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Effect of Foliar Application of Grape and Pumpkin Seed Oils on Productivity and Chemical Composition of Garlic Under Salinity Condition

Mallak M. Zikry*, Jackleen A. Khalil and Eman A. Elkamshoush

Medicinal and Aromatic Plants Researches Department, Horticulture Researches Institute, Agricultural Research Center, Giza, Egypt.

***Corresponding author:** mallak_src@yahoo.com

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Abstract: This research was carried out at Wadi El Natrun, Beheira Governorate, Egypt, during two seasons (2024-2025 and 2025-2026) in saline sandy soil. This study was carried out to evaluate the effect of grape seed oil (GO) and pumpkin seed oil (PO) at three concentrations (3, 6 and 9 ml/l) on productivity and phytochemical composition of *Allium sativum* L. under salinity stress. The experiments were designed with three replicates in a completely randomized blocks design. PO and GO at 9 ml of each showed highest values of vegetative growth and bulb yield/feddan. GO at 9 ml increased antioxidants, chlorophyll, and the K/Na ratio as K % increased. Gas chromatography mass spectrometry (GC-MS) analysis for the three garlic extract revealed organo sulfur compounds, fatty acids, and sugar derivatives. GO at 9 ml treatment showed the highest abundance of Trisulfide, di-2-propenyl (41.06 %) compared to control. GO at 9 ml treatment increase the stabilization of these compounds compared to control, also glucopyranoside derivatives and n-Hexa decanoic acid was detected. The variation in the concentration of these metabolites indicated the treatments have a vital role in the stability of the active phytochemicals. These results indicate that GO at 9 ml treatment as an eco-friendly, cost-effective bio stimulant for improving productivity and phytochemical quality of garlic in saline sandy soils.

Key words: *Allium sativum* L, soil salinity, grape and pumpkin-seed oil, Bio-stimulants, GC-MS

1. Introduction

Garlic (*Allium sativum* L.) is a vital crop with both financial and health advantages. It is the most significant vegetable in Egypt. Additionally, it is grown for medicinal purposes **(Collin, 2004; Awad-Allah *et al*., 2020).** *Allium sativum* L.'s high concentration of organo sulfur compounds, polyphenols, and flavonoids enhances its therapeutic qualities as an antioxidant, anti-cancer, anti-diabetic, and preventive agent against cardiovascular illnesses (***Batiha et al., 2020***). Abiotic stressors, such as salinity of the soil, pose a threat to the cultivation of *Allium sativum* L and pose a problem for food security in Egypt and around the world (**Munns, and Gilliham, 2015**). Through osmotic stress, salinity stress negatively impacts plant physiological systems, resulting in stunted growth, decreased photosynthetic efficiency, and decreased agricultural output (**Zulfiqar, and Ashraf, 2021; Shabala *et al*., 2020).**

By synthesizing suitable components and activating antioxidant systems, plants have defense mechanisms against these detrimental impacts. However, in salinity conditions, these reactions are inadequate. Therefore, mitigation methods must be used **(Hussain *et al.*, 2021).** Plant growth, productivity, and bulb quality are influenced by a variety of factors, including fertilizer and bio-stimulating chemicals **(El-Morsy, 2004)**. A useful strategy for protecting crops from salt stress is the foliar spray of bio stimulants produced from medicinal plants (**Gholami *et al*., 2021**). Extracts from aromatic and medicinal plants are rich in active phytochemicals. Antioxidants are the name given to these compounds. These antioxidants eliminate free radicals, reactive oxygen species, and chelated metal structures (**Maisuthisakul *et al*., 2008**).

Enzymes, vitamins, and amino acids are among the many phytochemicals found in aromatic and therapeutic plant oils. Aromatic medicinal herbs have antibacterial, antifungal, and anti-cancer effects because of these phytochemicals **(Abu-Dief, *et al.*, 2020; Abdel-Rahman *et al.*, 2022; Elkamshoush *et al.*, 2025).** When applied topically, these oils have a stimulating effect on plant growth and production (**Shalaby, and El-Ramady, 2014**). In many parts of the world, grapes are grown as a fruit crop. Due to its high phenolic chemical, fatty acid, and vitamin content, grape seed fixed oil is economically significant to the culinary, cosmetic, and pharmaceutical industries. Cardio protective, antibacterial, anti-inflammatory, and anticancer qualities are among the health benefits of grape seed oil. The components of grape seed fixed oil, such as linoleic acid, quercetin, carotenoids, and phytosterols, are responsible for these effects.

Eight to twenty percent of grape seed is oil (**Rombaut *et al.*, 2015**). The kind of solvent, the extraction method, and the harvesting year's environmental conditions all affect oil yield **(Duba, K.S., and Fiori, 2015)**. 15.4 % and 14.7 % had the highest oil concentrations **(Agostini *et al.*, 2012).** The most prevalent component of cold-pressed grape seed oils is linoleic acid. Vitamin E levels in grape seed oil range from 1 to 53 mg per 100 g of oil (**Fernandes *et al.*, 2013)**. Phytosterols are additional lipophilic components of grape seed oil. The high antioxidant potential of grape seed oil is caused by the combination of these phenolic compounds: gallic acid, catechin, epicatechin, procyanidins, and proanthocyanidins **(Shinagawa; *et al.*, 2015; Rombaut *et al*., 2015; Vivancos *et al.*, 2008; Khurana *et al.*, 2013).** Proanthocyanidins found in grape seed oil (GSO) provide the antioxidant activity (**Khalaf *et al.*, 2022**). These substances are in charge of preventing oxidative damage and maintaining the integrity of cellular membranes (**El-Sayed *et al.*, 2020**). Pumpkin seed oil (PSO) has antioxidant qualities because of its high phytosterol and fatty acid content (**Jabali *et al.*, 2021**). By eliminating free radicals, this oil functions as an antioxidant **(Amara; *et al.*, 2008).** Some illnesses have been treated using pumpkin extracts **(Fruhwirth, and Hermetter, 2007; Stevenson *et al.*, 2007**). Pumpkin seed oil contains omega 3 and 6, which are critical for both prostate issues and bodily functions. For medicinal purposes, numerous researchers investigate the extraction, separation, and characterization of oil bodies from pumpkin seeds **(Taylor *et al.*, 2006; Adams *et al.*, 2012; Kim *et al.*, 2012).** Fatty acids, vitamin E, and phytosterols found in pumpkin seed oils support their use in the medicinal sector **(Potocnik *et al.*, 2016; Morakul *et al.*, 2019, Wong et al., 2019).** Pumpkin seed oil is antioxidant **(Eraslan *et al.*, 2013; Boujemaa *et al.*, 2020),** anticancer **(Arslanbaş *et al.*, 2020; Nomikos *et al.*, 2021; Krishna *et al.*, 2012),** and preventive against prostate and cardiovascular issues (**Taha *et al.*, 2021**, **Amirzargar *et al.*, 2021).** Under salinity stress, the foliar application of these oils improves plant metabolism, increases chlorophyll, improves nutrient uptake, and boosts plant yield **(Sharma *et al.*, 2021)**. By applying biostimulants topically, many crops can withstand salt stress (**Taha *et al.*, 2021**). This study examines the effects of applying grape seed and pumpkin seed oils topically on the yield and chemical composition of *Allium sativum* L. under salt stress. Through increased productivity and secondary metabolite content, these bio stimulants improve *Allium sativum* L.'s biochemical and physiological tolerance to salinity. This research will provide a good and eco-friendly method for sustainable production of *Allium sativum* L. at saline sandy soils**.** The aim of present study is to**:** (a) Investigate the effect of GO and PO at 3 different concentrations on photosynthetic efficiency, antioxidant activity, growth, yield, proline content, and K^+/Na^+ ionic balance in garlic under salinity stress. (b) Screen the phytochemical profile of garlic extracts from our treatments using GC-MS analysis.

2. Materials and Methods

2.1. Experiment location and soil analysis

Field experiment carried out on *Allium sativum* L. Chinese purple garlic grown in saline sandy soils during two seasons (2024-2025 and 2025-2026) was applied at a farm located on Kilometer 113, Cairo, Alexandria desert road, Beheira Governorate (Wadi El Natrun), Egypt. Coordinates, Latitude: 30.558° East, and Longitude: 30.551° North, Egypt. Table 1 indicated the physical and chemical analysis of soil in the site of experimental; the soil samples were taken at 25 cm depth and dried in room temperature for analysis according to standard methods. Values of electrical conductivity (EC) (4.23 and 4.40 dS m^{-1} in the first and second seasons) respectively, confirmed saline soil conditions.

Table (1). Physical and chemical properties of soil in two seasons (2024-2025 and 2025-2026)

Properties	2024-2025 Season	2025-2026 Season
Physical properties		
Sand %	93.4	93.1
Silt %	3.75	3.9
Clay %	2.85	3
Soil texture	Sandy	Sandy
Chemical properties		
pH	8.10	8.22
EC (dS/m)	4.23	4.40
Cations (meq/L)		
K^+	1.18	1.23
Na^+	20.81	20.99
Mg^{++}	9.25	9.28
Ca^{++}	12.06	12.02
Anions (meq/L)		
SO_4^{2-}	21.27	21.32
Cl^-	19.96	19.99
HCO_3^-	1.98	1.95
CO_3^{2-}	0.09	0.07

2.2. Treatments and experiment design for two seasons (2024 - 2025 and 2025 - 2026)

The experiment was designed in a complete randomized block design at three replicates for each treatment. This experiment included 11 treatments. The unit area for each plot was 10.5 m^2 (5 rows, 3 m length and 70 cm width). *Allium sativum* L. cloves were selected uniform in shape and size. The cloves were sown at distance of 7 cm apart. Planting date was on October 5^{th} and 7^{th} during the first and the second seasons, respectively. The cultural practices were done according to the recommendation of Ministry of Agriculture, Egypt.

2.3. Fertilization system

The fertilization system was applied to introduce nutrients with the crucial stages of *Allium sativum* L. fertilizer treatments were carried out by using drip irrigation technique. The fertilizer NPK (20:20:20) was introduced (one kilogram /feddan). As *Allium sativum* L. moved toward the bulbing stage, micronutrients were applied twice a month. (Fe- EDTA, 0.5 kg /feddan), (Mn- EDTA, 0.5 kg/ feddan), and (Zn-EDTA, one kg /feddan) Concentrated on increasing bulb diameter and dry matter at the final stage, the following macronutrients ($MgSO_4$, four kilogram /feddan) and (K_2SO_4, four kilogram/ feddan) were included in the fertilization system. Also H_3PO_4 85 %, (two litre /feddan) and HNO_3 (One litre / feddan) were applied. Feddan = 4200 m^2 = 0.42 hectare

2.4. Biostimulant Treatments applied

Eleven foliar treatments were applied at 45 and 90 days after planting in both seasons (2024 / 2025 and 2025 / 2026). Table 2 give description of biostimulant treatments applied in this study. All foliar treatments were applied using a hand held pressure sprayer. Each treatment was replicated three times. Humic acid (K-humate at 6 kg/feddan) as a positive control is applied to garlic via soil drenching to leach sodium and enhance nutrient uptake. It was split into 3 times: at planting, during vegetative growth (35 day after planting) and at bulb initiation (70 day after planting).

Table (2). Description of treatments applied in the study in the two seasons (2024 - 2025 and 2025-2026)

Treatment	Description
GO 3	Grape seed fixed oil (3 mL/L)
GO 6	Grape seed fixed oil (6 mL/L)
GO 9	Grape seed fixed oil (9 mL/L)
PO 3	Pumpkin seed fixed oil (3 mL/ L)
PO 6	Pumpkin seed fixed oil (6 mL/ L)
PO 9	Pumpkin seed fixed oil (9 mL/ L)
HA	Humic acid (1 kg/ feddan) positive control
GO + PO 3	Mixture of GO and PO (1:1) (3 mL/ L)
GO + PO 6	Mixture of GO and PO (1:1) (6 mL/ L)
GO + PO 9	Mixture of GO and PO (1:1) (9 mL/ L)
Control	water

GO (grape seed fixed oil); PO (pumpkin seed fixed oil); HA (humic acid).

Using laboratory expeller press, cold pressed grape seed fixed oil was extracted. Seeds dried in oven (35 ºC, 6 h). One kg of seeds was pressed in each cycle of extraction. Oils were clarified by sedimentation. Extracted grape seed oil was stored in dark glass bottles under nitrogen at - 18 °C until further analysis (**Cecchi *et al.*, 2019**). Grape seeds containing 40 % fiber, 16 % oil, 11 % proteins, and 7 % complex phenols such as tannins.

Also cold pressed pumpkin seed fixed oil was extracted. Seeds dried and pressed. Extracted grape seed oil was stored in dark glass bottles until further analysis. The pumpkin seed oil contains a high amount of free fatty acids (oleic, linoleic,) are present with the relative distribution of 43.8 %, 33.1 % respectively, (**Badr *et al.*, 2011**). The oil content of dry pumpkin seeds is 47.03 % ((**Younis *et al.*, 2000**). Pumpkin seed oil is used as nutritional supplements for natural source of proteins, fatty acids, omega 3, 6 and 9, carotenes, lutein, vitamins, chlorophyll, zinc, and selenium (**Gossell-Williams *et al.*, 2006; Tsai *et al.*, 2006).**

2.5. Data Collection and Measurements

2.5.1. Vegetative and Yield parameters

At 150 days after planting (at harvesting date), random selections of ten plants from each plot were made to assess various parameters: fresh weight (g), dry weight (g), height (cm), leaf number, fresh leaves (g), dry leaves (g), bulb diameter (cm), clove number, fresh bulb (g), dry bulb (g), fresh weight of five cloves (g) , and dry weight of five cloves (g). To obtain the data for dry weight, various parts of the *Allium sativum* L. plant were dried at 70° C in an oven until a constant weight was achieved.

2.5.2. Chemical composition

a- Photosynthesis pigments

A 500 mg of *Allium sativum* L. Fresh leaves from every sample was macerated in 25 ml ethanol for 72 hour to obtain the extraction, chlorophyll A and B and carotenoid were determined in the clarified extract at 662 nm, 646 nm and 470 spectrophotometry and calculated according to **(Lichtenthaler *et al.*, 2006)** respectively.

b- Antioxidants activity %

According to the method described by **(Burits *et al.*, 2000)** the antioxidants activity was determined in ethanol extraction by DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay at 517 nm where ascorbic acid was the standard substrate, the antioxidants activity calculated as;

$$\text{Antioxidants activity \%} = (A_{blank} - A_{sample}/A_{blank}) \times 100 \qquad (1)$$

c- Proline content

At the end of experiment proline content was determined in fresh leaves of *Allium sativum* L. plant samples calorimetrically by the method described by **(Bates *et al.*, 1973)**

d- Sodium (Na), Potassium (K) content and K^+/Na^+ ratio

The samples of dried leaves of *Allium sativum* L. plant were used to determine the sodium and potassium content by flame photometry. While, the values of K^+/Na^+ ratio obtained by dived K^+ to Na^+ value for every treatment.

2.5.3. GC-MS (Gas chromatography mass spectrometry) analysis

GC system was used (GC–MS QQQ 7890B) to screen the active compound in *Allium sativum* L. cloves extracts for controls and the best treatment obtained from this study. Helium was the carrier gas, the column was agilent 1909 1S-433:1[Hp-5MS UI, 325°], (C: 30m x 250µm x 0.25 µm, in: front mm Inlet He and Out: Vacuum. The front MM inlet He While, The injector temperature 250 °C with injection volume 2 µL. According to the library of NIST and Wiley databases, run time 64 min. Retention time (Rt) and mass fragmentation of active compound was used to compare identified compound **(Kobacy *et al.*, 2025)**.

2.6. Statistical analysis

Statistical analyses were performed using appropriate software (MSTAT-C). Treatment means were compared using the Least Significant Difference (LSD) test at $p \leq 0.05$. All data were subjected to analysis of variance (ANOVA) using a completely randomized block design.

3. Results and Discussion

3.1. Vegetative Growth and Bulb Yield

During both the 2024/2025 and 2025/2026 growing seasons, the application of different bio-stimulants (GO, PO, Humic acid, and their combinations) significantly improved the vegetative growth parameters of *Allium sativum* L. plants (Tables 3 and 4). The treatment GO 9 ml/L consistently performed better than other individual treatments, recording the highest values for plant height, fresh/dry weights, and leaf number, followed by PO 9 ml/L. This increase in vegetative biomass is probably caused by the presence of protein hydrolysates or extracts that act as signaling molecules to initiate cell division **(Calvo *et al.*, 2014, Colla *et al.*, 2015).**

The significant rise in the dry weight of leaves suggests that these stimulants did more than just improve water intake; they actively promoted the production of structural components and carbon assimilation (**Yakhin *et al.*, 2017**). Additionally, the Humic acid and GO 9 treatment outperformed the control (Water), most likely functioning as a natural chelator that improves root membrane permeability and raises the bioavailability of micronutrients in the rhizosphere**, (Varanini *et al.*, 2001).** When compared to GO 9 alone, the treatment (GO+PO 9 showed substantial but marginally weaker responses, indicating potential minor antagonistic interactions at higher concentrations. Bio-stimulants encourage the "Source" (leaf canopy) to grow, which increases solar energy absorption. Then, through up-regulated phloem loading mechanisms, this energy is transferred to the bulb as sucrose. **(Nardi *et al.*, 2016)**. Increase in dry matter showed that these treatments prioritize the accumulation of carbohydrates.

All biostimulants treatments improved total yield, bulb weight and bulb diameter compared to the control. The application of GO 9 and PO 9 treatments increased bulb weight and diameter significantly in both seasons. The positive effect GO 9 and PO 9 treatments is may be attributed to the increase in plant nutrient contents, amino acids. Amino acids increase the metabolic processes and the levels of endogenous hormones, which affect the bulb weight and size **(Sarhan *et al.*, 2010).**

Table (3). Effect of treatments on the vegetative growth at (2024/2025 and 2025/2026 seasons)

Seasons	**2024/2025**						**2025/2026**					
Traits / **Treatment**	FW plant (g)	DW plant (%)	plant height (cm)	Leaves No/ plant	FW leaves (g)	DW leaves (%)	FW plant (g)*	DW plant (%)*	plant height (cm)*	Leaves No/ plant*	FW leaves (g)*	DW leaves (%)*
GO 3 ml / L	67.55 bc	39.95 bc	56.5 abc	9.0 b	19.5 abcd	6.88 ab	69.50 bc	41.89 bc	57.50 abc	11 bc	20.35 ab	7.82 ab
GO 6 ml/ L	83.00 ab	45.49 ab	57.5 ab	9.0 b	24.9 abc	8.99 a	84.80 ab	46.89 ab	57.50 abc	11 bc	25.75 a	9.40 a
GO 9 ml/ L	94.15 a	59.44 a	62.5 a	13.0 a	27.2 a	9.24 a	96.15 a	60.87 a	63.50 a	15 a	28.00 a	9.76 a
PO 3 ml/ L	46.05 cd	27.30 cd	49.0 d	9.0 b	15.1 cd	5.11 bc	46.65 cd	28.12 cd	50.00 d	10 bc	15.95 b	5.45 bc
PO 6 ml/ L	54.30 c	29.63 bcd	50.5 cd	9.0 b	15.9 cd	5.19 bc	55.05 c	31.30 bcd	51.50 cd	11 bc	16.40 b	5.78 bc
PO 9 ml/ L	93.75 a	57.78 a	61.5 a	10.0 b	26.1 ab	9.20 a	95.15 a	59.37 a	62.50 a	12 b	26.80 a	9.63 a
Humic acid	59.90 bc	33.81 bcd	53.5 bcd	9.0 b	16.65 bcd	5.78 bc	61.75 c	35.54 bcd	54.50 bcd	11 bc	17.05 b	6.59 bc
GO + PO 3 ml/ L	59.90 bc	32.99 bcd	52.75 bcd	9.0 b	16.55 bcd	5.66 bc	61.40 c	33.79 bcd	53.50 bcd	11 bc	16.50 b	6.00 bc
GO+PO 6 ml/ L	60.90 bc	35.97 bc	55.0 bcd	9.0 b	19.4 abcd	6.39 bc	62.35 c	37.78 bc	55.95 bcd	11 bc	19.95 ab	7.12 ab
G O+ PO 9 ml/ L	88.60 a	54.34 a	58.5 ab	10.0 b	25.8 ab	8.99 a	90.26 a	55.84 a	59.5 ab	11 bc	26.50 a	9.58 a
Water	33.50 d	19.76 d	42.5 e	8.0 b	12.1 d	3.92 c	35.15 d	21.07 d	42.00 e	9 c	12.75 b	4.23 c
LSD (p ≤ 0.05)	16.25	10.69	4.35	1.0	6.27	1.79	16.14	10.69	4.26	2	6.18	1.78

FW(fresh weight); DW (dry weight percentage). *Season (2025/2026 data). Means followed by different lowercase letters are significantly different at p ≤ 0.05 (LSD test).

Table (4). Effect of treatments on quality of bulbs at (2024/2025 and 2025/2026 seasons)

Seasons	2024/2025						2025/2026					
Traits Treatment	FW Bulb (g)	DW Bulb (%)	Bulb diameter (cm)	cloves No/ Bulb	FW 5 cloves (g)	DW 5 cloves (%)	FW Bulb (g)*	DW Bulb (%) *	Bulb diameter (cm)*	cloves No/ Bulb	FW 5 cloves (g)*	DW 5 cloves (%)*
GO 3 ml / L	48.15 bc	33.07 cd	4.93 bcd	17 ab	8.75 cd	6.69 bc	49.15 bc	34.07 cd	5.75 abc	17 ab	9.75 cd	7.69 bc
GO 6 ml/ L	53.70 a	45.09 ab	5.41 ab	19 ab	11.75 bc	8.61 bc	56.80 ab	37.49 bc	5.82 abc	19 ab	10.82 cd	8.21 bc
GO 9 ml/ L	55.8 ab	36.50 bc	5.17 abc	19 ab	9.82 cd	7.21 bc	69.35 a	51.24 a	6.44 a	22 a	17.60 a	13.94 a
PO 3 ml/ L	30.15de	22.11de	4.29ef	14ab	7.2cd	5.67c	30.15 de	22.11 de	4.67 e	14 b	8.20 cd	6.67 c
PO 6 ml/ L	37.65 cd	24.52 cde	4.45 de	17 ab	7.95 cd	5.94 c	38.65 cd	25.52 cde	4.95 de	17 ab	8.95 cd	6.94 c
PO 9 ml/ L	67.65 a	48.78 a	5.41 ab	20 ab	13.65 ab	10.28 b	68.65 a	49.78 a	6.11 ab	20 ab	14.65 ab	11.28 ab
Humic acid	44.8 bcd	30.18 cd	4.77 cde	17 ab	8.65 cd	6.64 bc	44.35 bc	28.42 cde	5.26 cde	17 ab	9.65 cd	7.32 bc
GO + PO 3 ml/ L	41.40 bcd	27.33 cde	4.61 de	17 ab	8.6 cd	6.14 bc	42.40 bcd	28.33 cde	5.03 de	17 ab	9.60 cd	7.14 bc
GO + PO 6 ml/ L	43.35 bcd	27.42 cde	4.77 cde	17 ab	8.65 cd	6.32 bc	45.80 bc	31.18 cd	5.55 bcd	17 ab	9.65 cd	7.64 bc
GO + PO 9 ml/ L	68.35 a	50.24 a	5.57 a	21 a	16.6 a	12.94 a	64.51 a	46.08 ab	6.01 ab	19 ab	12.75 bc	9.61 bc
Water	21.4 e	15.84 e	3.98 f	13 b	6.55 d	5.31 c	22.40 e	16.84 e	4.56 e	13 b	7.00 d	5.45 c
LSD ($p\leq 0.05$)	10.44	9.13	0.38	5	3.11	2.65	10.42	9.12	0.52	4.00	3.09	2.67

FW (fresh weight); DW (dry weight percentage). *Season (2025/2026 data). Means followed by different lowercase letters are significantly different at $p \leq 0.05$ (LSD test).

3.2. Photosynthetic Efficiency and Antioxidant Capacity

Chlorophyll A, B and beta-carotene levels have increased, according to data in Table 5. The high-dose GO 9 and PO 9 treatments showed the strongest antioxidant activity, Figures (1). Increased beta-carotene has two functions: it is a powerful antioxidant that quenches singlet oxygen, preventing Photosystem II from being photo-inhibited, and it is an accessory light-harvesting pigment (**Ertani *et al.*,2014**). In both seasons, the use of biostimulants greatly increased the amounts of photosynthetic pigment and antioxidant activity in garlic leaves. In addition to having the best antioxidant activity (66.68% DPPH inhibition), GO 9 mL/L had the highest amounts of β-carotene (29.36 μg/g FW), chlorophyll A (0.25 mg/g FW, season 2), and chlorophyll B (0.32 mg/g FW). The highest antioxidant capacity reached about 66.68% in the second season; this up-regulation of the antioxidant defense system suggests that bio-stimulants prime the plant to neutralize reactive oxygen species (ROS), thereby protecting cellular integrity and the photosynthetic apparatus. PO 9 mL/L achieved comparable pigment and antioxidant levels, while the distilled water control recorded the lowest values throughout. The increase in chlorophyll biogenesis and protection of thylakoid membranes from peroxidative damage (**Rouphael *et al.*, 2020**). There was a dose-dependent rise in secondary metabolites such beta-carotene and total antioxidant percentage. In the second season, the maximum antioxidant capacity was almost 66.68%. This up-regulation of the antioxidant defense system implies that bio-stimulants prepare the plant to counteract reactive oxygen species (ROS), hence preventing oxidative damage to the photosynthetic equipment and cellular integrity (**Bulgari *et al.*, 2019**).

Table (5). Effect of biostimulants on Chlorophyll, B- carotene and antioxidant at (2024/2025 and 2025/2026 seasons)

Seasons	2024/2025				2025/2026			
Traits Treatment	Chl. A	Chl. B	β-carotene	Antioxidant (%)	Chl. A*	Chl. B*	β-carotene*	Antioxidant (%)*
GO 3 ml /L	0.196 ab	0.216 e	26.63 c	60.68 d	0.22 bc	0.24 bc	27.63 c	61.68 d
GO 6 ml/ L	0.20 a	0.226 d	26.98 c	61.93 c	0.23 ab	0.27 b	27.98 c	62.93 c
GO 9 ml/ L	0.20 a	0.276 a	28.36 a	65.68 a	0.25 a	0.32 a	29.36 a	66.68 a
PO 3 ml/ L	0.126 d	0.156 g	19.86 g	52.74 h	0.13 e	0.16 d	20.25 f	53.74 h
PO 6 ml/ L	0.146 c	0.196 f	24.02 f	58.84 g	0.18 d	0.21 c	25.02 e	59.84 g
PO 9 ml/ L	0.20 a	0.256 b	27.77 b	62.57 b	0.24 a	0.32 a	28.53 b	63.57 b
Humic acid	0.190 b	0.196 f	25.59 e	59.52 f	0.21 bc	0.22 c	26.34 d	60.52 f
GO + PO 3 ml/L	0.190 b	0.196 f	25.30 3	58.9 g	0.20 c	0.22 c	26.30 d	59.9 g
GO + PO 6 ml/L	0.190 b	0.196 f	26.13 d	59.91 e	0.21 bc	0.23 c	26.66 d	60.91 e
G O+ PO 9 ml/L	0.20 a	0.246 c	27.49 b	62.54 b	0.23 ab	0.28 b	28.00 c	63.54 b
Water	0.096 e	0.150 g	16.54 h	48.88 i	0.10 f	0.15 d	16.79 g	49.93 i
LSD ($p \leq 0.05$)	0.0059	0.0078	0.36	0.21	0.015	0.029	0.42	0.22

Chl. (chlorophyll) (mg /g FW); β-carotene (µg/ g FW); Antioxidant (DPPH radical scavenging activity). *Season (2025/2026 data). Means followed by different lowercase letters are significantly different.

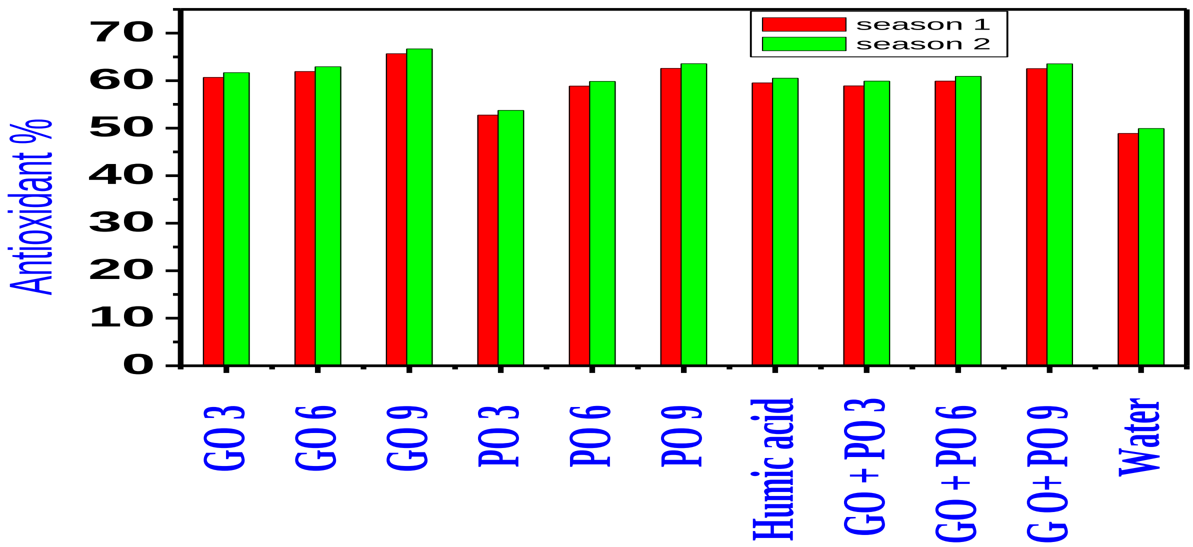

Fig (1). Effect of biostimulants on antioxidant % of *Allium sativum* L. leaves at (2024/2025 and 2025/2026 seasons)

3. 3 Osmotic Regulation and Ionic Balance

Proline and the K/Na ratio significantly increased in treated plants, as seen in Table 6. Bio-stimulants increase the potassium channels. GO 9 mL/L showed the best K^+/Na^+ ratio (0.29 and 0.37) and the maximum proline accumulation (20.94 and 21.94 µg /g FW in seasons 1 and 2), whereas the negative control showed the lowest values for both indicators, Figures (2). The plant avoids sodium toxicity and sustains the cytosolic enzyme activity necessary for protein synthesis by keeping a high K/Na ratio (**Bose *et al.*, 2014**).

As a "compatible solute," high proline levels help the plant sustain turgor pressure even in the face of varying ambient water potentials (**Hayat *et al.*, 2012**). Proline sheds light on the nutritional value and stress-tolerance processes of Allium sativum L. When high-dose stimulants (GO and PO 9 ml/L) were applied, proline elevated. Proline stabilizes proteins by acting as a molecular chaperone and essential osmoprotectant (**Ashraf *et al.*, 2007**). A favorable K/Na ratio resulted from an increase in K% and a controlled Na%. Protein synthesis and stomatal control both depend on potassium (K), an essential cofactor enzyme (**Du Jardin *et al.*, 2015**). Superior ionic homeostasis can be maintained by biostimulants, and a high K/Na ratio is a reliable sign of improved bulb quality and metabolic vigor.

Table (6). Effect of biostimulant on proline, K/Na ratio of *Allium sativum* L. leaves at (2024/2025 and 2025/2026 seasons)

Seasons	2024/2025				2025/2026			
Treatment Traits	Proline	K %	Na %	K / Na	Proline*	K % *	Na % *	K / Na*
GO 3 ml / L	16.27 c	0.76 c	3.20 c	0.24 c	17.27 c	0.83 b	3.01 c	0.28 bc
GO 6 ml/ L	16.65 b	0.77 c	3.32 b	0.25 bc	17.65 b	0.84 b	3.07 c	0.29 b
GO 9 ml/ L	20.94 a	0.92 a	3.75 a	0.29 a	21.94 a	1.11 a	4.20 a	0.37 a
PO 3 ml/ L	9.50 g	0.46 f	2.30 f	0.21 e	10.50 f	0.53 c	2.05 e	0.23 cd
PO 6 ml/ L	9.55 g	0.66 e	2.91 e	0.21 de	10.55 f	0.75 b	2.41 d	0.26 bc
PO 9 ml/ L	16.77 b	0.89 b	3.37 b	0.26 b	17.77 b	1.05 a	3.56 b	0.35 a
Humic acid	13.31 e	0.71 d	3.05 d	0.22 d	14.31 e	0.82 b	2.63 d	0.27 bc
GO + PO 3 ml/ L	10.44 f	0.69 d	2.96 de	0.22 de	10.84 f	0.76 b	2.61 d	0.26 bc
GO + PO 6 ml/ L	14.84 d	0.75 c	3.05 d	o.24 c	15.57 d	0.82 b	3.00 c	0.27 bc
G O+ PO 9 ml/ L	16.71 b	0.77 c	3.36 b	0.26 b	17.71 b	0.86 b	3.10 c	0.33 a
Water	7.54 h	0.46 f	2.21 g	0.15 f	8.54 g	0.53 c	2.03 e	0.20 d
LSD ($p \leq 0.05$)	0.212	0.024	0.077	0.0117	0.30	0.09	0.22	0.03

Proline (µg/ g FW); K (Potassium) Na (Sodium). *Season (2025/2026 data). Means followed by different lowercase letters are significantly different.

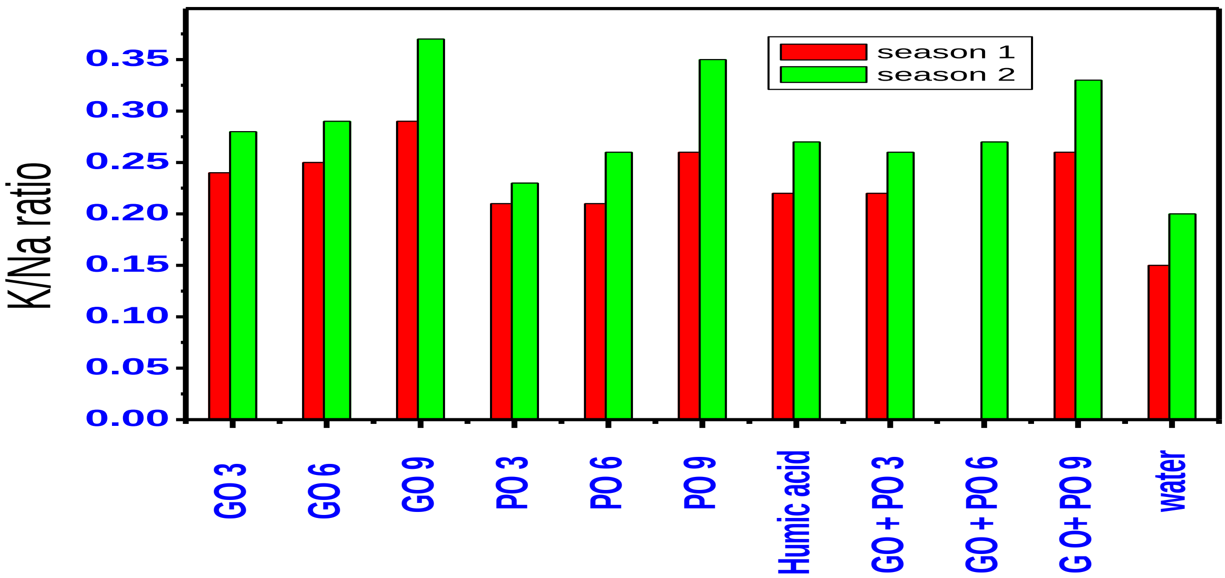

Fig. (2). Effect of biostimulant on K^+/Na^+ ratio of *Allium sativum* L. leaves at (2024/2025 and 2025/2026 seasons)

3.4. GC-MS Phytochemical Profiling of Garlic Extracts

Allium sativum L. extracts were examined using gas chromatography mass spectrometry to identify the active components in Tables (7, 8, 9) and Figures (3, 4, 5). According to the GC-MS analysis presented for the three garlic extract treatments (GO 9, HA, and control). The GC-MS analysis of *Allium sativum* L. extracts under three varying treatment conditions showed a intricate array of organ sulfur compounds, fatty acids, and sugar derivatives. The differences in the concentration and occurrence of these metabolites indicate that the treatment conditions influence the chemical yield and stability of the active components. A notable feature of all three extracts is the significant presence of organosulfur compounds(OSCs), including Trisulfide, di-2-propenyl (Diallyl trisulfide (DATS) and Diallyl disulfide (DADS). These compounds result from the enzymatic breakdown of allicin and are well-known for their strong antimicrobial and antioxidant effects. DATS is the predominant component in all samples, with GO 9 treatment exhibiting the highest relative abundance at 41.06%, while H A treatment showed 32.78% and control treatment recorded 34.75%. HA Treatment showed the greatest concentration of DADS (23.44%), whereas GO 9 treatment had the least (11.99%). The difference in the levels of these metabolites (DADS) and (DATS) suggests that GO 9 Treatment enhances the conversion or stabilization of these volatile compounds relative to the control group. The compounds that contain sulfur are the key indicators for assessing the quality of *Allium sativum* L. extract because of their proven antimicrobial and antioxidant properties. In *Allium sativum* L. chemistry, DATS is frequently mentioned as being more biologically active than DADS concerning its anticancer and antihypertensive effects. The elevated percentage in GO 9 treatment indicates a more effective conversion of allicin into higher-order sulfides **(Li *et al.*, 2022)**. Many studies have shown that DATS exhibits more effective anti-tumor and antioxidant properties than DADS, primarily because it contains a higher number of sulfur atoms that boost its capacity to trigger apoptosis in cancer cells and counteract free radicals **(Altonsy *et al.*, 2012; Kim *et al.*, 2014)**. The concentration of DADS was at its lowest in the GO 9 Treatment (11.99%). The elevated ratio of DATS in the GO 9 treatment indicates that this protocol promotes a more efficient or stable transformation of volatile thio sulfinates into more complex sulfides, which are favored in pharmaceutical, uses **(Liang *et al.*, 2015)**. Glycosides are found in extracts of *Allium sativum* L. GO 9 Treatment features peaks of alpha.-D-Glucopyranoside, O-.alpha.-D-glucopyranosyl-(1.fwdarw.3)-.beta.-D-fructofuranosyl, observed at RT 14.91, 27.77, and 31.31 min, comprising over 21% of the total area sum. In comparison, this tri saccharide was much lower in HA (2.83%) and minimal in Control. The retention of these carbohydrate derivatives in GO 9 treatment indicates that this particular treatment might more effectively stabilize the polar glycosidic fractions of *Allium sativum* L. extracts than the controls. Recent research indicates that D-glucopyranoside derivatives from *Allium sativum* L. may have potential inhibitory effects on enzymes linked to Type 2 diabetes, including α-glucosidase and α-amylase **(Chatterton *et al.*, 2024)**. The retention of these polar fractions in GO 9 treatment indicates a more thorough extraction profile that preserves the integrity of the bulb's carbohydrate structure. The identification of n-Hexadecanoic acid (Palmitic acid) at 6.04% in GO 9 treatment aligns with the standard GC-MS profiles for Allium species. This fatty acid is recognized for its roles in antimicrobial activity and inflammation reduction **(Pak *et al.*, 2018)**. The occurrence of Bis (2-ethylhexyl) phthalate was observed in GO 9 treatment (5.86 %) and peaked in the negative control C- (9.43%). Although frequently regarded as a plasticizer contaminant, research indicates that certain phthalic acid esters can occasionally be naturally produced by microorganisms or plants as secondary metabolites. The diminished amount of this compound in the GO 9 treatment compared to the negative control suggests a more refined phytochemical profile. GO 9 treatment is recognized as the best approach for optimizing the extraction of Diallyl trisulfide (41.06%), the main bioactive organosulfur compound in *Allium sativum* L. Additionally, the elevated levels of glucopyranosyl-fructofuranosyl derivatives in GO 9 treatment demonstrate a more thorough extraction of *Allium sativum* L.'s metabolic profile. These findings indicate that GO 9 treatment boosts the phytochemical efficacy of the extract, making it ideal for therapeutic or industrial use. The occurrence of 3-Cyclohexene-1-propanal (1.55%) is exclusive to the GO 9 treatment, while 8-Chlorooctyl 2-methylbutanoate was found in the control and HA. These differences in constituents serve as chemical that distinguish the best treatment from controls. GO 9 treatment provides a balance of organosulfur compounds and stabilized glycosidic fractions. This chemical profile aligns with high-quality *Allium sativum* L. extracts reported in pharmaceutical literature, enhancing therapeutic potential **(Kobacy *et al.*, 2025)**.

Table (7). Active compounds from *Allium sativum* L. extract with treatment (GO 9) analyze by gas chromatography mass spectrometry

Peak	Active compound	RT (min)	Area Sum%
1	3-Cyclohexene-1-propanal	6.936	1.55
2	Decanoic acid, 3-hydroxy-, methyl ester	8.285	1.34
3	Hexanoic acid, 2-phenylethyl ester	8.653	4.57
4	Diallyl disulphide	14.016	11.99
5	alpha.-D-Glucopyranoside, O-.alpha.-D-glucopyranosyl-(1.fwdarw.3)-.beta.-D-fructofuranosyl	14.91	2.47
6	1,2,3,4,5-Cyclopentanepentol	16.465	3.56
7	Trisulfide, di-2-propenyl	22.712	41.06
8	.alpha.-D-Glucopyranoside, O-.alpha.-D-glucopyranosyl-(1.fwdarw.3)-.beta.-D-fructofuranosyl	27.773	14.96
9	alpha.-D-Glucopyranoside, O-.alpha.-D-glucopyranosyl-(1.fwdarw.3)-.beta.-D-fructofuranosyl	31.319	4.26
10	n-Hexadecanoic acid	41.935	6.04
11	9,12-Octadecadienoic acid (Z,Z)-	46.573	2.35
12	Bis(2-ethylhexyl) phthalate	54.081	5.86

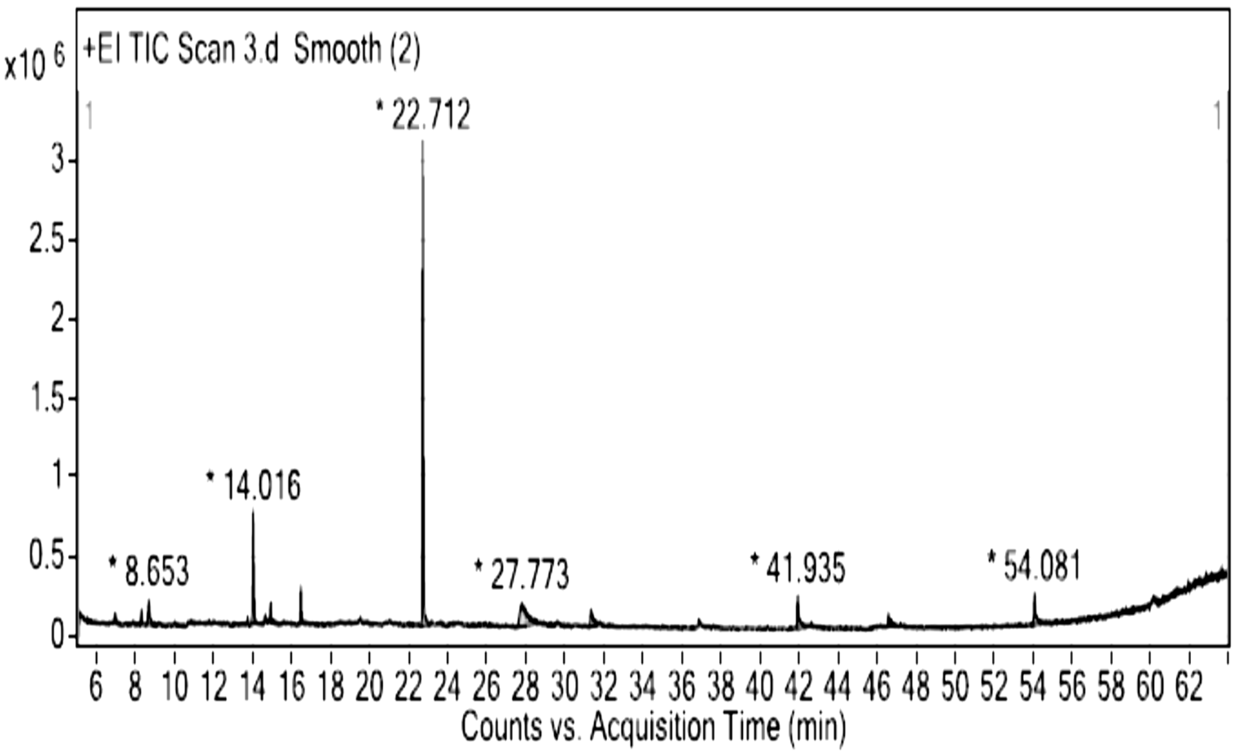

Fig. (3). Chromatograms for *Allium sativum* L. extract with GO 9 treatment analyzed by gas chromatography mass spectrometry

Table (8). Active compounds from *Allium sativum* L. extract with Humic Acid (HA) analyze by gas chromatography mass spectrometer

Peak	Active compound	RT (min)	Area Sum%
1	Decanoic acid, 3-hydroxy-, methyl ester	8.672	6.52
2	8-Chlorooctyl 2-methylbutanoate	8.993	6.98
3	Trimethyl((3E,6E)-nona-3,6-dienyloxy)silane	13.939	2.14
4	Diallyl disulphide	14.198	23.44
5	3,cis-(1,1-dimethylethyl)-4,cis-methoxycyclohexanol	15.072	5.21
6	Cyclohexane, 1,4-dimethoxy-2-methyl-, stereoisomer	16.599	6.32
7	3,cis-(1,1-dimethylethyl)-4,cis-methoxycyclohexanol	19.64	3.14
8	Trisulfide, di-2-propenyl	22.761	32.78
9	O-.alpha.-D-Glucopyranoside, O-.alpha.-D-glucopyranosyl-(1.fwdarw.3)-.beta.-D-fructofuranosyl	25.955	2.83
10	3,cis-(1,1-dimethylethyl)-4,cis-methoxycyclohexanol	31.38	4.77
11	Cyclohexanebutanoic acid	41.939	5.88

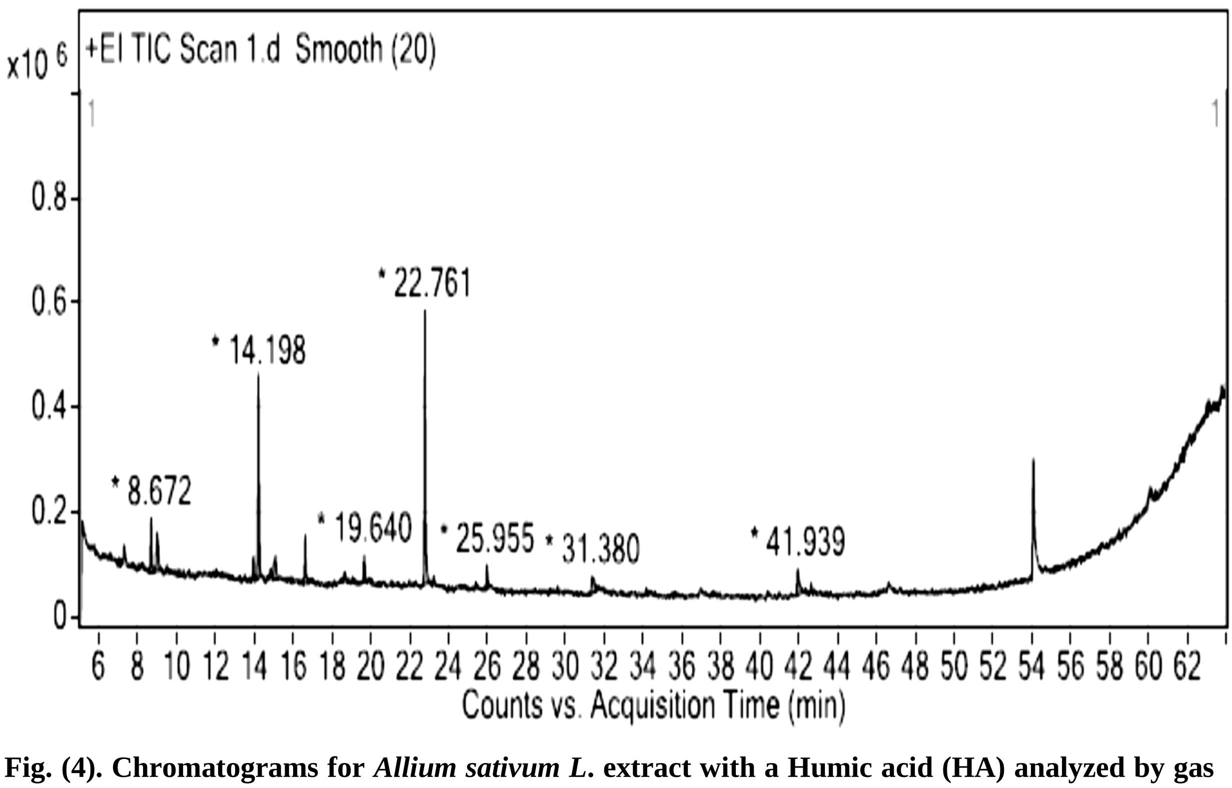

Fig. (4). Chromatograms for *Allium sativum L.* extract with a Humic acid (HA) analyzed by gas chromatography mass spectrometry

Table (9). Active compounds from *Allium sativum* L. extract with control analyze by gas chromatography mass spectrometry

Peak	Active compound	RT (min)	Area Sum %
1	Decanoic acid, 3-hydroxy-, methyl ester	8.354	3.64
2	8-Chlorooctyl 2-methylbutanoate	8.72	5.63
3	Decanoic acid, 3-hydroxy-, methyl ester	13.775	1.31
4	Diallyl disulphide	14.041	17.84
5	3,cis-(1,1-dimethylethyl)-4,cis-methoxycyclohexanol	14.926	6.08
6	Cyclohexane, 1,4-dimethoxy-2-methyl-, stereoisomer	16.48	3.48
7	3-trsns-(1,1-dimethylethyl)-4-trans-methoxycyclohexanol	19.565	2.04
8	Trisulfide, di-2-propenyl	22.719	34.75
9	Silane, (chloromethyl)ethenyldimethyl-	25.932	1.12
10	3,cis-(1,1-dimethylethyl)-4,cis-methoxycyclohexanol	31.336	5.77
11	n-Decanoic acid	41.934	7.12
12	3,cis-(1,1-dimethylethyl)-4,cis-methoxycyclohexanol	46.621	1.8
13	Bis(2-ethylhexyl) phthalate	54.074	9.43

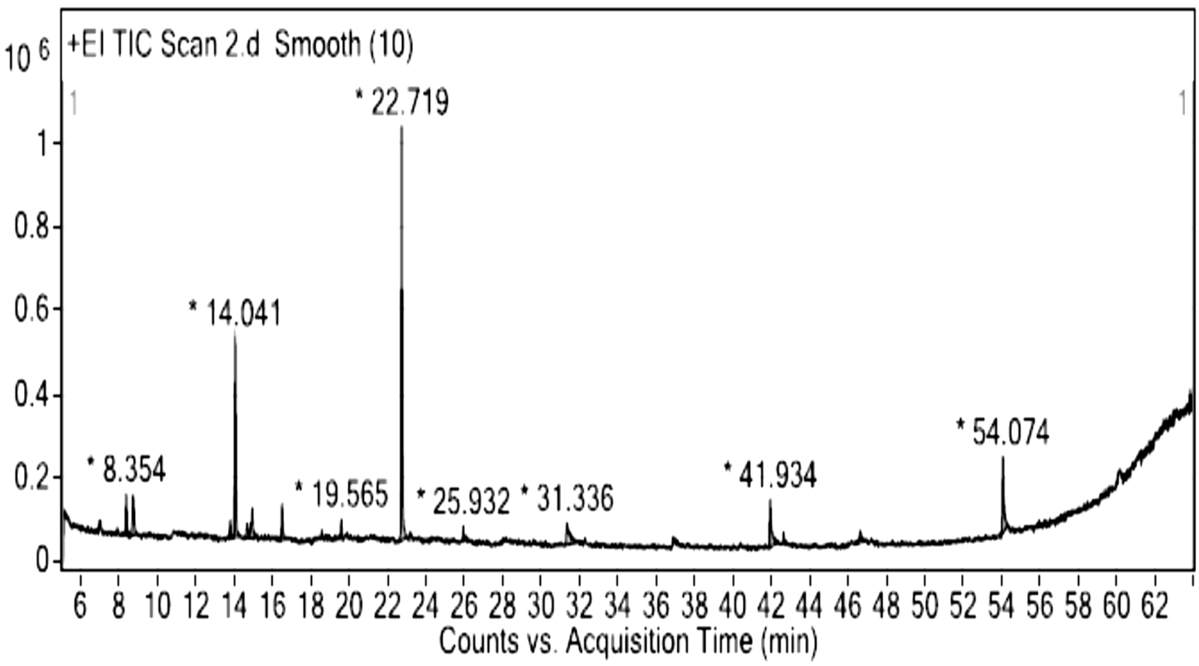

Fig. (5). Chromatograms for *Allium sativum* L. extract with control analyzed by gas chromatography mass spectrometry

3.5. Garlic Yield/ Feddan

The bulb yield /feddan is presented in Table 10 in both seasons (2024/2025 and 2025/2026). In season 1 (2024/2025), GO+PO 9 ml/L treatment produced a yield of approximately (5.86 ton/feddan), PO 9 with 5.80 ton/feddan and GO 9 with 4.78 ton/feddan. In season 2 (2025/2026), GO 9 ml/L achieved the highest yield, approximately (5.94 ton/feddan), These results are consistent with the field data recorded in Table 10 and confirm the superiority of GO 9 and PO 9 treatments as the best treatments across both seasons. The increasment in yield at GO 9 and PO 9 treatments is due to the effect of improved efficient nutrient uptake, photosynthetic capacity, and elevated antioxidant defense under saline sandy soil conditions. The high content of fatty acid and phenolic component of grape seed oil leading to increased dry matter accumulation and, more bulb weight/ plant and total yield/feddan (**Awad *et al.*, 2021**; **Mrid *et al.*, 2021)**. Confirmation of biostimulant foliar application increased total yield in garlic crops grown under salt-affected soils (**Taha *et al.*, 2022)**. The bulb yield/feddan was calculated based on the data of fresh bulb weight/plant (as shown in Table 4) and the density of plant, plant was sown distance of 7 cm apart between plants and 70 cm between rows, yielding approximately 85,714 plants/feddan **(Ali, *et al.*, 2023; Shalaby *et al.*, 2014).**

Table (10). Bulb yield (ton/feddan) of *Allium sativum* L. under treatments

Treatment	**Season 2024/2025 FW Bulb (g)**	**(ton/feddan)**	**Season 2025/2026 FW Bulb (g)***	**(ton/feddan)***
GO 3	48.15 bc	4.13 d	49.15 bc	4.21 d
GO 6	53.70 a	4.60 c	56.80 ab	4.87 c
GO 9	55.8 ab	4.78 b	69.35 a	5.94 a
PO 3	30.15de	2.58 h	30.15 de	2.58 h
PO 6	37.65 cd	3.23 g	38.65 cd	3.31 g
PO 9	67.65 a	5.80 a	68.65 a	5.88 a
Humic acid	44.8 bcd	3.84 e	44.35 bc	3.80 e
GO + PO 3	41.40 bcd	3.55 f	42.40 bcd	3.63 f
GO + PO 6	43.35 bcd	3.72 e	45.80 bc	3.93 e
GO + PO 9	68.35 a	5.86 a	64.51 a	5.53 b
water	21.4 e	1.83 i	22.40 e	1.92 i
LSD ($p \leq 0.05$)	10.44	0.15	10.42	0.24

Yield /feddan calculated from fresh bulb weight / plant × 85,714 plants/feddan (planting density: 7 cm × 70 cm, feddan = 4200 m²). Values represent estimated commercial fresh bulb yield.

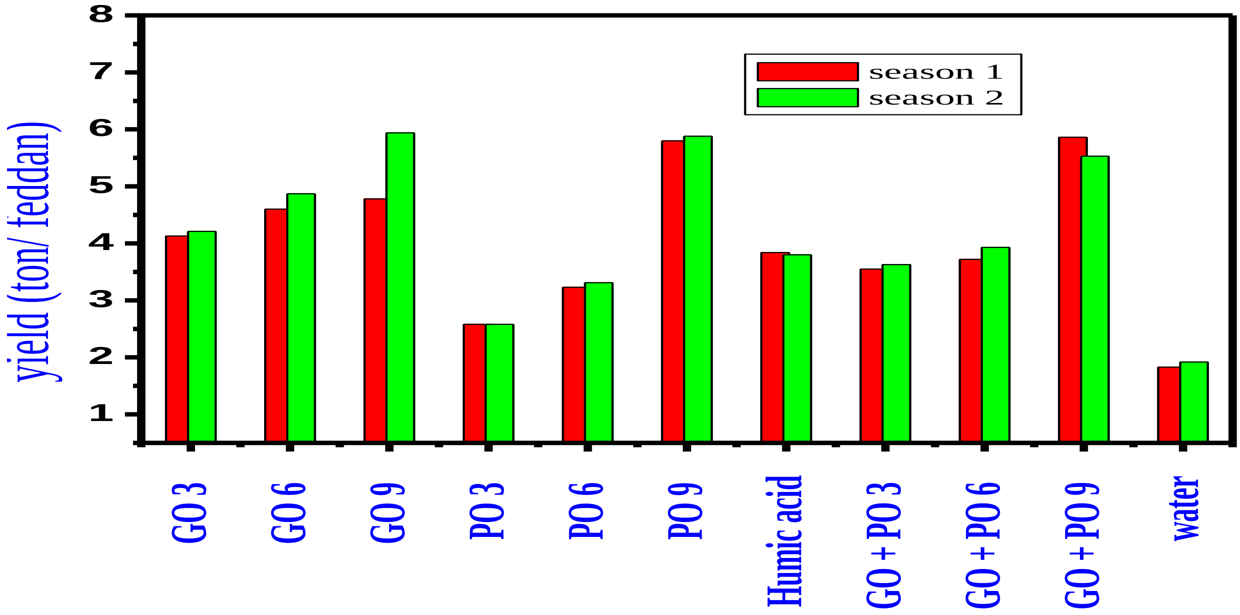

Fig. (6). Bulb yield (kg/feddan) of *Allium sativum* L. under different biostimulant treatments in both seasons (2024/2025 and 2025/2026)

4. Conclusion

According to this study, foliar application of GO 9ml/l and PO 9ml/l improves garlic's vegetative development, antioxidant defense, bulb yield, osmotic tolerance, photosynthetic efficiency, and phytochemical quality. Because of its abundance of fatty acids and phenolic antioxidants with physiological defensive mechanisms, GO 9 ml/l treatment was found to be the most effective treatment. The capacity of GO 9 ml/l to enhance glucopyranoside and diallyl trisulfide content was validated by GC-MS analysis, resulting in a phytochemically superior garlic extract with significant therapeutic potential.

For garlic development on salty sandy soils, foliar application of GO 9 ml/l twice is advised as an efficient, cost-effective, and environmentally beneficial biostimulant. GO 9 ml/l represents a good alternative. Future study will study the economic feasibility at large-scale, Mechanisms behind enhanced salinity tolerance via oil treatment, and the residual effects of oil applications on soil fertility.

Conflict of interest

The authors declare no conflicts of interest.

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تأثير الرش بزيوت بذور العنب والقرع العسلى على الانتاجية والتركيب الكيميائى للثوم تحت ظروف الملوحة

ملاك مجلع زكرى ، جاكلين عطية خليل، إيمان عادل الكمشوش

قسم بحوث النباتات الطبيه والعطريه، معهد بحوث البساتين، مركز البحوث الزراعية، الجيزة ،مصر

الملخص

تمت الدراسة على مدار موسمين (2024-2025 و 2025-2026) لمعرفة فاعلية المحفزات الحيوية (زيت بذور العنب و اليقطين) بتركيزات مختلفة ، من اجل تحسين إنتاجية ورفع الجودة الكيميائية والقدرة التنافسية لمحصول الثوم. تم تطبيق احدى عشر معاملة و استُخدم تصميم القطاعات كاملة العشوائية بثلاثة مكررات. تحليل GC-MS لمستخلصات الثوم كشفت عن مجموعة من مُركبات الكبريت العضوية، والأحماض الدهنية، ومُشتقات السكر. يُعدّ ثلاثي كبريتيد ثنائي-2-بروبينيل المُكوّن الرئيسي في جميع المستخلصات حيث أظهرت مُعاملة (GO 9) أعلى نسبة مُقارنةً بمُعاملة حمض الهيوميك و العينة الضابطة. يُشير التباين في تركيز هذه المُواد داخل مستخلص الثوم إلى أن مُعاملة GO 9 هي الأفضل . اوضحت النتائج أن مُعاملات GO 9, PO 9 حققت أعلى معدلات في الوزن الكلي للنبات وعدد الأوراق و حدوث زيادة كثافة للكلوروفيل وأكثر قدرة على تصنيع الغذاء . كما زادت نسبة مضادات الأكسدة ومادة البرولين و تحسين امتصاص البوتاسيوم مقابل الصوديوم و ضبط التنظيم الأسموزي ، مما يجعل الثوم قادر على مواجهة التقلبات والظروف البيئية الصعبة و منع الهدر في الطاقة والنمو و ادى كل ذلك الى تحسين الانتاجية مقارنة الكنترول. لذا نقدم نوصية لمزارعي الثوم باستخدام تلك المحفزات للحصول على انتاجية و جودة افضل و أبصال أكثر تحمل للتخزين والتداول وعائد اقتصادي اعلى للمحصول. وهذا يرفع قيمة هذه المحفزات الامنة بيئيا و يقلل استخدام الأسمدة التقليدية مما يحافظ على التربة.