. Recommend dose 100 kg of potassium sulfate (K₂O 50%, S 14.4%), every plant was fertilized with 2.4 g of potassium sulfate. The recommended dose of calcium superphosphate (15.5% P₂O₅), was applied at 4.7 g/plant. The above-mentioned chemical fertilizers obtained from factory of Kema Aswan. these quantities were applied as dressing beside each plant in three equal portions at monthly internals except calcium superphosphate dose which was added during soil preparation with compost 200 kg/fed.

6. Combination treatment of all aforementioned treatments i.e. (mycorrhiza, humic acid, seaweed and recommended dose of NPK) (T₆).

At the end of each season on 15th April in both seasons, the plants were harvested at the beginning of flowering by cutting the vegetative growth.

The experiment included six treatments with three replicates, each replicate contains 10 plants, 30 plants in each treatment

**Data measurements and recorded:**

The vegetative and yield parameters were measured and recorded at harvesting time on 15th April, 2019 and 2020 as follows:

1. Vegetative characteristics at the beginning of flowering:

### Table 1. Mechanical and Chemical analysis of the Experimental soil.

| Parameters | Mechanical properties | Chemical analysis |
|------------|-----------------------|------------------|
|            | 2018/2019 | 2019/2020 | Values | 2018/2019 | 2019/2020 | Values |
| Sand       | 68.55%    | 65.23%    |        |          |          |        |
| Silt       | 15.12%    | 16.33%    |        |          |          |        |
| Clay       | 16.33%    | 18.44%    |        |          |          |        |
| Textural class | Sandy loam | Sandy loam |        |          |          |        |
| Organic matter |          |          | 1.55 |          |          | 1.77 |
| CaCO₃      |          |          | 0.97 |          |          | 1.08 |
| Available nitrogen (mg kg⁻¹) |          |          | 0.69 |          |          | 0.74 |
| Available phosphorus (mg kg⁻¹) |          |          | 0.38 |          |          | 0.42 |
| Available potassium (mg kg⁻¹) |          |          | 133  |          |          | 144  |
| pH         |          |          | 7.13  |          |          | 7.32  |
| EC (dS/m)  |          |          | 0.84  |          |          | 0.93  |
- Plant height (cm): length of the main stem from soil surface to the plant apex using measuring tape.
- Number of branches.
- Number of leaves/plant.
- Stem diameter (cm).
- Herb Fresh weight/plant (g).
- Herb Dry weight/plant (g).

2. Flowering parameters: main inflorescences height/plant (cm):

3. Seed yield parameters:
- Seed yield/plant (g).
- Seed yield/fed (ton).

4. Chemical constituents:

Nitrogen, phosphorus, potassium and total carbohydrates were determined in dried chia leaves according to the methods described by Horneck and Miller (1998), Hucker and Catroux (1980), Horneck and Hanson (1998) and Chaplin and Kennedy (1994) respectively.

5. Fixed oil productivity:

The percent of fixed oil was calculated as weight/weight using the following equation:

\[
\text{Fixed oil percentage} = \frac{\text{Extracted fixed oil weight}}{\text{seeds sample weight}} \times 100.
\]

5. Fatty acids determination:

The components representing methylated fatty acids were analyzed by G.L.C. method according to (Stahl, 1967).

Statistical analysis:

Data from the studied factors were subjected to analyses of variance (ANOVA) with simple trials in a randomized complete block design. Data were passed through a test for analysis of variance (ANOVA) was used to assess the significance of the data at \( P \leq 0.05 \) and differences were evaluated using least significant differences (LSD), according to (Snedecor and Cochran, 1989).

RESULTS

1. Vegetative growth measurements at the beginning of flowering:

Data presented in Table (2) declared that, all biostimulants treatments succeeded in increasing vegetative growth measurements i.e. (plant height, number of branches, stem diameter (cm), No. of leaves and herb fresh and dry weights/plant (g) of \((S. \text{ hispanica} \ L.)\) when compared to untreated (T1: control) specially T6: the combined treatment of AMF, humic acid, seaweed and recommended dose of NPK in the two seasons. Although all the treatments had no significant differences in the thickness of the stem diameter in the second season only. T5: recommended dose of NPK (350:200:100 kg/ha) gave the second greatest values of parameters mentioned before in this context. On the contrast, the minimum values of vegetative parameters mentioned afore scored by T1 (control).

2. Flowering and seed parameters:

Data presented in Table (3) demonstrated that, T6: the combination of mycorrhiza, humic acid, seaweed and recommended dose of NPK showed to be the most effective treatment for increasing the height of main inflorescences height/plant, seed yield/plant g and seed yield/fed (ton) of plant when compared to untreated plant (T1) in both seasons. Also, T5 and T4 recorded the highest increments of parameters mentioned afore in both seasons. Irrespective to control, the minimum values of above-mentioned parameters were obtained by T3.

3. Chemical composition determinations:

Data presented in Table (4) reveal that all biostimulants treatments statistically increased leaf N, P, K and total carbohydrate contents (%) of \((S. \text{ hispanica} \ L.)\) when compared to control in the two seasons. Hence, T6 gave the highest values in this
Table 2. Effect of some biostimulants and chemical fertilizer treatments, on plant height (cm), No. of branches, stem diameter (cm), No. of leaves and herb fresh and dry weights/plant (g) of chia (*Salvia hispanica* L.) plant, during 2018/2019 and 2019/2020 seasons.

| Treatments                       | Plant height (cm) | No. of branches/plant | Stem diameter (cm) | No. of leaves | Herb fresh weight/g | Herb dry weight/g |
|----------------------------------|-------------------|-----------------------|--------------------|--------------|--------------------|------------------|
| **1st season**                   |                   |                       |                    |              |                    |                  |
| Control (T1)                     | 83.00             | 31.67                 | 3.23               | 77.33        | 405.00             | 103.00           |
| Mycorrhiza at 5 mg/l (T2)        | 92.33             | 40.00                 | 4.17               | 87.33        | 481.33             | 122.33           |
| Humic acid at 1.5 mg/l (T3)      | 87.00             | 34.67                 | 3.43               | 85.67        | 410.00             | 109.00           |
| Seaweed at 1 ml/l (T4)           | 88.33             | 42.00                 | 3.93               | 89.33        | 413.00             | 110.00           |
| Recommended* dose of N.P.K. (T5) | 101.67            | 40.33                 | 4.27               | 124.67       | 486.67             | 122.67           |
| T2+T3+T4+T5 (T6)                 | 106.00            | 46.33                 | 4.50               | 130.00       | 487.67             | 131.33           |
| L.S.D at 0.05                    | 1.032             | 3.026                 | 1.11               | 4.40         | 7.54               | 5.38             |
| **2nd season**                   |                   |                       |                    |              |                    |                  |
| Control (T1)                     | 84.67             | 34.00                 | 3.73               | 86.33        | 410.00             | 104.00           |
| Mycorrhiza at 5 mg/l (T2)        | 92.00             | 42.00                 | 4.50               | 90.33        | 483.33             | 132.33           |
| Humic acid at 1.5 mg/l (T3)      | 89.00             | 36.67                 | 4.40               | 87.67        | 413.00             | 111.67           |
| Seaweed at 1 ml/l (T4)           | 90.00             | 44.00                 | 4.50               | 91.00        | 416.00             | 112.33           |
| Recommended* dose of N.P.K. (T5) | 104.00            | 42.67                 | 4.50               | 133.33       | 491.00             | 127.33           |
| T2+T3+T4+T5 (T6)                 | 108.33            | 48.00                 | 4.60               | 135.33       | 494.33             | 134.67           |
| L.S.D at 0.05                    | 2.759             | 3.092                 | N.S.               | 3.73         | 7.71               | 5.85             |

* NPK at 350:200:100 kg/feddan

Table 3. Effect of some biostimulants and chemical fertilizers treatments, on main inflorescences height/plant, seed yield/plant, and seed yield/Fed of chia (*Salvia hispanica* L.) plant, during 2018/2019 and 2019/2020 seasons.

| Treatments                       | Main inflorescences height/plant | Seed yield/plant (g) | Seed yield/Fed (ton) |
|----------------------------------|----------------------------------|----------------------|----------------------|
| **1st season**                   |                                  |                      |                      |
| Control (T1)                     | 26.00                            | 34.30                | 1.15                 |
| Mycorrhiza at 5 mg/l (T2)        | 34.33                            | 73.16                | 2.46                 |
| Humic acid at 1.5 mg/l (T3)      | 34.00                            | 69.82                | 2.36                 |
| Seaweed at 1 ml/l (T4)           | 37.00                            | 76.22                | 2.56                 |
| Recommended* dose of N.P.K. (T5) | 36.33                            | 77.61                | 2.61                 |
| T2+T3+T4+T5 (T6)                 | 36.67                            | 80.78                | 2.71                 |
| L.S.D at 0.05                    | 1.55                             | 2.24                 | 0.081                |
| **2nd season**                   |                                  |                      |                      |
| Control (T1)                     | 30.33                            | 36.39                | 1.22                 |
| Mycorrhiza at 5 mg/l (T2)        | 37.00                            | 75.66                | 2.54                 |
| Humic acid at 1.5 mg/l (T3)      | 36.00                            | 71.38                | 2.40                 |
| Seaweed at 1 ml/l (T4)           | 38.67                            | 77.21                | 2.59                 |
| Recommended* dose of N.P.K. (T5) | 38.33                            | 78.99                | 2.65                 |
| T2+T3+T4+T5 (T6)                 | 40.00                            | 82.45                | 2.77                 |
| L.S.D at 0.05                    | 3.45                             | 1.93                 | 0.058                |

* NPK at 350:200:100 kg/feddan
concern, followed descendingly by T5 in both seasons. Moreover, the T3 gave the third values in this concern. The minimum values of parameters mentioned above were recorded by T1.

4. Fixed oil (%):

Data presented in Table (5) stated that, the fixed oil percentage of (Salvia hispanica L.) seeds were more affected by using all biostimulant treatments as compared to control (T1) in both seasons. However, the richest fixed oil % were scored by T6, followed in descending order with T5 in the first and second seasons. Moreover, the T2 mycorrhiza (5 mg/l) gave the third highest values in this concern on the contrast, the minimum values of this parameter were scored by T1.

5. Fixed oil content of chia (Salvia hispanica L.) seed:

Table (6) and Fig. (1) declare the data belonging to the effect of different treatments of biostimulants on the qualitative of the fixed oil compositions of chia (Salvia
combined of mycorrhiza, humic acid, seaweed and recommended dose of N.P.K registered the greatest values of α-linolenic acid as (53.28%) followed descendingly by T5: recommended dose of N.P.K (350:200:100 kg/ha) as (47.09%) and T4: seaweed (1 ml/l) gave 45.06%) as when compared to control as (43.34%) control (T1). Meanwhile, T3: humic acid (1.5 mg/l) gave the maximum values of linoleic acid (23.31%), followed by T4: seaweed (1 ml/l) (23.04%) Additionally, treatments mentioned afore registered decreases in the percentage of oleic acid from 20.83 in T1 (control) to 14.06% of T6.

**Discussion**

Biostimulants and NPK fertilizers succeeded in increasing plant growth compared to the control, as it improved plant height, number of branches and dry weight in all fertilization treatments higher than the untreated treatment. Mycorrhizal symbioses are essential for the sustainable management of agricultural ecosystems (Barrios, 2007; Smith and Read, 2008). Arbuscular mycorrhizal fungi (AMF) play an important role in plant growth, health and productivity. AMF fungi help plants to absorb nutrients, especially the less available mineral nutrients such as copper, molybdenum, phosphorus and zinc (Chanda et al., 2014). AMF have been shown to have benefits to host plants by increasing dry leavesivore tolerance, pollination, soil stability, and heavy metal tolerance (Hart and Trevors, 2005). AMF help plant species to uptake water and nutrients and make physiological changes to increase the growth and productivity of host plants, AMF fungi play a key role in soil fertility and plant nutrition, enhancing the uptake and translocation of mineral nutrients (P, N, S, K, Ca, Fe, Cu and Zn) from soil to host plants, by means of an extensive below ground hyphal network, which spreads from colonized roots into the soil environment (Smith and Read, 2008 and Raei and Weisany, 2013).

AMF inoculation causes physiological changes where the amount of mitochondria increases threefold, the nucleus increases in size, and increased numbers of mitochondria and plastids lead to an increase in energy production. (French, 2017). They can move towards the arbuscules, forming a net-like structure over the fungus. The number of plastids also increases and the strands become more abundant. (Buee et al., 2000; Lohse et al., 2005). Similar results of mycorrhizal fungi were obtained by Moghith et al., (2021) on (*Salvia hispanica* L.) plants. Mohamed (2020) on Dutch fennel.

In this concern, the role of humic acid on growth and development of the plants. Humic acid is one of the natural growths biostimulants as it is considered an important component of organic substances in aquatic systems. Humic acid has a great importance for the plant in terms of its effect on growth

---

**Table 6. Effect of different treatments on fixed oil contents of chia (*Salvia hispanica* L.) plant, during the second season 2019/2020.**

| Peak No. | Components       | T1   | T2   | T3   | T4   | T5   | T6   |
|-----------------|------------------|------|------|------|------|------|------|
| 1    | Palmitic acid   | 13.78| 14.83| 13.85| 12.55| 12.08| 10.77|
| 2    | Oleic acid      | 20.83| 18.42| 19.00| 19.36| 17.83| 14.06|
| 3    | Linoleic acid   | 22.08| 22.22| 23.31| 23.04| 23.01| 21.89|
| 4    | α-linolenic acid| 43.34| 44.54| 43.85| 45.06| 47.09| 53.28|
| -    | Total identified| 100  | 100  | 100  | 100  | 100  | 100  |

*T1: Control, T2: Mycorrhiza (5 mg/l), T3: Humic acid (1.5 mg/l), T4: Seaweed (1 ml/l), T5: Recommended dose of N.P.K (350:200:100 kg/ha), T6: The combined of mycorrhiza, humic acid, seaweed and recommended dose of N.P.K.*
The combined of mycorrhiza, humic acid, seaweed and recommended dose of N.P.K. (T₆)

Recommended dose of N.P.K (350:200:100 kg/feddan) (T₅)

Seaweed (1 ml/l) (T₄)

Mycorrhiza (5 mg/l) (T₂)

Control (T₁)

Fig. 1. G.L.C. of chia (*Salvia hispanica* L.) fixed oil content as affected by different treatments during the second season (2019/2020).
in addition to its clear importance to the soil as it works to increase the content of the soil and increase the rate of microbial activity as it enhances the absorption of many nutrients as a chelating agent. On the other hand, HA activates microorganisms in the soil and works to increase humic and organic matter, especially in alkaline soils containing little of the organic matter. It is known that humic materials increase the activity of root growth such as auxins, which in turn is reflected in the increased growth of the plant in various stages of growth, and leaf pigments (Berlyn and Russo., 1990; Graves et al., 2004; Frankenberger and Arshad, 1995; Pereira et al. (2019)).

The aforementioned results of humic acid concerning studied traits in parallel with those obtained Aiyafar et al. (2015) on cumin (Nigella sativa L.), El-Shayeb et al. (2015) on Salvia officinalis L. Hendawy et al. (2015) on Mentha piperita var. citrata plants, Nasiri et al. (2015) on Pelargonium graveolens plant and Awad (2016) on Carum carvi plant, El-Khateeb et al. (2017) on Majorana hortensis plants, Ibrahim and Helaly (2017) on fenugreek plants. Mohamed and Ghatas (2020) on chia (Salvia hispanica L.) plants. The results of safety biostimulants concerning yield parameters are in parallel with those obtained, Aiyafar et al. (2015) on Nigella sativa L., Ariafar and Forouzandeh (2017) on black cumin, Ibrahim and Helaly (2017) on fenugreek plants. Mohamed (2020) declared that the largest growth, yield and chemical components of Dutch fennel can achieve combinations of seaweed extract at a rate of 2 ml/l and AMF. Also, humic acid at 5.0 ml/l. Mohamed and Ghatas (2020) demonstrated that plants were sprayed with safe growth stimulants, which ranked the highest significant mean values of all vegetative growth, seed yield and chemical components of Salvia hispanica L. plants, especially humic acid at 5 ml/l. The aforementioned results of humic acid concerning chemical composition are in parallel with those obtained by Salachna et al. (2017) on Verbena bonariensis.

Seaweed extract are used as safe alternatives to some chemical fertilizers because they contain hormones, such as auxins, gibberellins and cytokinins. (Du Jardin, 2015). It also leads to increased absorption of both macro and micro elements, thus reducing fertilizer requirements (Ertani et al., 2012). Seaweed contains essential minerals and hormones as growth stimulants. It gained importance in the later period as a foliar spray for many crops including various herbs, grains, flowers, medicinal and aromatic plants (Crouch and Van Staden, 1994; Mooney and Van Staden, 1986).

Seaweed extracts are widely used as biostimulants and are widely used in all countries of the world (Du Jardin, 2015; Crouch and VanStaden, 1994). Some previous studies of marine seaweed extract, i.e., Mohamed et al. (2016) on basil (Ocimum basilicum, L. Ghatas et al. (2021)) on Artemisia annua.

Mineral and organic fertilization causes an increase in the production of many crops (Goudarzi et al., 2016). The aforementioned results of chemical fertilization concerning vegetative growth are in parallel with those obtained by Said-Al Ahl et al. (2015) on Anethum graveolens L., Ghatas (2020) on Coriandrum sativum plants in the two seasons in most cases., Abou El-Ghait et al. (2021) on chia plants.

The majors components of chia oil was stated by various studies like Ayerza and Coates (2005) found that the major component were α-linolenic (63.2%), linoleic (18%), oleic (3.4%), palmitic (7.25%) and stearic (3.4%); Segura-Campos et al. (2014) stated that chia oil contains α-linolenic (68.52%), linoleic (20.40%), oleic (2.43%), palmitic (7.74%) and stearic (0.29%) and Silva et al. (2016) studied quantification of fatty acids in the chia seed oils obtained with different solvents and stated that α-linolenic arranged (61.48 –
62.92%), linoleic (18.10 – 19.76%), oleic (6.9 – 6.87%), palmitic (9.13 – 9.95) and stearic (2.92 - 2.99%). Moghith et al. (2021) declared that the main components were α-linolenic acid (54.96 to 63.23%) linoleic acid (15.82 to 21.36), oleic acid (6.19 to 15.86%) and palmitic acid (6.30 to 8.15%). Mohamed and Ghatas (2020) on chia declared that the main component was α-linolenic acid (37.28 to 39.72%). The major components were α-linolenic acid, linoleic acid, oleic acid, and palmitic acid.

CONCLUSION

From the results obtained conclusively, it can be indicated that T6 (the combination of mycorrhiza, humic acid, seaweed, and recommended dose of N.P.K.) or T5 (recommended dose of N.P.K. at 350:200:100 kg/feddan) could be used to improve growth, seed yield, chemical constituents, fixed oil productivity and fixed oil constituents of chia (Salvia hispanica L.) plant.

REFERENCES

Abou-El-Ghait, E.M.; Bahloul, H.E.; Youssef, A.S.M. and Hamed, A.1. (2021). Partial substitution of chemical fertilization of chia plant (Salvia hispanica L) by organic fertilization in the presence of bio fertilization, Future J. Biol., 3:1-10.

Aiyafar, S.; Poudineh, H.M. and Forouzandeh, M. (2015). Effect of humic acid on qualitative and quantitative characteristics and essential oil of black cumin (Nigella sativa L.) under water deficit Stress. DAV. Int. J. Sci., 4:89-102.

Ariafar, S. and Forouzandeh, M. (2017). Evaluation of humic acid application on biochemical composition and yield of black cumin under limited irrigation condition. Bulletin de la Société Royale des Sciences de Liège, 86(1):13-24.

Awad, M.Y.M. (2016). Poultry manure and humic acid foliar applications impact on caraway plants grown on clay loam. J. Soil Sci. and Agric. Eng., Mansoura Univ., 7(1):1-10.

Ayerza, R. (2013). Seed composition of two chia (Salvia hispanica L.) genotypes which differ in seed color. Emirates Journal of Food and Agriculture, 25: 495-500.

Ayerza, R. and Coates, W. (2000). Dietary levels of chia influence on yolk cholesterol, lipid content and fatty acid composition, for two strains of hens. Poultry Science., 78:724-739.

Ayerza, R. and Coates, W. (2005). Ground chia seed and chia oil effects on plasma lipids and fatty acids in the rat. Nutrition Research., 25(11):995-1003.

Badr El-Din, S.M.S.; Attia, M. and Abo-Sedera, S.A. (1999). Evaluation of several substrates for mass multiplication of arbuscular mycorrhizal (AM) fungi grown on onion. Egyptian Journal of Microbiology, 34:57-61.

Barrios, E. (2007). Soil biota, ecosystem services and land productivity. Ecological Economics, 64:269-285

Berlyn, G.P. and Russo, R.O. (1990). The use of organic biostimulants to promote root growth. Below Ground Ecol., 2:12-13.

Black, C.A. (1965). Methods of Soil Analysis, Part 1. Physical and Mineralogical. ASA Madison, Wisconsin, USA, 1188 p.

Bresson, J.L.; Flynn, A.; Heinonen, M.; Hulshof, K.; Korhonen, H.; Lagiou, P.; Lovik, M.; Marchelli, R.; Martin, A.; Moseley, B.; Pryzembel, H.; Salminen, S.; Strain, J.J.; Strobel, S.; Tetens, I.; Berg, H.; Loveren, H. and Verhagen, H. (2009). Opinion on the safety of chia seeds (Salvia hispanica L.) and ground whole chia seeds as a food ingredient. The European Food Safety Authority Journal, 996:1–26.

Buee, M., Rossignol, M.; Jauneau, A.; Ranjeva, R. and Bécard, G. (2000). The
presymbiotic growth of arbuscular mycorrhizal fungi is induced by a branching factor partially purified from plant root exudates. Molecular Plant-Microbe Interactions, 13(6): 693–698.

Cahill, J.P. (2003). Ethnobotany of chia, *Salvia hispanica* L. (Lamiaceae). Econ. Bot., 57: 604–618

Cahill, J.P. (2004). Genetic diversity among varieties of chia (*Salvia hispanica* L.). Genetic Resources and Crop Evolution, 51:773–781.

Cahill, J.P. and Provance, M.C. (2002). Genetics of qualitative traits in domesticated chia (*Salvia hispanica* L.). Journal of Heredity, 93(1):52–55.

Chanda, D.; Sharma, G.D. and Jha, D.K. (2014). The potential use of arbuscular mycorrhiza in the cultivation of medicinal plants in Barak Valley, Assam: A Review. Current World Environment, 9(2):544-551.

Chaplin, M.F. and Kennedy, J.F. (1994). Carbohydrate Analysis: A Practical Approach, 2nd ed. Oxford University Press, New York, USA, 324 p.

Coorey, R.; Grant, A. and Jayasena, V. (2012). Effect of chia flour incorporation on the nutritive quality and consumer acceptance of chips. Journal of Food Research, 1: 85-95.

Crouch, I.J. and VanStaden, J. (1994). Commercial seaweed products as biostimulants in horticulture. Journal of Home and Consumer Horticulture 1:19–76.

Du Jardin, P. (2015). Plant biostimulants: definition, concept, main categories and regulation. Sci. Hort., 196: 3–14.

Eef, B.; Marlies, D.; Van Swam, K.; Veen, A. and Burger, L. (2018). Identification of the Seaweed Biostimulants Market (Phase1). The North Sea Farm Foundation: AD Den Haag, The Netherlands, 64 p.

El-Khateeb, M.A.; El-Attar, A.B.; and Nour, R.M. (2017). Application of plant biostimulants to improve the biological responses and essential oil production of marjoram (*Majorana hortensis*, Moench) plants. Middle East J. Agric. Res, 6(4):928-941.

El-Shayeb, N.S.A.; Abo El-Soud, I.H. and El-Shal, S.A. (2015). Effect of different levels of chemical fertilization and humic acid on *Salvia officinalis* L. plants, Egypt. J. of Appl. Sci., 30(11):727-758.

Engel, S.; Puglisi, M.P.; Jensen, P.R. and Fenical, W. (2006). Antimicrobial activities of extracts from tropical Atlantic marine plants against marine pathogens and saprophytes. Mar. Biol. 149:991-1002.

Ertani, A.; Nardi, S. and Altissimo, A., (2012). Long-term research activity on the biostimulant properties of natural origin compounds. Acta Hort. 1009:181–187.

Fageria, N.K. and Baligar, V.C. (2005). Enhancing nitrogen use efficiency in crop plants. Advan. Agron. 88: 97–185.

Frankenberger, W.T. and Arshad, M. (1995). Phytohormones in Soils: Microbial Production and Function. Marcel and Deckker, New York, 503 p.

French, K.E. (2017). Engineering mycorrhizal symbioses to alter plant metabolism and improve crop health. Frontiers in Microbiology, 8:1-8. https://doi.org/10.3389/fmicb.2017.01403

Ghatas, Y. (2020). Impacts of using some fertilization treatments in presence of salicylic acid foliar spray on growth and productivity of *Coriandrum sativum* L. Plant. J. of Plant Prod. Mansoura Univ. 11(2):119–125.

Ghatas, Y.; Ali, M.; Elsadek, M.; and Mohamed, Y. (2021). Enhancing growth, productivity and artemisinin content of *Artemisia annua* L. plant using seaweed extract and micronutrients. Industrial Crops and Products, 161:1-10. https://doi.org/10.1016/j.indcrop.2020.113202
Goudarzi, T.; Saharkhiz, M.; Rowshan, V. and Taban, A. (2016). Changes in essential oil content and composition of Tansy (Tanacetum vulgare L.) under foliar application of salicylic and orthophosphoric acids. J. Essent. Oil Res. 28:64–70.

Graves, A.; Matthews, R. and Waldie, K. (2004). Low external input technologies for livelihood improvement in subsistence agriculture. Adv. Agron., 82:473-555.

Hart, M.M. and Trevors, J.T. (2005). Microbe management: application of mycorrhizal fungi in sustainable agriculture. Frontiers in Ecology and the Environment, 3: 533-539.

Hatamian, M.; Rezaei Nejad, A.; Kafi, M.; Souri, M.K. and Shahbazi, K. (2020). Nitrate improves hackberry seedling growth under cadmium application. Heliyon, 6 (1): 1-7. https://doi.org/10.1016/j.heliyon.2020.e03247

Hendawy, F.S.; Hussein, M.S.; El-Gohary, A.E. and Ibrahim, M.A. (2015). Effect of foliar organic fertilization on the growth, yield and oil content of Mentha piperita var. citrata. Asian Journal of Agric. Res., 9:237-248.

Horneck, D.A. and Hanson, D. (1998). Determination of potassium and sodium by flame emission spectrophotometry. In: Kolra, Y.P. (ed.), Handbook of Reference Methods for Plant Analysis, Taylor and Francis Group, LLC., USA, pp. 153-155.

Horneck D.A. and Miller R.O. (1998). Determination of total nitrogen in plant tissue. In: Kolra, Y.P. (ed.), Handbook of Reference Methods for Plant Analysis, Taylor and Francis Group, LLC., USA, pp. 75-83.

Hucker, T. and Catroux, G. (1980). Phosphorus in sewage ridge and animal’s wastes slurries. Proceeding of the EEC Seminar, Haren (Gr): Gromingen Netherlands 12, 13 June.

Ibrahim, F.R. and Helaly, A.A.E. (2017). Growth and productivity response to nitrogen, potassium and humic acid of fenugreek (Trigonella foenum-graecum L.) plant. Middle East Journal of Agriculture Research, 6:1526-1535.

Ixtaina, V.Y.; Nolasco, S.M. and Tomas, M.C. (2008). Physical properties of chia (Salvia hispanica L.) seeds. Industrial Crops and Products, 28(3):286–293.

Jackson, M.L. (1973). Soil Chemical Analysis. Prentice-Hall of India Private Ltd. M-97, New Delhi, India, 498 p.

Jamali, Z.S.; Astaraei, A.R. and Emami, H. (2015). Effect of humic acid, compost and phosphorus on growth characteristics of basil herb and concentration of micro elements in plant and soil. J. Sci. and Tech., 6(2): 187-205.

Lohse, S.; Schliemann, W.; Ammer, C.; Kopka, J.; Strack, D. and Fester, T. (2005). Organization and metabolism of plastids and mitochondria in arbuscular mycorrhizal roots of Medicago truncatula. Plant Physiology, 139:329-340.

Moghith, W.M.A.; Youssef, A.S.M.; Abd El-Wahab, M.A.; Mohamed, Y.F.Y. and Abou El- Ghait, Eman M. (2021). Effect of Arbuscular Mycorrhizal Fungi And Some Phosphorus Sources on Growth, Seeds Yield, Chemical Compositions, Oil Productivity and Fixed Oil Constituents of chia (Salvia hispanica L.) Plant. Proceeding of the 5th International Conference on Biotechnology Applications in Agriculture (ICBAA), Benha University, 8 April 2021, Egypt (Conference Online), pp. 541-562 p.

Mohamed, Y.F.Y.; Zewail, R.M.Y. and Ghatas, Y.A.A. (2016). The role of boron and some growth substances on growth, oil productivity and chemical characterization of volatile oils in basil (Ocimum basilicum L.) cv. Genovese. Journal of Horticultural Science and Ornamental Plants, 8(2):108-118.
Mohamed, Y.F.Y and Ghatas, Y.A.A. (2020). Effect of some safety growth stimulants and zinc treatments on growth, seeds yield, chemical constituents, oil productivity and fixed oil constituents of chia (Salvia hispanica L.) plant. Scientific J. Flowers & Ornamental Plants, 7(2):163-183.

Mohamed, Y.F.Y. (2020). Impact of some growth stimulants in cooperation with arbuscular mycorrhizal fungi on growth, productivity and chemical constituents of dutch fennel plant. Scientific J. Flowers & Ornamental Plants, 7(3):303-319.

Mooney, P.A. and Van Staden, J. (1986). Algae and cytokinins. Journal of Plant Physiology, 123:1–2.

Nasiri, Z.; Khalighi, A. and Matlabi, E. (2015). The effect of humic acid, fulvic acid, and kristalon on quantitative and qualitative characteristics of geranium. International Journal of Biosciences, 6(5): 34-41.

Peiretti, P.G. and Gai, F. (2009). Fatty acid and nutritive quality of chia (Salvia hispanica L.) seeds and plant during growth. Animal Feed Science and Technology, 148(2-4): 267–275.

Peiretti, P.G. and Meineri, G. (2008). Effects on growth performance, carcass characteristics, and the fat and meat fatty acid profile of rabbits fed diets with chia (Salvia hispanica L.) seed supplements. Meat Science, 80(4):1116–1121.

Pereira, M.M.A.; Morais, L.C.; Marques, E.A.; Martins, A.D.; Cavalcanti, V.P.; Rodrigues, F.A.; Gonçalves, W.M.; Blank, A.F.; Pasqual, M. and Dória1, J. (2019). Humic Substances and Efficient Microorganisms: Elicitation of Medicinal Plants: A Review. Journal of Agricultural Science, 1(7):268-280.

Raei, Y. and Weisany, W. (2013). Arbuscular mycorrhizal fungi associated with some aromatic and medicinal plants. Bulletin of Environment. Pharmacology and Life Sciences, 2(11): 129-138.
manipulating plant phosphorus acquisition. Plant physiology, 156(3): 1050-1057.

Snedecor, G. W. and Cochran, W.G. (1989). Statistical Methods, 8th ed. Iowa State University Press, Ames., 503 p.

Stahl, E.E. (1967). Thin Layer Chromatography. A Laboratory Handbook. Springer Verlag, New York., USA, pp. 14-37.

Suresh, K.; Sneh, G.; Krishn, K. and Mool, C. (2004). Microbial biomass carbon and microbial activities of soils receiving chemical fertilizers and organic amendments. Arch. Agron. Soil Sci., 50:641–647.

Vafaei, N.; Samavat, S. and Ladan, A.R. (2015) Effects of different levels of humic acid, amino acid and fulvic acid on the growth and yield of basil plants Intern. Res. Journal of Applied and Basic Sciences, 9(10): 1732-1734.

Van der Heijden, M.G.A.; Rinaudo, V.; Verbruggen, E.; Scherrer, C., Bárberi, P., and Giovannetti, M. (2008). The significance of mycorrhizal fungi for crop productivity and ecosystem sustainability in organic farming systems. Proceedings of the 16th IFOAM Organic World Congress, Modena, Italy, June 16-20.

Veresoglou, S.D.; Shaw, L.J. and Sen, R. (2011). 

Zargar Shooshtari, F.; Souri, M.K.; Hasandokht, M.R. and Kalate Jari, S. (2020). Glycine mitigates fertilizer requirements of agricultural crops: case study with cucumber as a high fertilizer demanding crop. Chem. Biol. Technol. Agri., 7(1):1–10.

Sndecor, G. W. and Cochran, W.G. (1989). Statistical Methods, 8th ed. Iowa State University Press, Ames., 503 p.

Stahl, E.E. (1967). Thin Layer Chromatography. A Laboratory Hand Book. Springer Verlag, New York., USA, pp. 14-37.

Suresh, K.; Sneh, G.; Krishn, K. and Mool, C. (2004). Microbial biomass carbon and microbial activities of soils receiving chemical fertilizers and organic amendments. Arch. Agron. Soil Sci., 50:641–647.

Vafaei, N.; Samavat, S. and Ladan, A.R. (2015) Effects of different levels of humic acid, amino acid and fulvic acid on the growth and yield of basil plants Intern. Res. Journal of Applied and Basic Sciences, 9(10): 1732-1734.

Van der Heijden, M.G.A.; Rinaudo, V.; Verbruggen, E.; Scherrer, C., Bárberi, P., and Giovannetti, M. (2008). The significance of mycorrhizal fungi for crop productivity and ecosystem sustainability in organic farming systems. Proceedings of the 16th IFOAM Organic World Congress, Modena, Italy, June 16-20.

Veresoglou, S.D.; Shaw, L.J. and Sen, R. (2011).

Zargar Shooshtari, F.; Souri, M.K.; Hasandokht, M.R. and Kalate Jari, S. (2020). Glycine mitigates fertilizer requirements of agricultural crops: case study with cucumber as a high fertilizer demanding crop. Chem. Biol. Technol. Agri., 7(1):1–10.

Sndecor, G. W. and Cochran, W.G. (1989). Statistical Methods, 8th ed. Iowa State University Press, Ames., 503 p.

Stahl, E.E. (1967). Thin Layer Chromatography. A Laboratory Hand Book. Springer Verlag, New York., USA, pp. 14-37.

Suresh, K.; Sneh, G.; Krishn, K. and Mool, C. (2004). Microbial biomass carbon and microbial activities of soils receiving chemical fertilizers and organic amendments. Arch. Agron. Soil Sci., 50:641–647.

Vafaei, N.; Samavat, S. and Ladan, A.R. (2015) Effects of different levels of humic acid, amino acid and fulvic acid on the growth and yield of basil plants Intern. Res. Journal of Applied and Basic Sciences, 9(10): 1732-1734.

Van der Heijden, M.G.A.; Rinaudo, V.; Verbruggen, E.; Scherrer, C., Bárberi, P., and Giovannetti, M. (2008). The significance of mycorrhizal fungi for crop productivity and ecosystem sustainability in organic farming systems. Proceedings of the 16th IFOAM Organic World Congress, Modena, Italy, June 16-20.

Veresoglou, S.D.; Shaw, L.J. and Sen, R. (2011).

Zargar Shooshtari, F.; Souri, M.K.; Hasandokht, M.R. and Kalate Jari, S. (2020). Glycine mitigates fertilizer requirements of agricultural crops: case study with cucumber as a high fertilizer demanding crop. Chem. Biol. Technol. Agri., 7(1):1–10.

Sndecor, G. W. and Cochran, W.G. (1989). Statistical Methods, 8th ed. Iowa State University Press, Ames., 503 p.

Stahl, E.E. (1967). Thin Layer Chromatography. A Laboratory Hand Book. Springer Verlag, New York., USA, pp. 14-37.

Suresh, K.; Sneh, G.; Krishn, K. and Mool, C. (2004). Microbial biomass carbon and microbial activities of soils receiving chemical fertilizers and organic amendments. Arch. Agron. Soil Sci., 50:641–647.

Vafaei, N.; Samavat, S. and Ladan, A.R. (2015) Effects of different levels of humic acid, amino acid and fulvic acid on the growth and yield of basil plants Intern. Res. Journal of Applied and Basic Sciences, 9(10): 1732-1734.

Van der Heijden, M.G.A.; Rinaudo, V.; Verbruggen, E.; Scherrer, C., Bárberi, P., and Giovannetti, M. (2008). The significance of mycorrhizal fungi for crop productivity and ecosystem sustainability in organic farming systems. Proceedings of the 16th IFOAM Organic World Congress, Modena, Italy, June 16-20.

Veresoglou, S.D.; Shaw, L.J. and Sen, R. (2011).

Zargar Shooshtari, F.; Souri, M.K.; Hasandokht, M.R. and Kalate Jari, S. (2020). Glycine mitigates fertilizer requirements of agricultural crops: case study with cucumber as a high fertilizer demanding crop. Chem. Biol. Technol. Agri., 7(1):1–10.