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Impact Of Fulvic Acid and Stimulant Substances on Growth and Productivity of Roselle (*Hibiscus Sabdariffa* L.) Plant

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ABSTRACT

A field experiment was conducted during the 2023 and 2024 seasons at the Experimental Farm, Faculty of Agriculture, Al-Azhar University, Assiut, Egypt (27°12'16.67" N, 31°09'36.86" E), to investigate the effects of fulvic acid (FA) application methods and stimulant substances on roselle (*Hibiscus sabdariffa* L. cv. Sabhia 17, dark line) growth, yield, and chemical composition. FA treatments included soil addition (1 and 2 g/l water), foliar spray (500 and 1000 ppm), and combined applications (1 g/l soil addition + 500 ppm foliar spray and 2 g/l soil addition + 1000 ppm foliar spray). Stimulant substances comprised chitosan (250 and 500 ppm), vitamin E (50 and 100 ppm), garlic extract (10 and 20 ml/l water), and a control (no stimulant substances). The combined treatment of 2 g/l FA soil addition with 1000 ppm foliar spray was the most effective in enhancing vegetative growth (branch number, herb dry weight), yield components (sepal and seed yields), and chemical constituents (fixed oil production, anthocyanin and acidity), outperforming the control, which showed the lowest values. Foliar application of chitosan, vitamin E, and garlic extract at all concentrations also significantly improved these parameters, with garlic extract at 20 ml/l yielding the highest results. The interaction of 2 g/l FA soil addition + 1000 ppm foliar spray with 20 ml/l garlic extract was the most effective, highlighting the potential of FA and garlic extract as sustainable, eco-friendly practices for enhancing the productivity of roselle plants. **Conclusion**: The combined treatment of 2 g/l FA soil addition with 1000 ppm foliar spray was the most effective in enhancing vegetative growth (branch number, herb dry weight), yield components (sepal and seed yields), and chemical constituents (fixed oil production, anthocyanin and acidity).

Keywords: *Hibiscus sabdariffa* L.; Fulvic acid; Chitosan; Vitamin E; Garlic extract; Anthocyanin.

INTRODUCTION

Medicinal and aromatic plants, valued for their bioactive compounds, are influenced by synthetic fertilizers, with productivity measured by biomass and phytochemical content. *Hibiscus sabdariffa*, a significant herb from the Malvaceae family, is an annual shrub with a deep taproot, upright stems, and green-to-red, serrated leaves, either ovate or palmately lobed (Qi *et al*., 2005; Abou-Sreea *et al*., 2022). Its yellow flowers with a crimson center and fleshy, red-to-purple calyces encase ovoid capsules with numerous seeds (Juhi and Ela, 2014). Roselle is consumed as a vegetable, with young shoots used in salads, and its calyces, the most utilized part, processed into sauces or the traditional beverage zobo, popular in tropical regions (Ogundapo *et al*., 2014; Salami and Afolayan, 2021). Rich in phytochemicals such as anthraquinone glycosides, alkaloids, tannins, polyphenols, and anthocyanins (e.g., delphinidin-3-sambubioside), roselle exhibits antioxidant, antiseptic, diuretic, and purgative properties, aiding in the treatment of cancer, hypertension, infections, and other ailments (Ogundapo *et al*., 2014; Aganbi *et al*., 2017; Teye *et al*., 2019; Olawale, 2011). Its high ascorbic acid, calcium, iron, riboflavin, and niacin content, coupled with low sugar, enhances its nutritional value (Builders *et al*., 2010; Teye *et al*., 2019). Anthocyanins provide potent antioxidant activity, surpassing ascorbate, and contribute to protection against cancer, atherosclerosis, and liver damage, while roselle also shows hypoglycemic and hypolipidemic effects (Builders *et al*., 2010; Ellis *et al*., 2021; Sini *et al.*, 2011).

The use of organic fertilizers, particularly fulvic acid, is pivotal in the sustainable cultivation of medicinal plants, ensuring high-quality production, environmental protection, and enhanced community health (Aminifard *et al*., 2020). Fulvic acid, a key organic amendment, is derived from the microbial decomposition of organic matter, especially lignin-rich materials, during the humification process in soil (Malan, 2015; do Rosário Rosa *et al*., 2021). It is characterized by a high concentration of carboxylic groups, abundant phenolic compounds, and a relatively low presence of aromatic structures, distinguishing it from other humic substances (Canellas *et al*., 2015). As a water-soluble component of humus, fulvic acid plays a significant role in plant growth regulation, acting as a hormone-like substance that promotes vegetative development and enhances plant resilience to environmental stresses such as drought and heat (Abd El-Rheem *et al.*, 2021; Farruggia *et al*., 2024; Aytaç *et al*., 2022). Fulvic acid contributes to plant health by upregulating genes involved in the metabolic pathways of flavonoids, glutathione, and ascorbate, which are critical for mitigating oxidative stress caused by drought (Fang *et al*., 2020; Suh *et al*., 2014). These compounds enhance the plant's antioxidant capacity, protecting cellular structures and improving stress tolerance. Additionally, fulvic acid is rich in essential nutrients, which improve crop yields and also enhancing soil properties, including physical structure, chemical composition, and biological activity (Daur and Bakhashwain, 2013). By improving soil fertility and nutrient availability, fulvic acid supports robust plant growth and development. Research by Khalid *et al*. (2015) and Abd El-Rheem *et al*. (2021) on sweet fennel demonstrates that fulvic acid significantly increases fruit yield, volatile oil percentage, and oil content, underscoring its efficacy in enhancing the productivity of medicinal and aromatic plants.

Chitosan, the second most abundant natural polymer and a key plant elicitor, is produced via chitin de-acetylation and is widely used in agriculture to enhance soil fertility, mineral nutrient uptake, and biomass production, supporting sustainable practices (Pal *et al*., 2020; da Silva Lucas *et al*., 2020; Adamuchio-Oliveira *et al*., 2020; Muley *et al*., 2019). Its antiviral, antibacterial, and antifungal properties depend on its form, chemical composition, and polymerization degree (Naskar *et al.,* 2019; Shariatinia, 2019). As an elicitor, chitosan promotes the synthesis of secondary metabolites, including essential oils, phenolics, and flavonoids, enhancing plant resilience and quality. Poorghadir *et al*. (2020) found that 0.1% chitosan maximized gamma-terpinene in summer savory, while 0.5% optimized essential oil production. Ghasemi Pirbalouti *et al*. (2017) reported increased phenol content and antioxidant activity in *Ocimum ciliatum* and *O. basilicum* with varying chitosan concentrations. Vosoughi *et al*. (2018) showed that foliar chitosan improved essential oil quality, antioxidant activity, and phenolic/flavonoid levels in sage under drought stress.

Tocopherols, particularly α-tocopherol (vitamin E), are plant-synthesized antioxidants that protect against abiotic stresses like intense light, drought, salinity, and cold by neutralizing reactive oxygen species (ROS) and preventing lipid peroxidation in chloroplast thylakoid membranes (Hess, 1983; Fryer, 1992; Munné-Bosch, 2007). Located in the chloroplast envelope and stroma, vitamin E works synergistically with water-soluble antioxidants ascorbate and glutathione to enhance stress tolerance (Caretto *et al.,* 2002; Hussain *et al*., 2013; Jin and Daniell, 2014). Foliar application of vitamin E significantly improves plant growth and yield, with a six-fold fruit yield increase in Jonagold apples within 48 hours (Noga & Schmitz, 2000) and enhanced height and dry weight in cowpea under salinity stress at 200 ppm (Hussein et al., 2007). In bean plants, vitamin E at 0.1 ml/L and 0.3 ml/L boosts vegetative growth, yield, pod quality, chlorophyll, and nutrient levels (nitrogen, phosphorus, potassium), with stronger effects at higher concentrations (El-Tohamy and El-Greadly, 2007; El-Bassiouny *et al.,* 2005; Nour *et al.,* 2012). Endogenous vitamin E supports low-temperature adaptation and phloem nutrient transport (Maeda, 2006).

The application of plant extracts as biostimulants to promote growth represents a contemporary approach to enhancing crop productivity, improving plant resilience against environmental stresses, and providing natural pest control (Hanafy *et al*., 2012). Garlic extract, in particular, is rich in essential minerals and plant hormones that stimulate cell enlargement and cell division, thereby enhancing overall plant growth, fruit characteristics, and the production of essential oils in various medicinal plants. Numerous studies have investigated the efficacy of garlic extract in agriculture (Nour Eldeen, 2014; Ziedan and Eisa, 2016). Research has shown that garlic extract significantly improves growth parameters, flowering characteristics, fruit yield, and fruit quality (Mohamed and Akladious, 2014; Al-Obady, 2015). For instance, Ahmed *et al*. (2005) reported that treating pea plants (cv. Meteor) with garlic extract at a concentration of 10 g/L after inoculation led to a substantial increase in the number of pods. Additionally, Mady (2009) demonstrated that applying garlic extract at concentrations of 50% or 100% to *Majorana hortensis* and *Salvia officinalis* significantly increased fresh and dry weights and total essential oil content in both the first and second cuts.

This study aimed to investigate the impact of varying application methods of fulvic acid and foliar spraying with stimulant substances on the growth and productivity of *Hibiscus sabdariffa* L., dark line plants under the environmental conditions of Assiut, Egypt.

MATERIALS AND METHODS

This study was carried out at the Experimental Farm, Faculty of Agriculture, Al-Azhar University, Assiut, Egypt (27°12'16.67" N, 31°09'36.86" E) during the 2023 and 2024 growing seasons to evaluate the effects of different fulvic acid application methods, foliar spraying with stimulant substances (chitosan, vitamin E, and garlic extract), and their interactions on growth parameters, yield, and chemical composition of roselle (*Hibiscus sabdariffa* L. cv. Sabhia 17, dark line). Roselle seeds, sourced from the Horticulture Research Institute, Agricultural Research Center, Giza, Egypt, were sown 10th April in in the nursery during both seasons. 40 days after the sowing, seedlings were transplanted into loamy soil in plots measuring 3.2 x 1.8 m, each containing three rows (60 cm apart) with eight hills per row (40 cm apart), resulting in 24 plants per plot, equivalent to 16666 plants per fed. Soil physical and chemical properties were analyzed following Black *et al*. (1982) and are presented in Table (a).

Table (a): Mechanical and chemical properties of experimental farm soil (average of both growing seasons)

Mechanical analysis													**Texture**
Clay (%)			Silt (%)		Fine sand (%)			Coarse sand (%)			Loamy		
33.66			39.10		14.48			12.76					
Chemical analysis													
pH	EC (m.mohs/cm)	Organic matter (%)	Soluble cations (meq l^{-1})				Soluble anions (meq l^{-1})			Available (ppm)			
			Mg^{++}	Ca^{++}	K^{+}	Na^{+}	Cl^{-}	$HCO3^{-}$	$SO4^{--}$	N	P	K	
7.87	1.35	1.22	2.6	1.6	1.3	4.1	5.3	1.4	2.9	17.0	8.30	71.0	

This experiment was conducted using a split-plot design with three replicates, implemented within a randomized complete block design (RCBD). Main plots were assigned to seven fulvic acid (FA) treatments: control (T1, no FA), fulvic acid as soil addition (FSA) at 1 g/l water (T2) and 2 g/l water (T3), fulvic acid as foliar spray (FFS) at 500 ppm (T4) and 1000 ppm (T5), and combinations of T2 + T4 (T6) and T3 + T5 (T7). Subplots were allocated to seven foliar spraying treatments: chitosan at 250 and 500 ppm, vitamin E at 50 and 100 ppm, garlic extract at 10 and 20 ml/l water, and a control (sprayed with water).

Fulvic acid application: Fulvic acid (Fulvo Max, 60%, City Max Agrochemical Company, China) was applied with 0.1% Triton B as a wetting agent. Treatments were administered three times at three-week intervals, starting two weeks after transplanting, as soil additions to the root zone or foliar sprays on leaf surfaces.

Stimulant substance application: Chitosan was sourced from the National Research Center, Giza, Egypt, and amino acids from Techno Gene Company, Dokky, Giza, Egypt. Vitamin E (α-tocopherol) was obtained from El-Gomhoria Chemical Company, Assiut. Garlic extract was prepared from 100 g of fresh *Allium sativum* L. cloves, washed with sterile distilled water, ground in 100 mL of the same, filtered through double-layered muslin cloth and Whatman No. 1 filter paper, centrifuged at 4,000 rpm for 5 minutes, and passed through a sintered glass filter to yield a sterile 100% (w/v) extract (Bhatti, 1988), chemicals and minerals per 100 g of garlic extract are shown in Table (b). Plants were foliar sprayed to runoff with stimulant substances three times at three-week intervals, starting 21 days after transplanting, with a one-day interval between each substance.

Triton B at 0.1% was added to all sprays, and the control was sprayed with water containing Triton B at 0.1%. Other agricultural practices followed standard protocols.

Table b: Important chemicals and minerals per 100 g garlic extract authorized by (Bhatti, 1988)

Chemical composition	Concentrations	Chemical composition	Concentrations
Lysine (g)	0.273	Aspartic acid (g)	0.489
Carbohydrates (g)	33.07	Leucine (g)	0.308
Lipids (g)	0.50	Manganese (mg)	1672.0
Sodium (mg)	17.0	Calcium (mg)	181.00
Magnesium (mg)	32.0	Phosphorus (mg)	153.00
Calories Kcal	149.0	Potassium (mg)	401.00
Glutamic acid (g)	0.805	Vit. B 6 (mg)	1235.0
Argenine (g)	0.634	Vitamin C (mg)	31.0
Water (g)	59.0	Fiber (g)	2.10
Sulphur (mg)	70.0		

Recorded data: A random sample of ten plants was carefully collected each plot for analysis.

1. **Plant growth parameters**: At the flowering stage, branch number per plant, and herb dry weight (g) were measured.
2. **Yield parameters**: At harvest (the last week of October of both growing seasons), the dry weights of sepals and seeds (g) per plant were measured, and yield per fed. (kg) were determined by multiplying the per-plant weights in the number of plants per fed.
3. **Chemical constituents**: Fixed oil percentage in the dried seeds was extracted using a Soxhlet apparatus with hexane (boiling point 60-80°C) as the solvent, by A.O.A.C. (1980). The fixed oil yield was calculated as follows; fixed oil yield (ml/plant) = (fixed oil percentage × seed yield g/plant) / 100 and fixed oil yield (L/fed) = (fixed oil percentage × seed yield kg/fed) / 100. Anthocyanin percentage in the air-dried roselle sepals was determined using the method of Du and Francis (1973). Sepal acidity percentage the air-dried roselle sepals was measured following Diab (1968).

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) for a split-plot design. The least significant difference (LSD) test at a 5% probability level was applied to compare treatment means, following Gomez and Gomez (1984). Statistical analyses were conducted using Statistix version 9.0 software (Analytical Software, 2008).

RESULTS

1-Plant growth parameters

Data from Table 1 demonstrate that fulvic acid applications at all levels significantly enhanced the growth parameters (number of branches per plant and herb dry weight g/plant) of *Hibiscus sabdariffa* L. cv. Sabhia 17 in both 2023 and 2024 seasons. The highest growth values were recorded with treatment T7 (fulvic acid as soil addition at 2 g/l + foliar spray at 1000 ppm), yielding 15.47 and 16.04 branches and 255.0 and 256.4 g dried herb weight in the first and second seasons, respectively, followed by T6 (fulvic acid as soil addition at 1 g/l + foliar spray at 500 ppm), with 13.41 and 13.74 branches and 238.7 and 238.6 g dried herb weight in the first and second seasons, respectively.

Foliar spraying with stimulant substances (chitosan, vitamin E and garlic extract) at all concentrations also significantly increased branch number and herb dry weight compared to untreated plants. Garlic extract at 20 ml/l produced the highest values, with 13.46 and 13.70 branches and 239.8 and 240.8 g dried herb weight in the respective seasons. In contrast, unsprayed plants recorded the lowest values, with 10.84 and 11.15 branches and 206.9 and 207.7 g dried herb weight in the first and second seasons, respectively (Table 1).

The interaction between fulvic acid treatments and stimulant substances was significantly influenced growth parameters in the two seasons. All combinations significantly increased these traits, except for T1 combined with chitosan at 250 ppm or vitamin E at 50 ppm in both seasons and T4 (fulvic acid foliar spray at 500 ppm) with control stimulant substances in the first season for branch number. The most effective combination was T7 paired with garlic extract at 20 ml/l, achieving 17.57 and 18.12 branches and 273.8 and 276.0 g dried herb weight in the first and second seasons, respectively (Table 1).

Table 1: Influence of fulvic acid applications and stimulant substances and their interactions on branch number/plant and herb dry weight/plant (g) of roselle plant during both growing seasons

Stimulant substances (B)	**Fulvic acid applications (A)**							
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**Mean (B)**
Branch number/plant								
First season								
Control	9.20	10.23	10.67	9.90	10.43	11.87	13.57	**10.84**
Chitosan at 250 ppm	9.90	11.33	11.90	11.10	11.33	12.57	14.87	**11.86**
Chitosan at 500 ppm	10.10	11.77	12.33	11.43	12.00	13.43	15.47	**12.36**
Vitamin E at 50 ppm	9.77	11.10	11.67	10.67	11.43	12.57	15.13	**11.76**
Vitamin E at 100 ppm	10.47	11.80	12.33	11.33	11.87	13.77	16.13	**12.53**
Garlic extract at 10 ml/l	10.37	11.90	12.80	11.33	12.13	14.10	15.57	**12.60**
Garlic extract at 20 ml/l	11.00	12.23	13.10	12.00	12.77	15.57	17.57	**13.46**
Mean (A)	**10.11**	**11.48**	**12.11**	**11.11**	**11.71**	**13.41**	**15.47**	
LSD 0.05	**A: 0.40 B: 0.33 AB: 0. 0.87**							
Second season								
Control	9.42	10.46	10.89	10.22	10.99	12.09	14.01	**11.15**
Chitosan at 250 ppm	10.12	11.44	12.02	11.32	11.66	13.01	15.42	**12.14**
Chitosan at 500 ppm	10.20	11.89	12.56	11.67	12.12	13.66	15.79	**12.55**
Vitamin E at 50 ppm	9.89	11.22	11.79	10.89	11.66	13.01	15.79	**12.04**
Vitamin E at 100 ppm	10.58	11.91	12.56	11.56	12.21	13.99	16.68	**12.78**
Garlic extract at 10 ml/l	10.58	12.11	12.91	11.66	12.58	14.54	16.46	**12.98**
Garlic extract at 20 ml/l	10.99	12.44	13.22	12.32	12.89	15.89	18.12	**13.70**
Mean (A)	**10.25**	**11.64**	**12.28**	**11.38**	**12.01**	**13.74**	**16.04**	
LSD 0.05	**A: 0.34 B: 0.29 AB: 0.77**							
Herb dry weight/plant (g)								
First season								
Control	171.6	193.9	214.6	193.6	211.5	226.1	237.1	**206.9**
Chitosan at 250 ppm	193.3	220.0	231.9	203.9	221.0	235.1	253.1	**222.6**
Chitosan at 500 ppm	196.0	225.8	235.9	212.9	227.5	241.2	258.2	**228.2**

Vitamin E at 50 ppm	187.1	214.5	224.9	211.8	219.3	232.2	246.9	**219.5**
Vitamin E at 100 ppm	194.8	223.0	237.1	222.3	227.0	242.2	259.5	**229.4**
Garlic extract at 10 ml/l	193.0	229.8	237.4	221.6	225.9	239.7	256.3	**229.1**
Garlic extract at 20 ml/l	198.0	236.0	246.5	233.0	236.9	254.5	273.8	**239.8**
Mean (A)	**190.5**	**220.4**	**232.6**	**214.2**	**224.1**	**238.7**	**255.0**	
LSD 0.05	**A: 2.1 B: 1.9 AB: 5.1**							
Second season								
Control	173.4	196.3	214.6	193.6	211.5	226.1	238.1	**207.7**
Chitosan at 250 ppm	193.0	218.7	229.3	203.9	221.0	235.1	254.3	**222.2**
Chitosan at 500 ppm	200.4	225.8	235.9	212.9	227.5	241.2	260.0	**229.1**
Vitamin E at 50 ppm	195.3	214.5	230.1	211.8	221.3	232.2	249.2	**222.1**
Vitamin E at 100 ppm	196.6	227.3	237.1	222.3	228.6	242.2	259.6	**230.5**
Garlic extract at 10 ml/l	195.9	226.9	235.9	222.9	227.1	239.7	257.7	**229.5**
Garlic extract at 20 ml/l	202.5	236.0	246.5	233.0	236.9	254.5	276.0	**240.8**
Mean (A)	**193.9**	**220.8**	**232.8**	**214.3**	**224.8**	**238.6**	**256.4**	
LSD 0.05	**A: 2.5 B: 1.8 AB: 4.7**							

2- Yield parameters

Data from Tables 2 and 3 indicate that fulvic acid applications significantly enhanced sepal and seed yields (g/plant and kg/fed) of *Hibiscus sabdariffa* L. cv. Sabhia 17 in the 2023 and 2024 seasons, except for T4 in the first season for sepal yield, with yields increasing progressively with higher fulvic acid levels across all application methods compared to the control. The most effective treatment, T7 (fulvic acid as soil addition at 2 g/l + foliar spray at 1000 ppm), resulted in the highest increments: sepal yields increased by 15.2% and 17.0% (36.4 g/plant and 606.8 kg/fed in the first season; 37.8 g/plant and 629.2 kg/fed in the second season) and seed yields by 41.0% and 26.7% (45.7 g/plant and 762.3 kg/fed in the first season; 42.1 g/plant and 702.0 kg/fed in the second season) compared to the control.

Foliar spraying with stimulant substances (chitosan, vitamin E, and garlic extract) at all concentrations also significantly increased sepal and seed yields compared to the control in both seasons (Tables 2-3). Garlic extract at 20 ml/l yielded the highest values, boosting sepal weights by 15.0% and 15.6% (35.3 g/plant and 588.1 kg/fed in the first season; 36.3 g/plant and 604.7 kg/fed in the second season) and seed weights by 18.5% and 16.8% (40.3 g/plant and 671.8 kg/fed in the first season; 39.5 g/plant and 658.6 kg/fed in the second season) in relative to the control.

The interaction between fulvic acid treatments and stimulant substances was significantly affected sepal and seed yields in both seasons. The most combinations resulted a significant increase in these parameters for seasons compared to untreated plants. The most effective combination was T7 with garlic extract at 20 mL/L, yielding the highest sepal (39.0 g/plant and 650.0 kg/fed in the first season; 40.1 g/plant and 667.8 kg/fed in the second season) and seed yields (49.5 g/plant and 824.4 kg/fed in the first season; 46.8 g/plant and 780.0 kg/fed in the second season), followed by T7 with vitamin E at 100 ppm (38.7 g/plant and 644.4 kg/fed sepals, 48.7 g/plant and 812.2 kg/fed seeds in the first season; 39.1 g/plant and 651.1 kg/fed sepals, 44.4 g/plant and 740.5 kg/fed seeds in the second season) as shown in Tables 2-3.

Table 2: Influence of fulvic acid applications and stimulant substances and their interactions on sepals weight/plant (g) and fed (kg) of roselle plant during both growing seasons.

Stimulant substances (B)	Fulvic acid applications (A)							
	T1	T2	T3	T4	T5	T6	T7	Mean (B)
Sepals weight/plant (g)								
First season								
Control	27.1	30.4	31.2	28.1	30.0	33.6	34.3	**30.7**
Chitosan at 250 ppm	30.3	31.4	32.3	30.6	31.7	34.7	35.5	**32.3**
Chitosan at 500 ppm	33.9	34.1	35.3	34.1	34.7	36.2	35.8	**34.9**
Vitamin E at 50 ppm	31.3	33.3	33.8	31.5	32.5	33.9	35.5	**33.1**
Vitamin E at 100 ppm	34.4	34.2	34.5	33.9	34.1	36.3	38.7	**35.1**
Garlic extract at 10 ml/l	31.1	33.2	33.6	32.4	31.8	34.9	36.1	**33.3**
Garlic extract at 20 ml/l	33.3	34.1	34.9	34.2	34.6	36.8	39.0	**35.3**
Mean (A)	**31.6**	**33.0**	**33.6**	**32.1**	**32.8**	**35.2**	**36.4**	
LSD 0.05	**A: 0.6 B: 0.6 AB: 1.5**							
Second season								
Control	27.7	31.2	32.2	28.9	30.9	33.8	35.1	**31.4**
Chitosan at 250 ppm	31.0	32.1	33.3	31.4	32.8	34.9	36.7	**33.2**
Chitosan at 500 ppm	34.3	34.9	36.0	35.1	35.6	36.7	37.7	**35.8**
Vitamin E at 50 ppm	32.4	33.9	35.0	32.4	33.8	36.2	37.5	**34.5**
Vitamin E at 100 ppm	34.9	35.3	35.7	35.0	35.1	37.0	39.1	**36.0**
Garlic extract at 10 ml/l	31.7	34.1	34.5	33.4	32.7	35.4	38.2	**34.3**
Garlic extract at 20 ml/l	34.0	35.3	36.0	35.4	35.9	37.2	40.1	**36.3**
Mean (A)	**32.3**	**33.8**	**34.7**	**33.1**	**33.8**	**35.9**	**37.8**	
LSD 0.05	**A: 0.7 B: 0.8 AB: 2.1**							
Sepals weight/fed (kg)								
First season								
Control	451.6	506.6	519.4	468.3	500.0	559.4	571.6	**511.0**
Chitosan at 250 ppm	505.0	523.3	537.8	510.5	528.3	577.8	591.1	**539.1**
Chitosan at 500 ppm	564.4	568.3	587.8	568.9	578.3	603.3	597.2	**581.2**
Vitamin E at 50 ppm	521.1	555.0	562.8	524.4	541.6	565.5	591.6	**551.7**
Vitamin E at 100 ppm	572.8	569.4	575.0	564.4	568.3	604.4	644.4	**585.5**
Garlic extract at 10 ml/l	518.3	553.3	559.4	539.4	530.0	582.2	601.6	**554.9**
Garlic extract at 20 ml/l	555.0	568.9	582.2	569.4	577.2	613.9	650.0	**588.1**
Mean (A)	**526.9**	**549.3**	**560.6**	**535.1**	**546.2**	**586.6**	**606.8**	
LSD 0.05	**A: 9.7 B: 9.6 AB: 25.4**							
Second season								

Control	461.1	520.5	537.2	481.6	515.0	562.8	584.4	**523.2**
Chitosan at 250 ppm	516.6	534.4	555.0	522.8	547.2	581.6	611.6	**552.8**
Chitosan at 500 ppm	571.1	582.2	600.0	584.4	593.3	611.6	628.9	**595.9**
Vitamin E at 50 ppm	540.5	564.4	582.8	540.5	563.9	603.9	624.4	**574.3**
Vitamin E at 100 ppm	581.1	588.9	594.4	583.9	585.0	616.6	651.1	**600.1**
Garlic extract at 10 ml/l	527.8	567.8	574.4	557.2	544.4	590.5	636.1	**571.2**
Garlic extract at 20 ml/l	567.2	588.9	600.5	590.5	598.9	619.4	667.8	**604.7**
Mean (A)	**537.9**	**563.9**	**577.8**	**551.6**	**563.9**	**598.1**	**629.2**	
LSD 0.05	**A: 14.0 B: 13.4 AB: 35.4**							

Table 3: Influence of fulvic acid applications and stimulant substances and their interactions on seeds weight/plant (g) and fed (kg) of roselle plant during both growing seasons

Stimulant substances (B)	**Fulvic acid applications (A)**							
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**Mean (B)**
Seeds weight/plant (g)								
First season								
Control	29.9	30.9	33.9	30.4	32.9	39.2	41.1	**34.0**
Chitosan at 250 ppm	32.3	33.5	36.7	33.8	34.9	40.7	42.5	**36.4**
Chitosan at 500 ppm	32.5	36.8	37.5	34.8	36.1	45.3	46.3	**38.5**
Vitamin E at 50 ppm	31.3	33.2	35.4	32.2	34.2	43.5	45.5	**36.5**
Vitamin E at 100 ppm	33.5	36.9	39.3	35.9	37.6	45.8	48.7	**39.7**
Garlic extract at 10 ml/l	32.6	34.9	35.6	34.0	34.5	45.1	46.6	**37.6**
Garlic extract at 20 ml/l	34.8	37.1	39.7	36.7	38.5	46.0	49.5	**40.3**
Mean (A)	**32.4**	**34.7**	**36.9**	**34.0**	**35.5**	**43.7**	**45.7**	
LSD 0.05	**A: 0.9 B: 1.1 AB: 2.8**							
Second season								
Control	31.0	32.2	34.2	31.8	32.7	36.1	38.5	**33.8**
Chitosan at 250 ppm	32.7	34.1	35.8	33.4	34.3	37.5	39.1	**35.3**
Chitosan at 500 ppm	33.6	35.3	37.5	34.5	35.3	38.6	41.9	**36.7**
Vitamin E at 50 ppm	31.9	34.2	36.4	33.1	34.5	38.1	41.5	**35.7**
Vitamin E at 100 ppm	34.3	36.5	38.6	35.5	36.5	39.3	44.4	**37.9**
Garlic extract at 10 ml/l	33.4	34.7	36.9	34.2	35.8	38.2	42.6	**36.5**
Garlic extract at 20 ml/l	35.8	37.6	39.7	37.4	38.8	40.6	46.8	**39.5**
Mean (A)	**33.2**	**35.0**	**37.0**	**34.3**	**35.4**	**38.3**	**42.1**	
LSD 0.05	**A: 0.7 B: 0.8 AB: 2.2**							
Seeds weight/fed (kg)								
First season								

Control	497.8	514.4	565.0	506.1	548.3	653.9	684.4	**567.1**
Chitosan at 250 ppm	538.9	558.9	612.2	562.8	582.2	677.8	708.3	**605.8**
Chitosan at 500 ppm	542.2	612.8	625.0	580.5	601.6	755.0	772.2	**641.3**
Vitamin E at 50 ppm	521.6	552.8	590.0	536.1	570.0	725.5	758.3	**607.8**
Vitamin E at 100 ppm	558.9	615.0	654.4	598.3	627.2	763.9	812.2	**661.4**
Garlic extract at 10 ml/l	543.9	582.2	593.9	566.6	575.0	751.6	776.1	**627.0**
Garlic extract at 20 ml/l	580.0	617.8	662.2	611.1	641.1	766.1	824.4	**671.8**
Mean (A)	**540.5**	**579.1**	**614.7**	**565.9**	**592.2**	**727.7**	**762.3**	
LSD 0.05	**A: 16.1 B: 17.7 AB: 46.8**							
Second season								
Control	516.1	537.2	570.0	529.4	545.0	601.6	642.2	**563.1**
Chitosan at 250 ppm	544.4	568.9	596.6	557.2	571.6	624.4	652.2	**587.9**
Chitosan at 500 ppm	560.5	588.9	624.4	575.0	588.3	643.9	698.3	**611.3**
Vitamin E at 50 ppm	532.2	570.0	606.1	551.1	574.4	635.0	691.1	**594.3**
Vitamin E at 100 ppm	571.6	608.3	642.8	591.1	607.8	654.4	740.5	**630.9**
Garlic extract at 10 ml/l	556.6	578.3	615.5	570.5	597.2	636.1	709.4	**609.1**
Garlic extract at 20 ml/l	596.6	626.6	661.6	622.8	646.1	676.6	780.0	**658.6**
Mean (A)	**554.0**	**582.6**	**616.7**	**571.0**	**590.1**	**638.9**	**702.0**	
LSD 0.05	**A: 12.1 B: 13.9 AB: 36.7**							

Chemical constituents traits

1- Fixed oil (FO) production

Data presented in Table 4 demonstrate that fulvic acid treatments significantly enhanced fixed oil % and yield (ml/plant and l/fed) of *Hibiscus sabdariffa* L. cv. Sabhia 17 in the 2023 and 2024 seasons, except for T2 (fulvic acid soil addition at 1 g/l) in the first season and T4 in 1st and 2nd seasons for FO% and T4 (fulvic acid foliar spray at 500 ppm) in the first season concerning FO yield (ml/plant and l/fed) compared to the control (T1). The highest FO (% and yields) were achieved with T7 (fulvic acid soil addition at 2 g/l + foliar spray at 1000 ppm), with increases of 34.4 and 35.7% for FO% during both seasons, respectively, 89.2% and 71.7% over the control, yielding 10.63 and 10.13 m/plant and 177.22 and 168.88 l/fed in the first and second seasons, respectively, compared to T1 (5.62 ml/plant and 93.61 l/fed in the first season; 5.90 ml/plant and 98.32 l/fed FO in the second season).

Foliar applications of stimulant substances (chitosan, vitamin E, and garlic extract) at all concentrations significantly increased fixed oil production, except for 250 ppm chitosan, 50 ppm vitamin E and 10 ml/l garlic extract in the first season for FO%, compared to the control in both seasons (Table 4). Garlic extract at 20 ml/l resulted in the highest increase in fixed oil % by 8.9 and 11.2% regarding FO%, the yield was increased by 29.2% and 30.1% over the control, producing 8.28 ml/plant and 138.00 l/fed and 8.44 ml/plant and 140.61 l/fed FO in the first and second seasons, respectively (Table 4).

The interaction between fulvic acid treatments and stimulant substances was significantly affected fixed oil production traits in both seasons, the most combinations caused a significant elevate in these traits during the two seasons, in relative to control. The highest FO production parameters were recorded by T7 combined with garlic extract at 20 ml/l, achieving 24.47 and 25.03 FO%, 12.10 and 11.72 ml FO/plant and 201.72 and 195.32 l FO/fed in the first and second seasons, respectively, followed closely by T7 with vitamin E at 100 ppm (23.91 and 24.85 FO%, 11.65 and 11.04 ml FO/plant; 194.22 and 183.96 l FO/fed), with no significant differences between these combinations. The lowest percentage and yields (15.68 and 15.93 FO%, 4.70 and 4.93 ml FO/plant; 78.26 and 82.22 l FO/fed) were observed with T1 combined with control stimulant substances in both seasons (Table 4).

Table 4: Influence of fulvic acid applications and stimulant substances and their interactions on fixed oil percentage and yield/plant (ml) and fed (L) of roselle plant seeds during both growing seasons

Stimulant substance (B)	**Fulvic acid applications (A)**															
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**Mean (B)**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**Mean (B)**
	First Season								**Second Season**							
Fixed oil percentage																
Control	15.68	17.63	18.87	17.34	18.33	20.15	22.31	**18.61**	15.93	17.89	19.24	17.74	18.51	20.92	23.05	**19.04**
Chitosan at 250 ppm	16.82	18.79	19.08	17.96	18.29	20.85	22.64	**19.20**	17.19	19.47	19.71	18.56	18.97	22.03	24.05	**20.00**
Chitosan at 500 ppm	17.90	19.05	20.05	18.17	19.62	21.40	23.54	**19.96**	18.22	19.82	20.41	18.99	19.98	22.34	24.26	**20.58**
Vitamin E at 50 ppm	17.18	18.18	18.92	17.96	18.25	21.10	23.22	**19.26**	17.55	18.35	19.69	18.40	18.99	21.70	23.65	**19.76**
Vitamin E at 100 ppm	18.18	18.63	19.77	18.54	19.07	22.18	23.91	**20.04**	18.66	18.85	20.72	18.76	20.75	23.03	24.85	**20.80**
Garlic extract at 10 ml/l	17.01	17.66	19.19	17.62	18.89	21.45	22.43	**19.18**	17.54	18.06	19.62	17.92	19.33	22.54	23.30	**19.76**
Garlic extract at 20 ml/l	18.19	18.73	19.98	18.33	19.32	22.83	24.47	**20.26**	18.87	19.86	21.01	18.82	21.00	23.64	25.03	**21.18**
Mean (A)	**17.28**	**18.38**	**19.41**	**17.99**	**18.82**	**21.42**	**23.22**		**17.71**	**18.90**	**20.06**	**18.46**	**19.65**	**22.31**	**24.03**	
LSD 0.05	**A: 1.37 B: 0.76 AB: 2.02**								**A: 0.88 B: 0.57 AB: 1.52**							
Fixed oil yield/plant (ml)																

Control	4.70	5.44	6.40	5.26	6.04	7.89	9.16	**6.41**	4.93	5.77	6.58	5.64	6.05	7.56	8.87	**6.49**
Chitosan at 250 ppm	5.43	6.32	7.05	6.08	6.40	8.46	9.62	**7.05**	5.61	6.65	7.06	6.22	6.50	8.24	9.41	**7.10**
Chitosan at 500 ppm	5.82	6.95	7.51	6.33	7.10	9.70	10.91	**7.76**	6.14	7.01	7.65	6.54	7.06	8.63	10.16	**7.60**
Vitamin E at 50 ppm	5.37	6.03	6.70	5.77	6.24	9.21	10.56	**7.13**	5.60	6.28	7.17	6.08	6.55	8.26	9.81	**7.11**
Vitamin E at 100 ppm	6.10	6.87	7.76	6.66	7.18	10.17	11.65	**8.06**	6.41	6.88	7.99	6.65	7.57	9.05	11.04	**7.94**
Garlic extract at 10 ml/l	5.55	6.17	6.83	5.99	6.52	9.67	10.43	**7.31**	5.86	6.27	7.24	6.13	6.92	8.59	9.92	**7.28**
Garlic extract at 20 ml/l	6.34	6.94	7.94	6.72	7.43	10.48	12.10	**8.28**	6.75	7.47	8.35	7.03	8.14	9.60	11.72	**8.44**
Mean (A)	**5.62**	**6.39**	**7.17**	**6.12**	**6.70**	**9.37**	**10.63**		**5.90**	**6.62**	**7.43**	**6.33**	**6.97**	**8.56**	**10.13**	
LSD 0.05	**A: 0.56 B: 0.35 AB: 0.94**								**A: 0.34 B: 0.27 AB: 0.71**							
Fixed oil yield/fed (L)																
Control	78.26	90.65	106.59	87.74	100.61	131.45	152.62	**106.85**	82.22	96.12	109.68	93.98	100.91	126.01	147.88	**108.11**
Chitosan at 250 ppm	90.55	105.27	117.42	101.37	106.67	140.94	160.39	**117.51**	93.55	110.85	117.62	103.58	108.40	137.25	156.84	**118.30**
Chitosan at 500 ppm	96.99	115.89	125.24	105.50	118.29	161.60	181.79	**129.33**	102.26	116.89	127.51	109.08	117.64	143.82	169.40	**126.66**
Vita	89.	100	111	96.	104	153	175	**118**	93.	104	119	101	109	137	163	**118**

min E at 50 ppm	55	.45	.59	24	.05	.54	.97	**.77**	33	.62	.42	.41	.08	.70	.48	**.43**
Vitamin E at 100 ppm	101.73	114.57	129.36	110.97	119.63	169.51	194.22	**134.28**	106.75	114.64	133.14	110.89	126.08	150.77	183.96	**132.32**
Garlic extract at 10 ml/l	92.53	102.87	113.89	99.85	108.63	161.13	173.88	**121.83**	97.64	104.46	120.72	102.23	115.37	143.11	165.29	**121.26**
Garlic extract at 20 ml/l	105.65	115.71	132.29	112.01	123.87	174.72	201.72	**138.00**	112.53	124.49	139.09	117.19	135.70	159.96	195.32	**140.61**
Mean (A)	**93.61**	**106.49**	**119.48**	**101.96**	**111.68**	**156.13**	**177.22**		**98.32**	**110.29**	**123.88**	**105.48**	**116.17**	**142.66**	**168.88**	
LSD 0.05	**A: 9.28 B: 5.89 AB: 15.58**								**A: 5.63 B: 4.47 AB: 11.83**							

2- Total anthocyanin percentage

Data from Table 5 indicate that fulvic acid treatments significantly increased anthocyanin percentage in the sepals of *Hibiscus sabdariffa* L. cv. Sabhia 17, except for T2 and T4 (1st and 2nd seasons) and T5 (1st season), compared to the control during the 2023 and 2024 seasons. The highest anthocyanin percentages (2.32% and 2.41%) were recorded with T7 (fulvic acid soil addition at 2 g/l + foliar spray at 1000 ppm), followed by T6 (fulvic acid soil addition at 1 g/l + foliar spray at 500 ppm) with 2.24% and 2.30% in the first and second seasons, respectively, with no significant difference between T7 and T6 in the first season.

All tested stimulant substances (chitosan, vitamin E, and garlic extract) at all concentrations significantly increased anthocyanin percentage in roselle sepals compared to the control, except for chitosan at 250 ppm in both seasons and garlic extract at 10 ml/l in the first season. The highest anthocyanin percentages (2.28% and 2.32%) were achieved with garlic extract at 20 ml/l in the first and second seasons, respectively (Table 5).

The interaction between fulvic acid treatments and stimulant substances were significantly affected anthocyanin content. The combination of T7 with garlic extract at 20 ml/l yielded the highest values (2.41% and 2.49% in the first and second seasons, respectively), followed by T7 with vitamin E at 100 ppm (2.38% and 2.46%), with no significant differences between these combinations in the two seasons (Table 5). The lowest anthocyanin percentages (1.96% and 2.00%) were recorded for T1 (control) with no stimulant application in the first and second seasons, respectively.

3- Total acidity percentage

Data from Table 5 demonstrate that all fulvic acid levels among different application methods significantly and progressively enhanced the total acidity percentage in sepals of *Hibiscus sabdariffa* L. cv. Sabhia 17 during the 2023 and 2024 seasons, except for T4 in the second season compared to control. Treatment of T7 (fulvic acid soil addition at 2 g/l + foliar spray at 1000 ppm) resulted in the most substantial elevating total acidity by 6.7% and 4.8% compared to untreated plants in the first and second seasons, respectively.

Foliar application of the three stimulant substances (chitosan, vitamin E, and garlic extract) at all concentrations significantly increased total acidity percentage in roselle sepals in both seasons,

except for chitosan at 250 ppm in the first season comparing to no sprayed plants. Garlic extract at 20 ml/l was the most effective, increases acidity by 3.0% and 6.1% over the control in 2023 and 2024, respectively (Table 5).

Table 5: Influence of fulvic acid applications and stimulant substances and their interactions on total anthocyanin and total acidity percentages of roselle plant sepals during both growing seasons

Stimulant substances (B)	Fulvic acid applications (A)							
	T1	T2	T3	T4	T5	T6	T7	Mean (B)
Total anthocyanin %								
First season								
Control	1.96	2.07	2.14	2.00	2.08	2.16	2.27	**2.10**
Chitosan at 250 ppm	2.04	2.10	2.18	2.05	2.15	2.21	2.24	**2.14**
Chitosan at 500 ppm	2.14	2.17	2.21	2.14	2.17	2.22	2.33	**2.20**
Vitamin E at 50 ppm	2.07	2.12	2.17	2.11	2.13	2.22	2.34	**2.17**
Vitamin E at 100 ppm	2.13	2.18	2.29	2.18	2.22	2.31	2.38	**2.24**
Garlic extract at 10 ml/l	2.05	2.11	2.16	2.09	2.11	2.21	2.27	**2.14**
Garlic extract at 20 ml/l	2.15	2.26	2.30	2.24	2.25	2.32	2.41	**2.28**
Mean (A)	**2.08**	**2.15**	**2.21**	**2.12**	**2.16**	**2.24**	**2.32**	
LSD 0.05	**A: 0.12 B: 0.06 AB: 0.17**							
Second season								
Control	2.00	2.12	2.24	2.05	2.11	2.24	2.36	**2.16**
Chitosan at 250 ppm	2.06	2.15	2.27	2.08	2.21	2.27	2.38	**2.20**
Chitosan at 500 ppm	2.15	2.21	2.31	2.17	2.21	2.30	2.42	**2.25**
Vitamin E at 50 ppm	2.13	2.19	2.29	2.14	2.19	2.28	2.38	**2.23**
Vitamin E at 100 ppm	2.18	2.21	2.38	2.21	2.26	2.34	2.46	**2.29**
Garlic extract at 10 ml/l	2.12	2.17	2.31	2.14	2.22	2.30	2.40	**2.24**
Garlic extract at 20 ml/l	2.19	2.28	2.33	2.27	2.29	2.36	2.49	**2.32**
Mean (A)	**2.12**	**2.19**	**2.30**	**2.15**	**2.21**	**2.30**	**2.41**	
LSD 0.05	**A: 0.09 B: 0.05 AB: 0.14**							
Total acidity %								
First season								
Control	8.10	8.59	8.61	8.57	8.56	8.63	8.80	**8.55**
Chitosan at 250 ppm	8.32	8.61	8.65	8.60	8.62	8.68	8.90	**8.62**
Chitosan at 500 ppm	8.59	8.70	8.74	8.62	8.75	8.84	9.02	**8.75**
Vitamin E at 50 ppm	8.31	8.67	8.67	8.64	8.64	8.71	8.98	**8.66**
Vitamin E at 100 ppm	8.60	8.72	8.78	8.67	8.76	8.89	9.08	**8.79**
Garlic extract at 10 ml/l	8.33	8.62	8.66	8.59	8.64	8.72	8.90	**8.64**

Garlic extract at 20 ml/l	8.61	8.74	8.83	8.69	8.77	8.92	9.10	**8.81**
Mean (A)	**8.41**	**8.66**	**8.70**	**8.63**	**8.68**	**8.77**	**8.97**	
LSD 0.05	**A: 0.10 B: 0.08 AB: 0.22**							
Second season								
Control	8.15	8.39	8.44	8.25	8.31	8.49	8.55	**8.37**
Chitosan at 250 ppm	8.35	8.51	8.58	8.41	8.46	8.63	8.73	**8.52**
Chitosan at 500 ppm	8.62	8.77	8.84	8.69	8.74	8.91	8.96	**8.79**
Vitamin E at 50 ppm	8.35	8.52	8.64	8.40	8.49	8.76	8.86	**8.57**
Vitamin E at 100 ppm	8.63	8.79	8.86	8.70	8.77	8.92	9.01	**8.81**
Garlic extract at 10 ml/l	8.39	8.66	8.72	8.44	8.55	8.92	8.95	**8.66**
Garlic extract at 20 ml/l	8.72	8.83	8.90	8.79	8.89	8.96	9.06	**8.88**
Mean (A)	**8.46**	**8.64**	**8.71**	**8.53**	**8.60**	**8.80**	**8.87**	
LSD 0.05	**A: 0.08 B: 0.07 AB: 0.17**							

The interaction between fulvic acid and stimulant treatments was significantly influenced total acidity percentage in both seasons (Table 5). In most cases, the combination of T7 with any stimulant consistently gave higher acidity values compared to other combinations in both years. Notably, T7 combined with garlic extract at 20 ml/l or plus vitamin E at 100 ppm, registered the highest total acidity percentages in both seasons, compared to other combination treatments.

DISCUSSION

During the 2023 and 2024 growing seasons, a field study on *Hibiscus sabdariffa* L. cv. Sabhia 17 demonstrated that fulvic acid (FA) significantly enhanced vegetative growth, yield components, and chemical constituents, as detailed in Tables 1-6. FA's multifaceted role in plant growth promotion was attributed to its ability to increase cell membrane permeability, enabling rapid nutrient absorption through leaves and efficient translocation to sink tissues, thereby reducing nutrient competition among plant organs and enhancing essential nutrient availability (El-Desuki *et al*., 2010). This led to increased dry matter accumulation, supporting greater branch numbers and herb dry weight (Table 1). FA's content of plant signaling molecules, such as auxin-like compounds, modulated metabolic processes, while its upregulation of mitogen-activated protein kinases and increased protein content further promoted growth (Canellas *et al*., 2010; Calvo *et al.*, 2014; Che *et al.*, 2017; Li *et al*., 2019; Dawood *et al*., 2019). Due to its richness in carbon and nitrogen, FA enhanced water and nutrient absorption due to its high binding capacity, fostering robust vegetative development (Drobek *et al*., 2019). Additionally, FA improved soil physicochemical properties by darkening soil, raising root zone temperatures, stimulating root growth, and preventing soil cracking, which preserved root hairs and supported nutrient uptake (Halvin *et al*., 2005; Al-Jumaili, 2012). The results of this study demonstrating enhanced yield components with fulvic acid application, align with findings from other crops. Suh *et al*. (2014) reported an increased marketable yield in tomato production with higher FA rates, consistent with the significant improvements in roselle sepal and seed yields (Tables 2-3). Similarly, Kamel *et al*. (2014) observed improved yield quantity and quality in cucumber, along with enhanced soil microbial activity and control of powdery and downy mildews, following FA application. Moradi *et al*. (2017) further noted that higher FA rates increased seed number per head, 1000-seed weight, and overall seed yield in safflower, corroborating the positive impact of FA on roselle yield parameters in our study, particularly with the combined 2 g/l soil addition and 1000 ppm foliar spray treatment. FA increased fixed oil content in roselle seeds (Table 4) by regulating hormone levels and promoting secondary metabolite synthesis, consistent with findings in saffron and *Thymus vulgaris* (Santiago *et al*., 2008; Noroozisharaf and Kavian, 2018). In *Mentha piperita* var. citrata, foliar FA application enhanced essential oil percentage and constituents by improving nutrient absorption and stimulating enzyme activity critical for oil metabolism (Hendawy *et al*., 2015). FA boosts pigments and water content, enhances photosynthesis, and reduces stomatal opening and transpiration, promoting growth while minimizing water loss (Anjum *et al*., 2011;

Li *et al*., 2005). It enhances chlorophyll content by improving oxygen uptake and mRNA concentration, supporting enzyme and protein synthesis critical for metabolic processes (Dixon and Weed, 1989; Nardi *et al*., 1996). FA acts as a nutrient reservoir, potentially containing nitrogen within its humic substances, and improved soil properties to enhance nutrient availability (Taj Eldin and Al-Barakat, 2016). It reduced phosphorus deposition and adsorption on soil colloids, promoting P release and dissolution (Abdel Razzak and El Sharkawy, 2013), and enhanced potassium uptake by activating monovalent ion absorption and substituting H^+ ions with K^+ ions on soil exchange surfaces, reducing K fixation (Shahryari *et al*., 2011; Al-Jumaili, 2012). FA's high electronegative oxygen content increased cell membrane permeability, facilitating nutrient absorption, root activity, and ion uptake, while mitigating abiotic stresses like salinity and drought (Priya *et al*., 2014; Yang *et al.,* 2013; Kulikova *et al*., 2005). Phosphorus supported coenzyme activation for amino acid production, photosynthesis, glycolysis, respiration, and fatty acid synthesis, while potassium enhanced nitrogen, carbohydrate, lipid, starch, and protein metabolism (Zahra *et al*., 1984). FA's ability to improve nutrient-use efficiency, soil microbial activity, and physicochemical properties, while reducing reliance on inorganic fertilizers, underscores its role as an effective tool for sustainable, high-quality crop production (El-Serafy, 2018; Kamel *et al*., 2014; Suh *et al*., 2014).

Chitosan, a chitin-derived biopolymer, significantly enhances plant growth and productivity by stimulating metabolic processes, increasing dry matter accumulation, and accelerating development throughout the plant life cycle (Amin, 2013). Its structural similarity to chitin allows integration into plant cell walls, strengthening cellular integrity and enhancing resilience against stress (Salman and Al-Abadi, 2009). Chitosan's functional groups, amine, and primary and secondary hydroxyl groups at the second, third, and sixth carbon atoms, respectively, support plant health (Speiciene *et al*., 2007). Its hydrophilic nature regulates cellular water content, mitigates stress, and accelerates macromolecular processes (Chakraborty *et al*., 2020). In sweet basil under salinity stress, chitosan boosts photosynthetic pigment content by increasing cytokinin levels and providing amino compounds from its degradation, promoting chlorophyll synthesis (Chibu and Shibayama, 2001). It also elevates indole-3-acetic acid (IAA) levels by upregulating auxin-related gene expression and reducing IAA oxidase activity, enhancing enzyme activity and growth (Li *et al.,* 2018). Additionally, foliar chitosan application increases essential oil production by improving nutrient uptake, promoting growth cycles, and potentially modifying leaf oil gland populations and monoterpene biosynthesis (Ghasemi Pirbalouti *et al*., 2014).

The application of α-tocopherol (vitamin E) significantly enhanced growth, yield, and chemical constituents of roselle due to its potent antioxidant properties. Vitamin E protects chloroplast membranes from photooxidation, creating an optimal environment for photosynthesis, and accumulates under abiotic stresses such as drought, salinity, and high light to mitigate oxidative damage (Munné-Bosch and Alegre, 2002; Hess, 1983; Zhang *et al*., 2000; Havaux *et al*., 2000). Foliar application of α-tocopherol improves membrane stability, supports intracellular signaling, enhances electron transport in photosystem II, and provides photoprotection (Munné-Bosch and Falk, 2004). In medicinal plants, it increases chemical constituent levels (Ayad *et al*., 2009), while in faba bean, it boosts growth parameters, yield components, and chlorophyll a, b, and carotenoid content (El-Bassiouny *et al.*, 2005). Vitamin E also supports plant growth, development, and senescence, prevents lipid peroxidation, and interacts with signaling cascades to mediate responses to abiotic and biotic stresses (Baffel and Ibrahim, 2008; Sattler *et al*., 2004).

Foliar application of garlic extract significantly enhanced the growth, yield, and chemical constituents of roselle, attributed to its rich nutrient profile and protective effects against pathogens (Lampkin, 1994). Garlic extract contains growth-promoting compounds, including macro- and micronutrients (Na, K, Zn, P, Mn, Mg, Ca, Fe), gibberellic acid, enzymes, vitamins B and C, proteins, carbohydrates, saponins, alkaloids, flavonoids, and free sugars (sucrose, fructose, glucose), which support plant development (Sheren *et al*., 2015; Bhandari *et al.,* 2014). Organosulfur compounds like allicin and diallyl disulfide further enhance its efficacy (Puvača *et al*., 2014; Martins *et al*., 2016). In our study, garlic extract significantly increased roselle's vegetative growth (branch number, herb dry weight, Table, 1), yield components (sepal and seed yields, Table, 2-3), and chemical constituents [fixed oil (Table 4), anthocyanin, (Table, 5) and acidity, (Table, 5)], by stimulating photosynthetic pigments and soluble sugar content (Slattery *et al*., 2017). These findings align with previous studies reporting enhanced growth in *Majorana hortensis*, *Salvia officinalis*, *Schefflera arboricola*, and pepper (Mady, 2009; Hanafy *et al*., 2012; Sikandar *et al*., 2018), improved flower numbers in summer squash (Helmy, 1992; El-Desouky *et al*., 1998) and increased pod number in pea (Ahmed *et al*., 2005), and garlic extract's allelochemical properties promote sustainable agriculture by boosting growth and yield (Wang *et al*., 2014), with a strong correlation between morphological traits and yield/chemical constituent improvements in roselle.

CONCLUSION

The combined use of soil-applied fulvic acid at 2 g/l and foliar application at 1000 ppm, alongside foliar spraying with garlic extract at 20 ml/l recorded the optimum treatment to enhance vegetative growth, yield, and quality attributes of roselle (*Hibiscus sabdariffa* L. cv. Sabhia 17). This approach improves nutrient uptake, enhances soil properties, and mitigates environmental stresses, promoting robust plant development. By reducing reliance on chemical fertilizers, this sustainable and eco-friendly practice supports high-quality crop production while optimizing productivity and quality.

REFERENCES

1. A.O.A.C. (1980). Official Methods of Analysis,12th Ed. Association of official analysis chemists: Washington, D.C., USA.
2. Abd El-Rheem, K.M., El-Tanahy, A.M.M., Aboud, F.S. and Abdel-Kader, H.H. (2021). Effect of different rates of fulvic acid and compost on growth, yield and nutritional status of fennel plant grown in sandy soil. World Journal of Agricultural Sciences, 17(3): 252-258.
3. Abdel Razzak, H.S. and El Sharkawy, G.A. (2013). Effect of bio-fertilizer and humic acid applications on growth, yield, quality and storability of tow garlic (*Allium Sativum* L.) Cultivars. Asian Journal of Crop Science, 5, 48-64.
4. Abou-Sreea, A.I.B., Roby, M.H., Mahdy, H.A., Abdou, N.M., El-Tahan, A.M., El-Saadony, M.T., El-Tarabily, K.A., and El-Saadony, F.M. (2022). Improvement of selected morphological, physiological, and biochemical parameters of roselle (*Hibiscus sabdariffa* L.) grown under different salinity levels using potassium silicate and Aloe saponaria extract. *Plants*, 11(4), 497
5. Adamuchio-Oliveira, L.G., Mazaro, S.M., Mógor, G., Sant'anna-Santos, B.F. and Mógor, Á.F. (2020). Chitosan associated with chelated copper applied on tomatoes: Enzymatic and anatomical changes related to plant defense responses. Scientia Horticulturae, 271, 109431.
6. Aganbi, E., Onyeukwu, B.O., Avwioroko, J.O. and Tonukari, J.N. (2017). Effect of fermentation on sensory, nutritional and antioxidant properties of mixtures of aqueous extracts of *Hibiscus sabdariffa* (zobo) and *Raphia hookeri* (raffia) wine. Nigerian Journal of Science and Environment, 15(1), 66-74.
7. Ahmed, S., Iqbal, J. and Attauddin, I. (2005). Time of application effect of phytobiocides on powdery mildew and yield in pea. Sarhad J. Agriclture, 21, 729-731.
8. Al Jumaili, M.O.S. (2012) The combined effect of spraying with humic acid (humic and fulvic acid) and potassium fertilization method on the growth and yield of potatoes. PhD Dissertation, College of Agriculture, University of Baghdad, Baghdad, Iraq.
9. Al-Obady, M.R. (2015). Effect of foliar application with garlic extract and Licorice root extract and salicylic acid on vegetative growth and flowering and flower set of tomato and under unheated houses. J. of Applied Sci. and Res., 3(1), 11-22.
10. Amin, M.M. (2013). Chitosan. *Journal of Environmental Studies,* 38, 1-8.
11. Aminifard, M.H., Aroiee H., Nemati, H., Azizi, M. and Jaafar, H.Z.E. (2012). Fulvic acid affects pepper antioxidant activity and fruit quality. African Journal of Biotechnology, 11(68): 13179-13185.
12. Analytical Software (2008). Statistix Version 9, Analytical Software, Tallahassee, Florida, USA.
13. Anjum, S. A., Wang, L., Farooq, M., Xue, L. and Ali, S. (2011). Fulvic acid application improves the maize performance under well-watered and drought conditions. Journal of Agro. Crop Sci. 197 (6),409-417.
14. Ayad, H.S., Gamal El-Din, K.M. and Reda, F. (2009). Efficiency of stigmasterol and a-tocophrerol application on vegetative growth, essential oil pattern, protein and lipid peroxidation of geranium (*Pelargonium graveolens*, L.). J. Appl. Sci. Res., 5(7), 887-892.
15. Aytaç, Z., Gülbandılar, A. and Kürkçüo˘glu, M. (2022). Humic acid improves plant yield, antimicrobial activity and essential oil composition of Oregano (*Origanum vulgare* L. subsp. *hirtum* (Link.) Ietswaart). Agronomy *12* (9), 2086.
16. Baffel, S.O. and Ibrahim, M.M. (2008). Antioxidants and accumulation of α-tocopherol induce chilling tolerance in *Medicago sativa*. International Journal of Agriculture and Biology, 10(6), 593-598.

17. Bhandari, S.R., Yoon, M.K. and Kwak, J.H. (2014). Contents of phytochemical constituents and antioxidant activity of 19 garlic (*Allium sativum* L.) parental lines and cultivars. Hortic. Environ. Biotechnol., 55, 138-147.
18. Bhatti, B.S. (1988). Utilization of toxic plants for the control of nematode pest of economic crop. Final Technical Report April 1, 1983 to March 31, 1988, HAU Hissar, India 1988, 56.
19. Black, C.A., Evans, D.O., Ensminger, L.E. White, J.L., Clark, F.E. and Dinauer, R.C. (1982). Methods of Soil Analysis. part 2. Chemical and microbiological properties. 2nd Ed. Soil Sci., Soc. of Am. Inc. Publ., Madison, Wisconsin, U. S.A.
20. Builders, P.F., Ezeobi, C.R., Tarfa, F.D. and Builders, M.I. (2010). Assessment of the intrinsic and stability properties of the freeze-dried and formulated extract of *Hibiscus sabdariffa* Linn. (Malvaceae). African Journal of Pharmacognosy and Pharmacology, 4(6), 304-313.
21. Calvo, P., Nelson, L. and Kloepper, J.W. (2014). Agricultural uses of plant biostimulants. Plant Soil, 383, 3-41.
22. Canellas, L.P., Olivares, F.L., Aguiar, N.O., Jones, D.L., Nebbioso, A., Mazzei, P. and Piccolo, A. (2015). Humic and fulvic acids as biostimulants in horticulture. Sci. Hortic. *196*, 15–27.
23. Canellas, L.P., Piccolo, A., Dobbss, L.B., Spaccini, R., Olivares, F.L., Zandonadi, D.B. and Façanha, A.R. (2010). Chemical composition and bioactivity properties of size-fractions separated from a vermicompost humic acid. Chemosphere, 78, 457–466.
24. Caretto, S., Paradiso, A., D'Amico, L. and De Gara, L. (2002). Ascorbate and Glutathione Metabolism in Two Sunflower Cell Lines of Differing *α*-Tocopherol Biosynthetic Capability. Plant Physiol. Biochem., 40 (6), 509−513.
25. Chakraborty, M., Hasanuzzaman, M., Rahman, M., Khan, M.A., Bhowmik, P., Mahmud, N.U., Tanveer, M. and Islam, T. (2020). Mechanism of plant growth promotion and disease suppression by chitosan biopolymer. Agriculture, 10,1-30.
26. Che, R., Huang, L., Xu, J.W., Zhao, P., Li, T., Ma, H. and Yu, X. (2017). Effect of fulvic acid induction on the physiology, metabolism, and lipid biosynthesis-related gene transcription of *Monoraphidium* sp. *FXY-10.* Bioresour. Technol., 227, 324–334.
27. Chibu, H. and Shibayama, H. (2001). Effects of chitosan applications on the growth of several crops. In: Uragami T., K. Kurita, T. Fukamizo (eds.), Chitin and Chitosan in Life Science, Japan, pp. 235-239.
28. da Silva Lucas, A.J., Oreste, E Q., Costa, H.L.G., López, H.M., Saad, C.D.M. and Prentice, C. (2020). Extraction, physicochemi-cal characterization, and morphological properties of chitin and chitosan from cuticles of edible insects. Food Chemistry, 343 (128550), 1-42.
29. Daur, I. and Bakhashwain A.A. (2013). Effect of humic acid on growth and quality of maize fodder production. Pak. J. Bot., 45: 21-25.
30. Dawood, M.G., Abdel-Baky, Y.R., El-Awadi, M.E.S. and Bakhoum, G.S. (2019). Enhancement quality and quantity of faba bean plants grown under sandy soil conditions by nicotinamide and/or humic acid application. Bull. Natl. Res. Cent., 43, 28.
31. Diab, M.A. (1968). The chemical Composition of *Hibiscus sabdariffa*, L. M.Sc. Thesis, Fac. Agric., Cairo Univ.
32. Dixon, J.B. and Weed, S.B. (1989). Minerals in Soil Environments; Soil Science Society of America: Madison, WI, USA, p. 95.
33. do Rosario Rosa, V., Dos Santos, A.L.F., da Silva, A.A., Sab, M.P.V., Germino, G.H., Cardoso, F.B. and de Almeida Silva, M. (2021). Increased soybean tolerance to water deficiency through biostimulant based on fulvic acids and *Ascophyllum nodosum* (L.) seaweed extract. Plant Physiol. Biochem. 158, 228–243.
34. Drobek, M., Fra, C.M. and Cybulska, J. (2019). Plant biostimulants: Importance of the quality and yield of horticultural crops and the improvement of plant tolerance to abiotic stress: A review. Agronomy, 9, 335.
35. Du, C.T. and Francis, F.J. (1973). Anthocyanins of roselle. Amherst, Mass. J. Food Sci., 38(5), 310-312.
36. El Desuki, M., Hafez, M.M., Mahmoud, A.R. and Abd Albaky, F.S. (2010). Effect of organic and bio-fertilizers on the plant growth, green pod yield , quality of pea. International Journal of Academic Research, 2, 87-92.

37. El-Bassiouny, H.M., Gobarah, M.E. and Ramadan, A.A. (2005). Effect of antioxidants on growth, yield, and favism causative agents in seeds of *Vicia faba* L. plants grown under reclaimed sandy soil. Journal of Agronomy, 7(4), 653-659.
38. El-Desouky, S.A., Wanas, L.A. and Khedr, Z.M. (1998). Utilization of some natural plant extracts of garlic and yeast as cloves-soaking materials to squash (*Cucurbita pepo* L.). I. Effect on growth, sex expression and fruit yield and quality. Ann., Agric. Sci. Moshtohor, 36,839-854
39. Ellis, L.R., Zulfiqar, S., Holmes, M., Marshall, L., Dye, L. and Boesch, C. (2021). A systematic review and meta-analysis of the effects of *Hibiscus sabdariffa* on blood pressure and cardiometabolic markers. Nutrition Reviews, 80(6), 1723–1737.
40. El-Serafy, R.S. (2018). Growth and productivity of roselle (*Hibiscus sabdariffa* L.) as affected by yeast and humic acid. Sci. J. Flowers Ornam. Plants, 5, 195-203.
41. El-Tohamy, W.A. and El-Greadly, N.H.M. (2007). Physiological responses, growth, yield and quality of snap beans in response to foliar application of yeast, vitamin E and zinc under sandy soil conditions. Australian Journal of Basic and Applied Sciences 1: 294–299.
42. Fang, Z., Wang, X., Zhang, X., Zhao, D. and Tao, J. (2020). Effects of fulvic acid on the photosynthetic and physiological characteristics of *Paeonia ostii* under drought stress. Plant Signal. Behav.. 15 (7), 1774714.
43. Farruggia, D., Di Miceli, G., Licata, M., Leto, C., Salamone, F. and Novak, J. (2024). Foliar application of various biostimulants produces contrasting response on yield, essential oil and chemical properties of organically grown sage (*Salvia officinalis* L.). Front. Plant Sci. 15, 1397489.
44. Ghasemi Pirbalouti, A., Malekpoor, F., Salimi, A. and Golparvar, A. (2017). Exogenous application of chitosan on biochemical and physiological characteristics, phenolic content and antioxidant activity of two species of basil (*Ocimum ciliatum* and *Ocimum basilicum*) under reduced irrigation. Scientia Horticulturae, 217, 114–122.
45. Gomez, K.A. and Gomez, A,A. (1984). Statistical procedures for agricultural research, John Wiley & Sons.
46. Hanafy, M.S., Saadawy, F.M., Milad, S.M.N. and Ali, R.M. (2012). Effect of Some Natural Extracts on Growth and Chemical Constituents of *Schefflera arboricola* Plants, Journal of Horticultural Science & Ornamental Plants, 4(1), 26-33.
47. Havaux, M., Bonfils, J. P., Lutz, C. and Niyogi, K.K. (2000). Photodamage of the photosynthetic apparatus and its dependence on the leaf developmental stage in the npq1 Arabidopsis mutant deficient in the xanthophyll cycle enzyme violaxanthin de-epoxidase. Plant Physiology, 124(1), 273-284.
48. Havlin, J.L., Beaton, J.D., Tisdal, S.L. and Nelson, W.L. (2005) Soil fertility and fertilizers, an introduction to nutrient management. 7th ed., Upper Saddle River New Jersey. USA, 515 p.
49. Helmy, E.M.S. (1992). Response to summer squash application methods of fresh garlic extracted by different solvents. Alex. J. Agric., Res., 37, 125-142.
50. Hendawy, S.F., Hussein, M.S., El-Gohary, A.E. and Ibrahim, M.E. (2015). Effect of foliar organic fertilization on the growth, yield and oil content of *Mentha piperita* var. citrata. Asian J. Agric. Res., 9(5), 237-248.
51. Hess, J.L. (1983). Vitamin E, á-Tocopherol. In: Antioxidants in higher plants. R.G. Alscher and J.L. Hess (eds.). CRC press, Inc., Boca Raton., pp. 111-134.
52. Hussain, N., Irshad, F., Jabeen, Z., Shamsi, I.H., Li, Z. and Jiang, L. (2013). Biosynthesis, Structural, and Functional Attributes of Tocopherols in Planta; Past, Present, and Future Perspectives. J. Agric. Food Chem., 61 (26), 6137-6149.
53. Hussein, M.M., Balbaa, L.K. and Gaballah, M.S. (2007). Developing a Salt Tolerant Cowpea Using Alpha Tocopherol. Journal of Applied Sciences Research, 3, 1234-1239.
54. Jin, S. and Daniell, H. (2014) Expression of γ-Tocopherol Methyltransfer-ase in Chloroplasts Results in Massive Proliferation of the Inner Envelope Membrane and Decreases Susceptibility to Salt and Metal-Induced Oxidative Stresses by Reducing Reactive Oxygen Species. Plant Biotechnol. J., 12 (9), 1274−1285.
55. Juhi, A. and Ela, D. (2014). Current scenario of *Hibiscus sabdariffa* (Mesta) in India. International Journal of Social Science and Humanity Invent, 1(3), 129-135.
56. Kamel, S.M., I.Afifi, M.M., El-shoraky, F. and El-Sawy. M.M. (2014). Fulvic acid: a tool for controlling powdery and downy mildews in cucumber plants. Inter. J. Phytopathology, 3 (2),101-108.

57. Khalid, K.A., Omer, E.A., El Gendy, A.G. and Hussein, M.S. (2015). Impact of organic compost and humic acid on essential oil composition of sweet fennel (*Foeniculum vulgare* var. Dulce) under sandy soil conditions in Egypt. World J. Pharm. Sci., 3, 160-166.
58. Kulikova, N.A., Stepanova, E.V. and Koroleva, O.V. (2005). Mitigating activity of humic substances: Direct influence on biota. In: Use of humic substances to remediate polluted environments: From theory to practice (Ed.: I.U. Perminova). NATO Science Series IV. Earth and Environmental Series. Kluwer Academic Publishers, 285-309.
59. Lampkin, N. (1994). Organic farming. Published by Farming Press Books and Videos Wharfedale Road, Ipswich Ip14 LG, UK., pp, 13-85.
60. Li, M.S., Li, S. and Zhang, B.L.C. (2005). Physiological effect of new FA antitranspirant on winter wheat at ear filling stage. J. Agric. Sci. China. 11, 820-825.
61. Li, R.; He, J.; Xie, H.; Wang, W.; Bose, S.K.; Sun, Y.; Hu, J. and Yin, H. (2018). Effects of chitosan nanoparticles on seed germination and seedling growth of wheat (*Triticumae stivum* L.). International Journal of Biological Macromolecules, 126, 91-100.
62. Li, X., Li, X., Han, B., Zhao, Y., Li, T., Zhao, P. and Yu, X. (2019). Improvement in lipid production in *Monoraphidium* sp. QLY-1 by combining fulvic acid treatment and salinity stress. Bioresour. Technol., 294, 122179.
63. Mady, A. (2009). Effect of certain medicinal plant extracts on growth, yield and metabolism of some medicinal aromatic and plants. M.Sc., Al–Azher. Univ. Fac. of Sci. Botany and Microbiology Dep.
64. Maeda, H., Song, W., Sage, T.L. and DellaPenna, D. (2006). Tocopherols Play a Crucial Role in Low-Temperature Adaptation and Phloem Loading in Arabidopsis. The Plant Cell, 18, 2710-2732.
65. Malan, C. (2015). Review: humic and fulvic acids. A Practical Approach. In Sustainable soil management symposium. Stellen bosch, 5-6 November, Agrilibrium Publisher.
66. Martins, N., Petropoulos, S. and Ferreira, I.C.F.R. (2016). Chemical composition and bioactive compounds of garlic (*Allium sativum* L.) as affected by pre- and post-harvest conditions: A review. Food Chem. 2016(211), 41-50
67. Mohamed, H.I. and Akladious, S.A. (2014). Influence of garlic extract on enzymatic and non-enzymatic antioxidants in soybean plants (*Glycine max*) grown under drought stress. J. Life Sci., 11(3), 46-58.
68. Moradi. P., Babak, P. and Fayyaz, F. (2017). The effects of fulvic acid application on seed and oil yield of safflower cultivars. Agronomy for Sustainable Development, 584-597.
69. Muley, A.B., Shingote, P.R., Patil, A.P., Dalvi, S.G. and Suprasanna, P. (2019). Gamma radiation degradation of chitosan for application in growth promotion and induction of stress tolerance in potato (*Solanum tuberosum* L.). Carbohydrate Polymers, 210, 289–301.
70. Munné-Bosch, S. (2007). A-Tocopherol: A Multifaceted Molecule in Plants. *Vitam. Horm. Ser.*, *76*, 375−392.
71. Munné-Bosch, S. and Alegre, L. (2002). The function of tocopherols and tocotrienols in plants. Critical Reviews in Plant Sciences, 21(1), 31-57.
72. Munné-Bosch, S. and Falk, J. (2004). New insights into the function of tocopherols in plants. Planta, 218(3), 323-–326.
73. Nardi, S., Concher, G. and Agnola, G.D. (1996). Biological Activity of Humus. In Humic Substances in Terrestrial Ecosystems; Piccolo, A., Ed.; Elsevier: Amsterdam, The Netherlands, pp., 361–406.
74. Naskar, S., Sharma, S. and Kuotsu, K. (2019). Chitosan-based nanopar-ticles: An overview of biomedical applications and its preparation. Journal of Drug Delivery Science and Technology, 49, 66–81.
75. Noga, G. and Schmitz, M. (2000). Tocopherol and Its Potential for Improving Fruit Quality in Apple. Abstract from International Symposium on Growth and Development of Fruit Crops.
76. Noroozisharaf, A. and Kaviani, M. (2018). Effect of soil application of humic acid on nutrients uptake, essential oil and chemical compositions of garden thyme (*Thymus vulgaris* L.) under greenhouse conditions. Physiol. Mol. Biol. Plants, 24, 423–431.
77. Nour Eldeen, E.A. (2014). Effect of bio-fertilizers and plant extracts on growth and chemical constituents under water stress conditions of sage (*Salvia officinalis* L.) plant. Ph. D. Thesis, Fac. Agric., Mansoura Univ.

78. Nour, K.A., Mansour, N.T. and Eisa, G.S. (2012). Effect of Some Antioxidants on Some Physiological and Anatomical Characters of Snap Bean Plants under Sandy Soil Conditions. New York Science Journal, 5(5), 1-9.
79. Ogundapo, S.S., Onuoha, J.C., Olekanma, C.N., Okon, A.B., Soniran, O.T., Omoboyowa, D.A. and Okor, D.A. (2014). Alteration in biochemical parameters of *Hibiscus sabdariffacalyces* (zobo) supplemented with commercial flavor additive. Journal of Natural Product, 7, 116-123.
80. Olawale, A.S. (2011). Studies in concentration and preservation of sorrel extract. African Journal of Biotechnology, 10(3), 416-423.
81. Pal, P., Pal, A., Nakashima, K. and Yadav, B.K. (2020). Applications of chitosan in environmental remediation: A review. Chemosphere, 128934, 1–19.
82. Poorghadir, M., Torkashvand, A.M., Mirjalili, S.A. and Moradi, P. (2020). Interactions of amino acids (proline and phenylalanine) and biostimulants (salicylic acid and chitosan) on the growth and essential oil components of savory (*Satureja hortensis* L.). Biocatalysis and Agricultural Biotechnology, 30, 1–23.
83. Priya, B.N.V., Mahavishnan, K.,. Gurumurthy, D.S, Bindumadhava, H., Upadhyaya, A.P. and Sharmaa, N.K. (2014). Fulvic Acid (FA) for enhanced nutrient uptake and growth: Insights from biochemical and genomic studies. J. Crop Improvement, 28(6), 740-757.
84. Puvaˇca, N., Ljubojević, D., Lukač, D., Borojević, M., Kostadinović, L., Teodosin, S. and Stanaćev, V. (2014). Bioactive compounds of garlic, black pepper and hot red pepper. In: Proc XVI Int Symp, Feed Technology. Novi Sad, Serbia, pp., 116-122.
85. Qi, Y., Chin, K. L., Malekian, F., Berhane, M. and Gager, J. (2005). Biological characteristics, nutritional and medicinal value of roselle, *Hibiscus sabdariffa*. *Circular-urban Forestry Natural* Resources and Environment, 604, 1-2.
86. Salami, S.O. and Afolayan, A.J. (2020). Assessment of antimicrobial activities and toxicological effects of green and red cultivars of roselle – *Hibiscus sabdarifa* L. European Journal of Medicinal Plants, 31(15), 11-22.
87. Salman, D. and Al Abadi, I.M.K. (2009). Evaluation of some physiochemical and functional properties of ketosan prepared with an alkaline treatment of shrimp crusts. Iraqi Journal of Agricultural Sciences, 40, 63-75.
88. Santiago, A., Quintero, J.M., Carmona, E. and Delgado, A. (2008). Humic substances increase the effectiveness of iron sulfate and vivianite preventing iron chlorosis in white lupin. Biol. Fertil. Soils, , 44, 875–883.
89. Sattler, S.E., Gilliland, L.U., Magallanes-Lundback, M., Pollard, M. and DellaPenna, D. (2004). Vitamin E is essential for seed longevity and for preventing lipid peroxidation during germination", The Plant Cell, 16(6), pp. 1419-1432.
90. Shahryari, R., Khayatnezhad, M. and Bahari, N. (2011) Effect of two humic fertilizers on germination and seedling matter and a proposed modification of the chromic acid titration method. Soil Science, 34, 29-38.
91. Shariatinia, Z. (2019). Pharmaceutical applications of chitosan. Advances in Colloid and Interface Science, 263, 131–194.
92. Sheren, A., Abd, E. and El-Amary, E.I. (2015). Improving Growth and Productivity of "Pear" Trees Using Some Natural Plants Extracts under North Sinai Conditions. IOSR J. Agric. Vet. Sci., 8, 01–09.
93. Sikandar H, Ahmad H, Ali M, Kashif H, Khan MA, Cheng Z, Smith FMA, Gilles JK, Hamilton P (2018) Aqueous garlic extract as a plant biostimulant enhances physiology, improves crop quality and metabolite abundance, and primes the defense responses of receiver plants. Appl. Sci., 8(5),15.
94. Sini, J.M., Umar, I A. and Inuwa, H. M. (2011). The beneficial effect of the extract of Hibiscus sabdariffa calyces in Alloxan diabetic rats: Hypoglycaemic and Hypolipidaemic activities. Journal of Medicinal Plant Research, 5(11), 2182-2186.
95. Slattery, R.A., VanLoocke, A., Bernacchi, C.J., Zhu, X.G. and Ort, D.R. (2017). Photosynthesis, Light Use Efficiency, and Yield of Reduced-Chlorophyll Soybean Mutants in Field Conditions. Front. Plant Sci., 8, 549.
96. Speiciene, V., Guilmineau, F.U. and Kulazik, L.D. (2007) The effect of chitosan on the properties of emulsions stabilized by whey proteins. Food Chemistry, 102, 1048-1054.

97. Suh, H.Y., Yoo, K.S. and Suh, S.G. (2014). Effect of foliar application of fulvic acid on plant growth and fruit quality of tomato (*Lycopersicon esculentum* L.). Horticulture, Environ. Biotechnol., 55(6), 455–461.
98. Taj Eldin, Majid, M. and Al Barakat, H.N.K. (2016). The effect of bio-fertilization, foliar spray, and ground addition of humic and fulvic acid on the growth and yield of maize (*Zea mays* L.). Al-Muthanna University Journal for Agricultural Sciences, 4, 75 - 68.
99. Teye, M., Victor, Y.A.B. and Faustina, F. (2019). Phytochemical, proximate and mineral analyses. Africa Journal of Biochemistry and Research, 13(7), 90-95.
100. Vosoughi, N., Gomarian, M., Ghasemi Pirbalouti, A., Khaghani, S. and Malekpoor, F. (2018). Essential oil composition and total phenolic, flavonoid contents, and antioxidant activity of sage (*Salvia offici-nalis* L.) extract under chitosan application and irrigation frequencies. Industrial Crops and Products, 117, 366–374.
101. Wang, H., Li, X., Shen, D., Oiu, Y. and Song, J. (2014). Diversity evaluation of morphological traits and allicin content in garlic (*Allium sativum* L.) from China. Euphytica, 198, 243–254.
102. Yang, C.M., Wang, M.H., Lu, Y.F., Chang, I.F. and Chou, C.H. (2013). Humic substances affect the activity of chlorophyllase. Chem. Ecol. J., 30, 1057-1065.
103. Zahra, M.K., Monib, M., Abbdel-Al, Sh.I. and Heggo, A. (1984). Significance of soil inoculation with silicate bacteria. Zentralblatt fur Mikrobiologie, 139, 349-357.
104. Zhang, W.F., He, Y.L., Zhang, M.S., Yin, Z. and Chen, Q. (2000). Raman scattering study on anatase TiO_2 nanocrystals. Journal of Physics D: Applied Physics, 33(8), 912.
105. Ziedan, E.H. and Eisa, E.A. (2016). The use of some micronutrients and plant extracts of resistance topowdery mildew and nutrition dill plants in the Gharbiyah Governorate. J. Plant Prot. and Path., Mansoura Univ., 7(9), 579-586.