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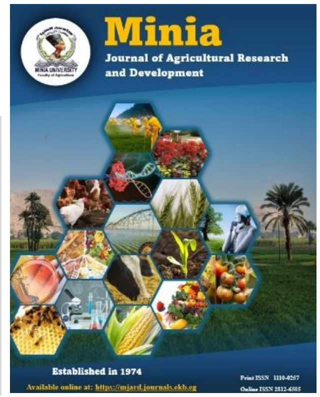

Effects of humic acid and biofertilizers on chia plants

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ABSTRACT

This study was conducted over two consecutive growing seasons of 2022/2023 and 2023/2024 to investigate the effects of humic acid (HA) and some biofertilizers on chia plants with respect to vegetative growth characteristics, seeds/yield, fixed oil content and chemical composition. Concerning this, two factors were examined the first factor (A) was HA at 3, 6 and 9 ml/L, while the second factor (B) was biofertilizers effective microorganisms (EM) or Minia azotein (MA). Highest main branch number/plant, herb dry weight, seed yield, fixed oil percentage and yield as well as,, photosynthetic pigments were significantly promoted by fertilizing chia plants with HA compared with control treatment. The best values for all previous characters were obtained from the high level of HA. Also, the same results were obtained with the treatments of EM or MA at different concentrations however, the best treatment was EM at 50% or MA at 25%. The best interaction treatment for the highest yield of chia plants was recorded with the HA at 9 ml/L combined with EM at 50% or MA at 25%.

Keywords: saline soil, Identification, nitrogen, Azotobacter, Phosphorous, Bacillus

INTRODUCTION

Salvia hispanica is native to Guatemala and central and southern Mexico. It belongs to the *Lamiaceae* family. In pre-Columbian times, civilizations such as the Aztecs and Mayans consumed chia seeds, appreciating their outstanding nutritional value. They used the seeds as a primary food source, for medicinal purposes, and in religious rituals **(Ayerza** and **Coates, 2005).** The name " Chia " derives from the Nahuatl word " Chitan " meaning oily, emphasizing that the seeds are rich content of essential fatty acid **(Ali *et al*., 2012** and **Muńoz *et al*., 2013).**

Chia seeds are small, oval, and may be either black or white in color. Despite their small size, they are high in nutrients and contain a significant concentration of

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Omega-3 fatty acids, which are known to have heart-healthy and anti-inflammatory effects **(Nieman *et al*., 2009, Moghith, 2019** and **Mohammed et al., 2019)**. According to **Reyes-Caudillo *et al*. 2008)**, it has a high dietary fiber content, protein, and nutrients including calcium, phosphorus, magnesium, and manganese. Its high antioxidant content also contributes to the reduction of oxidative stress and inflammatory reactions. These attributes make chia seeds popular as a functional food ingredient in health-conscious diets globally. It has a role in reducing cardiovascular risk factors, managing blood sugar levels and improving digestive health **(Nieman *et al*., 2009, Ulbricht *et al*., 2009 and Vuksan *et al*. 2010).**

Humic acid was shown to enhanced plant growth, which may be associated with in improved nutrient uptake through improved membrane permeability and root interactions **(Canellas *et al*., 2015).** Many authors investigate the role of HA on numerous plants **(Jamali *et al*., 2015, Mahsa *et al*., 2015, Taghipour *et al*., 2017, Hassan 2019, Karimian *et al*., 2019, Prasanna *et al*., 2021 a** and **b, Massoud *et al.*, 2022, Mirzapour *et al*., 2022** and **Abd El-Raouf** and **Ashour, 2023).**

Lactic acid bacteria, such as *Lactobacillus plantarum*, *Lactobacillus casei*, and *Streptococcus lactic*, as well as photosynthetic bacteria, yeasts, and algae, are among the more than 60 varieties of helpful microorganisms that produce EM culture. These microorganisms are known to produce lactic acid, that helps enhance microbial environments (**Formowitz *et al*., 2007**). Several studies have indicated that the application of EM technology may enhance the yield and quality of many plants by promoting growth and increasing the concentration of essential chemical compounds in plant raw materials (**Javaid, 2006** and **Singh, 2007, Mohamed** and **Ghatas, 2020**).

To produce clean and healthy crops, a lot of research has been done on using biofertilizers, such as N-fixing bacteria, instead of chemical ones. Through the natural processes of nitrogen fixation, phosphorus solubilization, and growth-promoting chemical synthesis, biofertilizers provide nutrients to plants. According to **Kapoor *et al*., (2015)**, the microbes in biofertilizers are essential for restoring the soil's natural nutrient cycle and building soil organic matter. Additionally, biofertilizers do not include any chemicals that are detrimental for the living soil **(Vessay, 2003).**

Therefore, the aim of this study was to evaluate the effect of HA and some biofertilizers; EM and MA treatments on growth and productivity of chia plants.

MATERIALS AND METHODS

The current study was conducted at the Nursery of Ornamental Plants, Faculty of Agriculture, Minia University during the two successive seasons of 2022/2023 and 2023/2024. Seeds of chia plant were obtained from nursery of ornamental plants, Fac. of Agric., Minia Univ., Egypt. The layout of the investigation was a randomized complete block design in a split-plot design with three replicates. The main plots (A) included HA at 0, 3, 6 and 9 ml/L, while, the sub-plots (B) involved biofertilizers (control + three concentrations from EM or MA. These concentrations were prepared using 250, 500, and 750 ml from the stock culture of these microorganisms and complete that volume to 1 L. Effective microorganisms and MA each with 1 ml containing 10^{-7} bacterial cells were sourced from the Biofertilizers Laboratory in the Dept. of Gene., Fac. of Agric., Minia Univ.

Therefore, the study including 28 interaction treatments (A × B).

The seeds of chia plants were sown on 21st Nov. of both seasons. Seeds were directly sown in hills at the rate of 7 seeds/hill with 30 cm between hills (Thinned to 2 plants/hill, after complete germination) and 70 cm between rows. The main plot was 6 × 14.70 m^2 each included 3 rows for each treatment. Plants received three foliar sprays of HA concentrations while the control treatment treated with top water. The primary treatment was applied after 43 days from sowing date, them 30 days intervals, in the two growing seasons.

Effective microorganisms or MA was applied to the soil next to the plants at a concentration of 50 ml three times The first application of biofertilizers occurred 45 days after sowing, followed by additional applications at monthly intervals during both growing seasons, each time followed by immediate irrigation.

Table (a): The physical and chemical analysis of the soil used during both seasons (2022/2023 and 2023/2024)

Soil character	Value	
	First season (2022/2023)	**Second season (2023/2024)**
Sand %	28.30	28.75
Silt %	30.50	30.00
Clay %	41.20	41.25
Soil type	Clay loam	Clay loam
Organic matter %	1.74	1.64
Ca Co_3 %	2.07	2.11
pH (1 : 2.5)	7.81	7.77
E. C. (m. mhos / cm)	1.05	1.07
Total N (%)	0.09	0.07
Available P (%)	15.20	15.46
Exch. K (mg. / 100 g.)	2.12	2.26
Exch. Ca (mg. / 100 g.)	31.60	31.40
Exch. Na (mg. / 100 g.)	2.37	2.50
DTPA Ext		
Fe (ppm)	8.46	8.39
Zn (ppm)	2.78	2.86
Mn (ppm)	8.29	8.19
Cu (ppm)	2.05	2.03

Harvesting time:

The plants reached full physiological maturity (complete senescence) by the last week of April in each growing season.

Data recorded:

Vegetative growth parameters: plant height, herb dry weight per plant, stem diameter, and number of main branch per plant.

Yield characteristics. Number of inflorescences/plant and seed yield/plant, seed yield/fed.

Fixed oil productivity: Fixed oil percentage, fixed oil yield (g/plant and kg/fed.)

Fixed oil determination:

Fixed oil content was determined according to (**A.O.A.C, 2000)** using a Soxhlet apparatus, fixed oil was extracted from 2.0 g of each seed sample, four samples were placed individually in the extraction chamber after being coarsely ground, and 250 ml of (Hexane) was added as the solvent. The extraction process lasted for a full day. Next, the fixed oil percentage and oil yield were calculated.

Chemical analysis:

Photosynthetic pigments content:

Three photosynthetic pigments were obtained from fresh leaves samples three weeks after the final treatment in both seasons. Sample weight of 0.5 g of the fresh leaves was taken and extracted by methanol alcohol according to the methods of **Moran (1982**). Then the absorbance was estimated using the Spectrophotometer at wavelength of 656, 665 and 452.5 nm, respectively, the concentration of three photosynthetic pigments were according to the following.

Chl. a mg/g = [(16.5 × E 665) - (8.3 × E 656)] * 0.5* 10/1000

Chl. b mg/g = [(33.3 × E 656) - (12.5 × E 665) *0.5*10/1000

Carot. = [(14.2 × E 452.5) - 0.264 × chl. - A- 0.496 CHL. B)] * 0.5* 10/1000

E = optical density at the given wavelength.

Statistical analysis:

Data obtained were statistically analyzed using **MSTAT-C (1986)** analytical software and the means were compared using a least significant difference **(L.S.D.)** test based on **Gomez and Gomez (1984).**

RESULTS

1- Vegetative characteristics:

Data in Table (1) showed that HA at three tested levels significantly stimulated plant height, stem diameter, number of branch/plant and herb dry weight/plant in comparison with check treatment in the two seasons. The highest values were produced as a result of adding of HA at high rate (9 ml/L). The improvement role of HA was emphasized by **Abd El-Raouf** and **Ashour (2023), Abou-El-Ghait *et al*. (2021)** and **Mohamed** and **Ghatas (2020)** on chia plants, **Tina *et al*. (2015)** and **El-Sayed *et al*. (2015)** on basil plants, **Sajadi *et al*. (2015)** on *Menthe piperita*, **Ali (2013)** on calendula plants, **Abdou *et al*. (2009 a)** borage plants, **Abd El-Satar (2020)** and **Said Al Ahl (2015)** on dill plants.

As for biofertilizers concentration rates, results listed in Table (1) clear that all treatments of EM or MA significantly increased the above-mentioned parameters. The EM treatments at 50% followed by MA at 25% were the most effective concentrations in this regard, in both seasons compared with non-treated plants. Biofertilization efficacy outcomes on vegetative growth parameters are in a similar pattern with those reported by **Ali (2013)** on calendula plants, **Abdou *et al*. (2009a)** on borage plants and **Mohamed (2009)** on guar plants. The beneficial effects of MA on vegetative growth parameters were cleared by **Abd El-Kader** and **Ghaly (2003), Abo-Aly** and **Gomaa (2002)** on coriander plants and **Safwat** and **Badran (2002)** on cumin plants.

There were significant interaction between HA and biofertilizers for vegetative growth characters in the two seasons. The highest values of plant height were obtained due to the supplying of HA at 9 ml/L in combination with EM at 50% or MA at 25%, in both seasons. Also, the better interaction treatment for stem diameter was HA at 9 ml/L plus EM 50%. Moreover, the superior interaction treatment overall were

HA at 9 ml/L plus EM 50% for total number of main branches/plant.

HA= Humic acid, EM = Effective microorganisms, MA= Minia Azotein

2-Yield characteristics:

Table (2) cleared that yield characteristics (number of inflorescences/plant and seeds yield) were increased by all concentration treatments of HA against the control one, in the two seasons. Ascending increased in previous two characters with the increase in HA levels. So, the highest number of inflorescences/plant (43.34 and 41.67) in the two seasons, respectively and the heaviest seed yield/fed. (526.07 kg and 600.36 kg) in both seasons, respectively were achieved following the application of the highest concentration of HA.

The best performance metrics compared to the control were obtained at the highest HA concentration (9 ml/L). These results align with the well-documented growth-promoting and yield-enhancing effects of HA on plant be productivity, as demonstrated by multiple studies including **Abd El-Raouf** and **Ashour (2023)** and **Mohamed** and **Ghatas (2020)** on chia plants, **Mohamed *et al*. (2022)** and **Sharaf El-Deen et al., (2012)** on fennel plants, **Ayyat *et al*. (2021)** on black cumin plants, **Beyzi *et al*. (2017)** on *Coriander sativa* plants and **Said Al Ahl *et al*. (2015)** on dill plants.

Regarding the effect of biofertilizer treatments, in both seasons, the number of inflorescences, seed yield per plant, andseed yield per fed. were all significantly obtained by all applied treatments of EM or MA. As indicated in Table (2), the MA treatment at 25% produced the heaviest seed yield/plant and/fed., (555.73 and 539.16 kg seed yield per fed in the two seasons, respectively), while the 50% EM concentration produced the highest number of inflorescences (44.30 and 42.42 for both seasons respectively).

Ahmed *et al*. (2024) and **Ahmed *et al*. (2019)** on chia plants**, El-Mekawy** and **Ali (2005)** on *Nigella sativa*, **Abdou *et al*. (2012c)** on fennel, **Badran *et al*. (2007)** on cumin, **Mathatora *et al*. (2006)** on coriander, and **El-Khayat** and **Zaghloul (1999)** on *Carum carvi* plants were all in agreement with these results.

During both growing seasons, the number of inflorescences, seed production per plant, and seed yield per feddan were all significantly increased by the interaction between the two parameters. Additionally, as shown in Table (2), plants fertilized with a high concentration of HA at 9 ml/L along with 25% MA produced the highest seed yield per plant and per feddan, while plants treated with 9 ml/L of HA combined with 50% EM produced the greatest number of inflorescences

Table (1): Effect of humic acid, some biofertilizers on some vegetative growth parameters of chia plants in the two growing seasons (2022/2023 and 2023/2024)

Biofertilizers concentrations (B)	The 1st season (2022/2023)					The 2nd season (2023/2024				
	Plant height (cm)									
	Humic acid ml/l (A)									
	0.0	3	6	9	**Mean (B)**	0.0	3	6	9	**Mean (B)**
Control	70.50	73.00	80.00	85.00	**77.10**	73.50	74.50	82.50	83.50	**78.90**
EM at 25%	79.00	81.50	85.00	87.00	**83.12**	85.50	89.60	92.30	93.30	**90.17**
EM at 50%	87.00	88.00	90.50	92.00	**84.37**	90.00	93.50	96.00	101.50	**95.25**
EM at 75%	75.00	82.50	85.50	89.00	**83.00**	82.60	90.50	91.35	92.50	**89.23**
MA at 25%	76.00	78.00	80.00	81.00	**78.80**	86.60	91.50	94.00	95.00	**91.77**
MA at 50%	74.00	77.00	78.00	79.00	**77.00**	82.70	89.33	91.50	94.00	**89.38**
MA at 75%	73.00	75.00	76.00	77.00	**75.30**	67.70	84.70	89.50	93.00	**83.72**
Mean (A)	**76.35**	**79.28**	**82.14**	**84.28**		**81.22**	**87.66**	**91.02**	**93.25**	
L.S.D at 5%	**A: 0.77**		**B: 2.03**		**AB: 4.06**	**A: 1.375**		**B: 1.30**		**AB: 2.60**
	Stem diameter (mm)									
Control	0.40	0.45	0.50	0.55	**0.47**	0.40	0.45	0.50	0.55	**0.47**
EM at 25%	0.45	0.50	0.55	0.60	**0.52**	0.60	0.60	0.65	0.70	**0.63**
EM at 50%	0.50	0.70	0.80	0.90	**0.72**	0.65	0.70	0.70	0.80	**0.71**
EM at 75%	0.40	0.50	0.60	0.70	**0.55**	0.40	0.50	0.60	0.70	**0.55**
MA at 25%	0.45	0.50	0.55	0.60	**0.52**	0.60	0.70	0.75	0.80	**0.71**
MA at 50%	0.40	0.45	0.50	0.55	**0.47**	0.55	0.60	0.65	0.70	**0.62**
MA at 75%	0.40	0.40	0.45	0.50	**0.43**	0.40	0.50	0.55	0.65	**0.52**
Mean (A)	**0.42**	**0.50**	**0.56**	**0.62**		**0.51**	**0.57**	**0.62**	**0.70**	
L.S.D at 5%	**A: 0.03**		**B: 0.04**		**AB: 0.07**	**A: 0.02**		**B: 0.03**		**AB: 0.05**
	Number of branches/plant									
Control	11.0	11.5	12.0	13.0	**11.90**	8.0	9.0	11.0	12.0	**10.00**
EM at 25%	12.0	13.0	13.5	14.0	**13.10**	13.0	13.0	14.5	15.0	**13.70**
EM at 50%	13.5	14.5	15.0	16.0	**14.80**	13.0	14.0	14.5	15.0	**14.30**
EM at 75%	11.0	13.0	14.0	15.0	**13.30**	12.0	12.5	13.0	14.0	**12.80**
MA at 25%	12.5	13.0	14.0	14.5	**13.50**	11.0	13.0	14.0	15.0	**13.15**
MA at 50%	12.5	13.0	13.5	14.5	**13.37**	10.5	13.0	13.5	14.0	**12.65**
MA at 75%	12.0	12.5	13.5	14.0	**13.00**	10.0	11.5	13.0	13.0	**12.00**
Mean (A)	**12.07**	**12.92**	**13.64**	**14.42**		**11.07**	**12.28**	**13.35**	**14.00**	
L.S.D at 5%	**A: 0.05**		**B: 0.67**		**AB: 1.34**	**A: 0.55**		**B: 0.83**		**AB: 1.67**
	Herb dry weight/plant (g)									
Control	20.14	22.69	25.14	27.68	**23.91**	20.16	20.18	22.71	25.24	**22.07**
EM at 25%	30.25	35.26	40.28	45.32	**37.77**	37.68	40.20	42.78	45.38	**41.51**
EM at 50%	37.93	45.35	47.91	50.44	**45.40**	40.32	40.46	50.53	57.99	**47.32**
EM at 75%	30.47	32.75	37.99	45.35	**36.64**	37.91	40.41	45.43	45.52	**42.31**
MA at 25%	60.47	65.61	70.59	85.63	**70.57**	60.48	60.50	65.52	70.58	**64.27**
MA at 50%	35.35	37.85	45.40	75.33	**48.48**	55.52	55.54	63.06	68.06	**60.54**
MA at 75%	30.21	32.81	40.34	60.33	**40.92**	50.55	53.01	58.08	65.53	**56.79**
Mean (A)	**34.97**	**38.90**	**43.95**	**55.72**		**43.23**	**44.32**	**49.73**	**54.04**	
L.S.D at 5%	**A: 6.82**		**B: 4.97**		**AB: 9.94**	**A: 5.22**		**B: 6.35**		**AB: 12.69**

Table (2): Effect of humic acid and some biofertilizers on some yield characteristic of chia plants in the two growing seasons (2022/2023 and 2023/2024)

Biofertilizers concentrations (B)	The 1st season (2022/2023)					The 2nd season (2023/2024)				
	Number of inflorescences/plant									
	Humic acid ml/l (A)									
	0.0	3	6	9	**Mean (B)**	0.0	3	6	9	**Mean (B)**
Control	33.04	34.51	36.00	39.12	**35.66**	24.03	27.05	33.04	36.08	**30.05**
EM at 25%	36.06	39.09	40.59	42.10	**39.46**	37.57	39.06	43.08	43.51	**40.80**
EM at 50%	40.60	43.51	45.05	48.06	**44.30**	39.06	42.08	43.54	45.03	**42.42**
EM at 75%	33.03	39.07	42.09	45.03	**39.80**	36.92	37.57	40.53	42.01	**39.25**
MA at 25%	37.60	39.06	42.00	43.51	**40.54**	33.07	39.07	42.05	44.04	**39.55**
MA at 50%	37.22	39.04	40.54	43.50	**40.07**	31.57	37.54	40.71	42.04	**37.96**
MA at 75%	36.18	37.55	40.21	42.10	**39.01**	30.10	34.55	39.05	39.05	**35.68**
Mean (A)	**36.24**	**38.83**	**40.92**	**43.34**		**33.18**	**36.70**	**40.28**	**41.67**	
L.S.D at 5%	**A: 1.34**	**B: 2.003**		**AB: 4.005**		**A: 1.84**	**B: 2.27**		**AB: 4.53**	
	Seed yield/plant (g)									
Control	13.21	14.35	14.97	15.43	**14.49**	13.64	14.19	15.21	15.76	**14.70**
EM at 25%	15.19	16.23	16.75	18.15	**16.58**	14.23	15.75	16.81	17.36	**16.03**
EM at 50%	15.24	16.23	17.46	18.55	**16.87**	15.28	16.34	16.86	17.89	**16.59**
EM at 75%	15.47	16.04	16.59	17.30	**16.35**	14.82	15.88	15.95	17.44	**16.02**
MA at 25%	17.45	18.04	18.28	19.35	**18.28**	16.42	16.98	18.00	19.54	**17.73**
MA at 50%	15.19	15.37	16.26	17.38	**16.05**	14.87	15.42	15.97	16.51	**15.69**
MA at 75%	14.32	15.03	15.32	15.79	**15.11**	14.32	15.37	15.42	15.86	**15.24**
Mean (A)	**15.25**	**15.89**	**16.51**	**17.42**		**14.79**	**15.70**	**16.31**	**17.19**	
L.S.D at 5%	**A: 0.16**	**B: 0.53**		**AB: 1.064**		**A: 0.67**	**B: 0.78**		**AB: 1.57**	
	Seed yield/fed. (kg)									
Control	401.68	436.23	455.08	469.07	**440.51**	414.93	431.37	462.48	478.59	**446.84**
EM at 25%	462.38	493.36	539.60	526.52	**505.46**	432.69	478.89	511.12	527.74	**487.61**
EM at 50%	463.29	493.39	530.78	563.92	**512.79**	464.61	496.73	512.54	543.95	**504.45**
EM at 75%	470.28	487.61	504.43	526.04	**497.09**	450.62	482.85	484.88	530.17	**487.13**
MA at 25%	530.48	548.41	555.71	588.42	**555.73**	499.26	516.19	547.20	594.01	**539.16**
MA at 50%	460.77	467.24	454.30	528.45	**487.94**	452.14	468.86	485.58	502.01	**477.14**
MA at 75%	435.32	457.01	465.62	480.11	**459.51**	435.42	467.34	468.86	482.14	**463.44**
Mean (A)	**460.60**	**483.32**	**500.78**	**526.07**		**449.95**	**477.46**	**496.09**	**600.36**	
L.S.D at 5%	**A: 7.513**	**B: 20.89**		**AB: 41.78**		**A: 20.23**	**B: 23.83**		**AB: 47.65**	

HA= Humic acid, EM = Effective microorganisms, MA= Minia Azotein

3-Fixed oil productivity:

The fixed oil parameters including oil %, oil yield (g/plant) and (kg/fed.) for all spraying treatments during two grown seasons are shown in Table (3). Available results show significant influence of HA treatments on the investigated traits. Humic acid at 9 ml/L was superior to other used treatments as it gave the highest values (33.68%, 6.73 g/plant and 204.80 kg/fed.) for the these traits respectively in the first season, and 37.04% , 7.40 g/plant and 225.21 kg/fed. in the second one respectively.

Similar results has been observed by **Abd El-Raouf** and **Ashour (2023)** and **Abou El-Ghait *et al.* (2021)** on salvia plants, **Sajadi *et al.* (2015)** on *Mentha piperita* plants, **El-Sayed *et al.* (2015)** and **Befrozfar *et al.* (2013)** on *Ocimum basillicum* plants, **El-Shayeb *et al.* (2015)** on *Salvia officinallis* plants, **Beyzi *et al.* (2017)** on coriander plants, **Said Al Ahl *et al.* (2015)** on dill plants, **Sharaf El-Deen *et al.* (2012)** on fennel plants and **Ibrahim** and **Helaly (2017)** on fenugreek plants.

Data presented in Table (3) showed that supplying chia plants with biofertilizers (EM or MA) significantly enhanced the percentage of essential oils, soil treatment of MA at 25% or EM 50% in both seasons resulted in the highest oil yield/plant and oil yield/fed chia plants. The role of EM on increasing fixed oil percentage, oil yield/plant and/fed. were found by **Hellal *et al.* (2011)** on dill plants, **Zand *et al.* (2013)** on anise plants, and **Hamed (2004)** on *Salvia officinalis* and *Origarnum syriacum* plants. While the role of MA in promoting fixed oil was stated by **Jafari *et al.* (2015)** on *Salvia officinalis* plants, **Abd El-Naeem (2008)** and **Al-Shareif (2006)** on caraway plants, **Badran *et al.* (2003)** on anise plants, **Abdou *et al.* (2004)** and **Ibrahim (2000)** on fennel plants and **Harridy *et al.* (2001)** on lemongrass plants.

The interaction between the two factors was significant for fixed oil yield, with the highest values obtained from 9 ml/L of HA combined with 25% MA, followed by 50% EM, in both seasons.

4-Photosynthetic pigments (mg/g FW):

Table (4) shows that chlorophyll a, b and carotenoids content were increased as a result of using HA in two experimental seasons. The high level resulted significantly the highest chl. a, b and carotenoids contents over other treatments including control. These results are similar to those obtained by **Abd El-Raouf** and **Ashour (2023), Abou El-Ghait *et al.* (2021)** and **Mohamed** and **Ghatas (2020)** on chia plants, **Abou El-Salehein *et al.* (2021)** on basil plants, **Shahin *et al.* (2014)** on merrmia plants and **Tina *et al.* (2015)** and **Azzaz *et al.* (2007)** on *Calendula officinalis* plants.

Table (4) shows that the photosynthetic pigments; chlorophyll a, b and carotenoids contents in the fresh leaves of chia plants were favorably and significantly affected by fertilizing the plants with EM or MA in the first season and second one. The best contents of the three pigments were recorded on plants received 25% of MA followed by 75% EM treatments while the three treatments of EM (25, 50 and 75%) or MA (25, 50 and 75%) separately increased the chlorophylls and carotenoids comparing with control plants.

Ibrahim (2018) on cumin plants, **Fouad (2017)** and **Abdou *et al.* (2009a)** on borage plants, **Abdou *et al.* (2009b)** and **Abdou *et al.* (2012)** on guar plants, **Patil (2010)** on *Sativa rebandiana*, and **Ibrahim (2014)** on khilla plants additionally reported the improvement in the production of photosynthetic pigments brought about by the EM obtained in this study.

Many investigators explored the beneficial influence of MA on photosynthetic pigments for examples **Ahmed (2007)** on black cumin plants, **Badran *et al.* (2007)** on cumin plants, **Soliman (2002)** and **Gomaa** and **Abo-Aly (2001)** on anise plants, , **Kandeel (2004)** on basil plants, **Samuel (2008)** and **Abdou *et al.* (2004)** on fennel plants and **Al-Shreif (2006)** on caraway plants

Table (4) clearly demonstrates a significant interaction between the studied factors (HA and biofertilizers) on three photosynthetic pigments content across the two seasons. As compared to all other combination treatments in both seasons, applying HA at 9 ml/L in addition to MA at 25% and then HA at 9 ml/L along with EM at 75% produced the highest levels of chlorophyll a, chlorophyll b and carotenoid contents.

Table (3): Effect of humic acid and some biofertilizers on fixed oil productivity of chia plants in the two growing seasons (2022/2023 and 2023/2024)

Biofertilizers concentrations (B)	The 1st season (2022/2023)					The 2nd season (2023/2024)				
	Fixed oil (%)									
	Humic acid ml/l (A)									
	0.0	3	6	9	**Mean (B)**	0.0	3	6	9	**Mean (B)**
Control	21.00	21.10	24.20	26.25	**23.13**	31.00	35.15	36.15	36.25	**34.63**
EM at 25%	28.25	30.45	36.20	36.40	**32.82**	35.15	36.70	36.95	37.30	**36.52**
EM at 50%	27.25	28.95	30.65	33.50	**30.08**	34.90	35.90	36.40	36.70	**35.97**
EM at 75%	27.00	28.85	29.55	33.45	**29.71**	34.40	35.05	35.55	35.65	**35.16**
MA at 25%	33.80	36.00	36.35	36.65	**35.70**	35.80	36.40	37.75	38.50	**37.11**
MA at 50%	24.10	28.60	29.20	36.00	**29.47**	34.70	36.00	37.10	37.60	**36.35**
MA at 75%	23.10	28.50	26.80	33.55	**27.98**	31.90	32.70	36.55	37.30	**34.61**
Mean (A)	**26.35**	**28.92**	**30.42**	**33.68**		**33.97**	**35.41**	**36.63**	**37.04**	
L.S.D at 5%	**A: 1.25**	**B: 2.55**	**AB: 5.10**			**A: 0.9**	**B: 1.10**	**AB: 2.19**		
	Oil yield/plant (g)									
Control	4.20	4.22	4.84	5.25	**4.62**	6.20	7.03	7.23	7.25	**6.92**
EM at 25%	5.65	6.09	7.24	7.28	**6.56**	7.03	7.34	7.39	7.46	**7.30**
EM at 50%	5.45	5.79	6.13	6.70	**6.01**	6.98	7.18	7.28	7.34	**7.19**
EM at 75%	5.40	5.77	5.91	6.69	**5.94**	6.88	7.01	7.11	7.13	**7.03**
MA at 25%	6.76	7.20	7.27	7.33	**7.14**	7.16	7.28	7.55	7.70	**7.42**
MA at 50%	4.82	5.72	5.84	7.20	**5.89**	6.94	7.20	7.42	7.52	**7.27**
MA at 75%	4.62	5.70	5.36	6.71	**5.59**	6.38	6.54	7.31	7.46	**6.92**
Mean (A)	**5.27**	**5.78**	**6.08**	**6.73**		**6.79**	**7.08**	**7.32**	**7.40**	
L.S.D at 5%	**A: 0.30**	**B: 0.51**	**AB: 1.01**			**A :0.17**	**B: 0.22**	**AB: 0.44**		
	Oil yield/fed. (kg)									
Control	127.67	128.28	147.13	159.59	**140.66**	188.47	198.36	209.65	220.39	**204.21**
EM at 25%	171.75	185.13	220.09	221.30	**199.56**	213.70	223.13	224.65	226.78	**222.06**
EM at 50%	165.67	176.01	186.34	203.67	**182.90**	212.18	218.26	221.30	223.13	**218.71**
EM at 75%	164.15	175.40	172.36	203.37	**178.82**	209.14	213.10	216.14	216.74	**213.78**
MA at 25%	205.50	218.87	221.01	222.82	**217.05**	217.66	221.30	229.51	234.07	**225.63**
MA at 50%	146.52	173.88	177.53	218.87	**179.02**	210.97	218.87	225.56	228.60	**221.00**
MA at 75%	140.44	173.27	162.94	203.98	**170.15**	193.94	198.81	222.22	226.78	**210.43**
Mean (A)	**160.24**	**152.97**	**183.91**	**204.80**		**206.58**	**213.11**	**221.29**	**225.21**	
L.S.D at 5%	**A: 9.18**	**B: 15.48**	**AB: 30.96**			**A: 7.65**	**B: 7.84**	**AB: 15.68**		

HA= Humic acid, EM = Effective microorganisms, MA= Minia Azotein

Table (4): Effect of humic acid and some, biofertilizers on three photosynthesis pigments (chlorophyll a, b and carotenoids) contents of chia plants in the two growing seasons (2022/2023 and 2023/2024)

Biofertilizers concentrations (B)	The 1st season (2022/2023)					The 2nd season (2023/2024				
	Chlorophyll a									
	Humic acid ml/l (A)									
	0.0	3	6	9	**Mean (B)**	0.0	3	6	9	**Mean (B)**
Control	2.069	2.450	2.464	2.546	**2.382**	2.225	2.400	2.402	2.437	**2.366**
EM at 25%	2.756	2.307	2.362	2.696	**2.530**	3.224	3.332	3.562	3.580	**3.424**
EM at 50%	3.307	3.328	3.503	3.760	**3.474**	3.400	3.452	3.585	3.542	**3.494**
EM at 75%	3.456	3.481	3.503	3.653	**3.523**	3.401	3.592	3.563	3.670	**3.581**
MA at 25%	3.542	3.592	3.612	3.903	**3.662**	3.675	3.685	3.863	3.890	**3.778**
MA at 50%	3.448	3.477	3.492	3.492	**3.477**	3.532	3.653	3.752	3.886	**3.705**
MA at 75%	3.413	3.425	3.429	3.448	**3.429**	3.338	3.347	3.434	3.738	**3.464**
Mean (A)	**3.141**	**3.151**	**3.195**	**3.356**		**3.256**	**3.351**	**3.451**	**3.534**	
L.S.D at 5%	**A: 0.126**	**B: 0.149**		**AB: 0.298**		**A: 0.204**	**B: 0.211**		**AB: 0.422**	
	Chlorophyll b (mg/g FW)									
Control	1.070	1.148	1.252	1.311	**1.195**	1.077	1.111	1.311	1.336	**1.209**
EM at 25%	1.130	1.314	1.338	1.395	**1.294**	1.203	1.357	1.508	1.583	**1.413**
EM at 50%	1.299	1.384	1.492	1.555	**1.432**	1.467	1.535	1.564	1.727	**1.573**
EM at 75%	1.343	1.455	1.502	1.559	**1.465**	1.547	1.594	1.647	1.791	**1.645**
MA at 25%	1.468	1.503	1.584	1.751	**1.576**	1.387	1.549	1.765	1.925	**1.656**
MA at 50%	1.179	1.219	1.320	1.446	**1.291**	1.125	1.355	1.607	1.648	**1.433**
MA at 75%	1.002	1.148	1.312	1.435	**1.224**	1.104	1.241	1.557	1.631	**1.383**
Mean (A)	**1.213**	**1.310**	**1.400**	**1.493**		**1.273**	**1.391**	**1.565**	**1.663**	
L.S.D at 5%	**A: 0.104**	**B: 0.138**		**AB: 0.275**		**A: 0.115**	**B: 0.125**		**AB: 0.249**	
	Carotenoids (mg/g FW)									
Control	1.079	1.130	1.191	1.505	**1.226**	1.226	1.267	1.369	1.435	**1.324**
EM at 25%	1.082	1.238	1.316	1.520	**1.289**	1.280	1.354	1.458	1.567	**1.415**
EM at 50%	1.126	1.327	1.430	1.525	**1.352**	1.289	1.418	1.454	1.598	**1.440**
EM at 75%	1.217	1.425	1.522	1.605	**1.442**	1.313	1.401	1.503	1.732	**1.487**
MA at 25%	1.303	1.428	1.522	1.558	**1.453**	1.460	1.559	1.641	1.655	**1.579**
MA at 50%	1.177	1.336	1.507	1.529	**1.387**	1.305	1.418	1.441	1.619	**1.446**
MA at 75%	1.130	1.270	1.391	1.426	**1.304**	1.148	1.298	1.367	1.545	**1.339**
Mean (A)	**1.159**	**1.307**	**1.415**	**1.524**		**1.289**	**1.388**	**1.462**	**1.593**	
L.S.D at 5%	**A: 0.098**	**B: 0.089**		**AB: 0.18**		**A: 0.175**	**B: 0.174**		**AB: 0.348**	

DISCUSSION

The improvement effects of HA recorded in this study may lead to the physiological and biological effects as well as functions of humic acid, which were explained by many researchers as follows.

By improving the uptake of essential major and trace elements, activating enzymes, changing membrane permeability, promoting protein synthesis, and encouraging biomass development, humic acid aids in the growth of plants **(Ulukan, 2008).**

Humic acid is used specifically to reduce or counteract the negative effects of certain chemical compounds in the soil and mineral fertilizers. As a result, humic substances have a beneficial effect on soil quality and encourage plant growth while demonstrating strong hormonal activity. According to **Brunetti *et al*. (2005)**, these compounds increase the levels of micronutrients in plant tissues in addition to increasing the content of macronutrients and ion absorption. By making plant cell membranes more permeable, they enhance the uptake of nutrients. Humic acid can also help the soil absorb nitrogen while promoting the uptake of calcium, magnesium, phosphorus, and potassium, increasing the mobility and accessibility of these nutrients for plant roots.

Humic matter has been questioned for its hormone-like activities in favor of significant effects on the plasma membrane H^+-ATPase **(Varanine** and **Pinüon, 2001)** or increased availability of microelements (Fe and Zn) **(Clap *et al*., 2001)**.

It has been shown to improve the uptake of mineral nutrients by plants through modifying the permeability of root membranes. Moreover, humic acid treatments were found to partially reduce the negative consequences of high Na in saline soil conditions. **(Türkmen *et al*. 2005, Asik *et al*. 2009,** and **Cimrin *et al*. 2010)**. This may be because the administration of humic acid has increased root growth, changed mineral uptake, and decreased membrane damage, there by developing salt tolerance in plants.

Finally, humic acids are a commercial product that contains a lot of nutrients. This increased the nutrients phyto-avalibility, which in response affected the production and growth of plants.

Effect of EM biofertilizer:

EM operates in physiological and biological roles in improving vegetative growth characteristics, yield and yield components, essential oil productivity, and some chemical compositions of chia plants, the results obtained in this study demonstrate the positive and advantageous effects of biofertilizers as EM.

Effective microorganism includes beneficial organisms, such as photosynthetic bacteria, lactic acid bacteria, yeasts and additional types; it is recently introduced in many countries to organic farming system. It reduces the amount of agro-chemicals. **Ho** and **Hwan (2000)** is EM are reported to in enhance soil properties and promote yield of fruits, as well as, augmenting tolerance.

Multiple studies have demonstrated the efficacy of EM enhancing plant growth, yield, oil and biochemical composition in various plants. For instance, research on coriander plants by **Abdalla (2009)**, **El-Houssini (2009)** on borage plants, **Ashour (2010)** on jojoba, **Helmy (2008)** on *Nigella sativa* plants, **Ali (2013)** on *Calendula officinalis*, plants and **Abd El-Salam (2015)** on coriander plants.

Effect of Minia Azotein:

The addition of MA a fertilizer containing various strains of nitrogen-fixing bacteria on chia plants in this study demonstrated significant improvements in vegetative growth, yield components, fixed oil content and chemical constituents. Many authors found that the effect of N-fixing bacteria causes an increase of available N by fixing the atmospheric N, resulting in increased biosynthesis of growth-stimulating metabolites that enhance vegetative development and enhancing Meristematic activities in cells and tissues. Additionally, these bacteria produce phytohormones such as Indole acetic acid, gibberellins and cytokinins, which expand root surface area, encourage root hair branching, and ultimately improve N availability. (**Sperenat, 1990, El-Hadded *et al*. 1993** and **El-Mandoh** and **Abd El-Magid, 1996**). The phytohormones released by N- fixing bacteria further enhance nutrient absorption, photosynthesis and overall plant growth (**Reynders** and **Vlassak, 1982, Fayez *et al*., 1985, Mostafa** and **Omar, 1993, El-Hadded *et al*., 1993** and **Hegde *et al*., 1999**).

Nitrogen-fixing bacteria strains produce same amino acids, such as aspartate, glutamate, serine **(Hartmann, 1989)** they enhance the plants water status, stimulate nitrate reductase activity, and produce siderophores that are improve the plants iron nutrition (**Hegde *et al*., 1999**) and produce antibacterial and antifungal substances (**Sorial *et al*., 1992**). Also, **Haweka (2000)** discovered that nitrogen-fixing bacteria normally benefit their host plants in a number of ways, such as fixing nitrogen, producing organic acids or growth-promoting compounds, enhancing nutrient absorption, and defense of regarding plant diseases.

CONCLUSION

The obtained results showed that spraying chia plants treated with HA and EM and MA to the soil significantly enhanced vegetative development and yield parameters, oil yield and photosynthetic pigments content compared to untreated plants. The greatest values of all studied characteristics were obtained by HA at 9 ml/L combined with biofertilizers (EM at 50% or MA at 25%). Therefore, we recommended that spraying chia plants with 9 ml/L and 25% MA could be used for obtaining higher yield and quantity of fixed oil.

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الملخص العربي

استجابة نباتات الشيا لحمض الهيومك وبعض الأسمدة الحيوية

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أجريت هذه التجربة خلال موسمين 2022\ 2023 و 2023\ 2024 في مزرعة كلية الزراعة – جامعة المنيا . بهدف دراسة استجابة نباتات الشيا لحمض الهيومك وبعض معاملات الأسمدة الحيوية وتأثيرها علي صفات النمو الخضري ومحصول البذور والزيت الثابت وكذلك المحتوي الكيميائى.

وفي هذا الخصوص تم دراسة عاملين. العامل الأول (أ) وهو حمض الهيومك بمعدلات 3-6 و 9مل/لتر والعامل الثاني (ب) كان السماد الحيوي (الميكروبات الدقيقة النشطة أو المنيا ازوتين) بالإضافة الي معاملة الكنترول . كما تم اختبار التداخل بين العاملين أ ×ب

أدت معاملات التسميد بحمض الهيومك (الثلاث مستويات) بالمقارنة بمعاملة الكنترول إلي زيادة معنوية في ارتفاع النبات – عدد الفروع – محصول البذرة للنبات وللفدان والنسبة المئويه للزيت الثابت ومحصول الزيت الثابت للنبات وللفدان بالإضافة الي صبغات البناء الضوئى الثلاث . وبصفة عامه تفوق المستوي العالي من حمض الهيومك لكل الصفات. كما تم الحصول علي نفس النتائج مع معاملات التسميد الحيوي اي ام او منيا ازوتين بالتركيزات المختلفة للصفات المدروسة . وكان أفضل تركيز هو اي ام 50 % او منيا ازوتين 25 % وكانت افضل معاملة تداخل للحصول علي اعلي انتاجية لنبات الشيا هي المعاملة لحمض الهيومك 9 مل/لتر متحدة مع اي ام بتركيز 50 % او منيا ازوتين 25 % .