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Glutamic acid and sulfur increases medicinal substance in *Allium sativum* L. and its antibiotic activity against *Staphylococcus aurous*

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The experiment was conducted during the winter season (2017 –2018) in the Department of Horticulture and Forestry / Najaf Governorate. The experiment included 15 treatments to study the effect of spraying five concentrations of the amino acid Glutamic acid (0, 50, 100, 150 and 200) mg. L^{-1} with three Concentrations of agricultural sulfur (0, 2.5 and 5) g / L on the growth of some physiological properties and the chemical content of Garlic (*Allium sativum L.*) with study the inhibitory activity of garlic water extract and antibiotic against pathogen *Staphylococcus aurous* bacteria. By using Factorial experiment randomized complete block design (R.C.B.D) with three-replicated. Treatment means were compared with the use of Duncan's Multiple Range Test (DMRT) at the probability level of 0.05. The results showed that increased concentrations of amino acid (Glutamic acid) and (sulfur) improved and increased the most studied parameters :-(A) The results of vegetative growth showed a significant increase in the interaction treatment of G4 (spray of the Glutamic acid at the concentration of 200 mg.L^{-1}) and S_2 (sulfur spray at a concentration of 5 g.L^{-1}) by gives the highest rate of dry weight (15.3 gm). However, the results showed a significant improvement in the interaction treatment of G_3 (spray of the Glutamic acid at the concentration of 150 mg. L^{-1}) and S_2 (sulfur spray at a concentration of 5 g.L^{-1}) which gaves the higher rate of leaf area which reached (258.2 cm^2.$plant^{-1}$) compared with the lowest rate by interaction treatment of S_0G_0 (spray distilled water) at (138.7 cm^2.$plant^{-1}$). (B) The results of the yield and its components also showed significant superiority in the interaction treatment G_4 (spray of the Glutamic acid at the concentration of 200 mg. L^{-1}) and S_2 (sulfur spray at a concentration of 5 g.L^{-1}) which gaves the highest rate in the characters (number of lobes, lobe weight (44.0 lobe.$plant^{-1}$, 3.45 gm) respectively. (C) the results of the chemical content of the lobes, also showed a significant superiority in the interaction treatment of G_4 (spray of the Glutamic acid at the concentration of 200 mg. L^{-1}) and S2 (spray of sulfur at a concentration of 5 g. L^{-1}) which gave the highest content of the lobes from Allicin, glutamic acid, and leaf content chlorophyll (238.05, 1209.7) µg.$mL^{-1}$and (70.14) mg / 100g dry weight respectively **.**(D) The experiment of testing the effectiveness of the water extracts of garlic lobes and antibiotic in inhibiting the growth of Pathological causes. The results showed that the water extract of the garlic lobes was superior by interaction treatment G_4 (spray of the Glutamic acid at the concentration of 200 mg. L^{-1}) and S_2 (sulfur spray at a concentration of 5 g. L^{-1}) which gives the highest inhibitory area of the Gram positive bacteria compared with the lowest in inhibitory which gives from the interaction treatment of S_0G_0 (spray distilled water), which was (27 and 21) mm respectively , while antibiotic Vancomycin gave the highest rate inhibition of positive bacteria was (14) mm.

Keywords: garlic, amino acid, glutamic acid, sulfur, pathogens.

INTRODUCTION

Garlic (Allium sativum L.) is the second plant of the Amaryllidaceous Huber (2003). This family contains more than 90 plant species, followed by about 1200 species (Metlub et al., 1989). The native plant is believed to be South Thompson And Kelly, 1957 and Hafez, 1992).Garlic is one of the most important winter vegetable plants in Iraq and the world for its high nutritional value and its many uses and medicinal benefits (Hassan, 2000). And because of its medicinal and nutritional benefits (Safadi et al., 1998). It has been used in many epidemiological and experimental studies that provide evidence that garlic affects the most dangerous factors such as triglycerides, LDL associated with heart disease, and can reduce serum cholesterol by not less than 9% (Bergener 2001, Green et al., 2001) It was also used as an adjuvant in World War I and II to prevent wound contamination and prevent its injury to Gangrene (Tattelman, 2005). Garlic cloves contain special compounds called sulfa compounds that protect the liver cells from the effects of carbon (Shafi'i, 2001).

Al-Jumaili (2016) found a significant increase in the number of lobes of the garlic plant with the effect of spraying the amino acids Valen and Arginine at a concentration of 100 mg per liter. For each acid, the addition of valine and arginine amino acids increased the value of 8.14, 7.19 respectively compared to the comparison treatment of 5.81 (CFA-1). Farooqui et al., (2009) obtained significant differences in the vegetative growth of the effect of sulfur at four levels of 0, 20, 40 and 60 kg. 1 ha on garlic plant, and the increase was at 60 kg. Ha-1. Mishu and others (2013) found it when added to five levels of sulfur (80, 60, 40, 20, 0) kg. The results showed that the level of paper and dry weight of the vegetative group of onion plant showed that the level (40 kg .1e) of sulfur has recorded the best results in the mentioned qualities. As a number of studies have indicated, the efficiency of the garlic extract of garlic cloves has been discouraged against Staphylococcus aureus (Nu'man and Chalabi, 2001). The effectiveness of garlic cloves is attributed to its containment of allicin inhibiting the growth of germs by destroying the SH group necessary for cell interaction (Slusarenko et al., 2008). The objective of the study was to improve plant growth and its physiological and medicinal properties and to study the water extract of its branches in inhibiting the positive bacteria of the Kram dye. It is generally noted that medicinal plants are the source of many therapeutic substances because they contain a large number of compounds with biological efficacy.

MATERIALS AND METHODS

The experiment was carried out during the 2018-2017 seasons at the site of the Department of Horticulture and Forestry, affiliated to the Department of Agriculture in Najaf Governorate. The experiment included (15) treatment, which is an overlap of five concentrations of Glutamic acid (0, 50, 100, 150 and 200) (G_0, G_1, G_2, G_3 and G_4) with three concentrations of agricultural sulfur (0, 2.5 and 5 g / L) with symbol (S_0, S_1 and S_2). Chemical and physical analyzes of soil samples and irrigation water was conducted for the site as shown in Table (1).

Plants were sprayed twice a morning, the first when the number of leaves in the plant was 4-6 sheets, 50 days after germination (Singh et al., 1983, Gad et al., 1997) and the second tranche ten days after the first

The quantitative and qualitative estimation of the Allicin and Glutamic acid in the garlic clove extract was obtained by using the Japanese high-performance liquid chromatography (HPLC) of Japan Koyoto of liquid chromatography Shimadzu (LC-6A) by injecting the standard solution, (Fürst et al., 1990, Fierabraci et al., 1991). The total chlorophyll dye in the green leaves was obtained using a UV-visible spectrophotometer in a manner (Goodwin, 1976) The biological efficacy of the garlic clove extract on the Gram-positive bacteria was tested using the well drilling method in the center of the Hardwoods (Harley and Prescott, 2002) in the Advanced Microbiology Laboratory - Life Sciences Department / Girls College of Education in the preparation of Petri dishes for the purpose of planting the bacteria Staphylococcus aureus .

RESULTS AND DISCUSSION

Leaf area (cm^2. plant $^{-1}$)

The results of Table (2) show that the interaction between the glutamic acid and sulfur fermentation agents has a significant effect. The highest surface area of the paper was treated with G_3 (spray of the Glutamic acid at the concentration of 150 $mg.L^{-1}$) with S_2 (sulfur spray at a concentration of 5 $g.L^{-1}$) 258.2 $cm^2.plant^{-1}$, which differed significantly from all interference factors, while the lowest rate was treated with S_0G_0 (distilled water spray) at 138.7 $cm^2.plant^{-1}$

Table. (1) some chemical and physical properties of the soil and water of the experiment

Water	Soil	Unit		Type of Analysis	
7.1	7.5	without unit	-	**PH**	**1**
4.2	3.07	dsi-samnes. M^{-1}	$ds.m^{-1}$	**degrees of salinity EC**	**2**
111	96.5	mg. L^{-1}	Ppm	**Sodium ion Na^{+}**	**3**
641	495	mg. L^{-1}	Ppm	**Potassium ion K^{+}**	**4**
460	420	mg. L^{-1}	Ppm	**Calcium ion Ca^{+2}**	**5**
591	603	mg. L^{-1}	Ppm	**Sulfur ions SO_4**	**6**
674	710	mg. L^{-1}	Ppm	**Chloride CL^{-}**	**7**
2.8	3.6	mg. L^{-1}	%	**Nitrogen N**	**8**
0.04	0.70	mg. L^{-1}	%	**PPhosphorus**	**9**
Sand mix				**Texture**	**10**
-	100	g. L^{-1}	**Clay**	**Soil separators**	**11**
-	170	g. L^{-1}	**Silt**		
-	730	g. L^{-1}	**Sand**		

Note: ions were estimated as soluble

The dry weight of the vegetative total (gm.plant^{-1})

The results of Table (3) showed that the interaction between the acid and glutamic acid splits had a significant effect. The highest weight was observed in the treatment of interference G_4 (spray of the Glutamic acid at the concentration of 200 mg.L-1) 15.3 gm.plant^{-1}with S_2 (sulfur spray at a concentration of 5 g.L^{-1}) 1.3 gm.plant^{-1} which differed significantly from all interference treatments, while the lowest weight was obtained when the S_0G_0 (distilled water) was treated 6.9 gm.plant^{-1}.

The content of the leaves of total chlorophyll (mg / 100 g)

It is clear from the results of Table (4) that the interaction between the parameters of glutamic acid and sulfur has a significant effect. This was the highest content of chlorophyll when treated with G_4 (spray of the Glutamic acid at the concentration of 200 mg.L^{-1}) with S_2 (sulfur spray at a concentration of 5 g.L^{-1}) 70.14 mg / 100g while the lowest treatment was S_0G_0 (distilled water spray) at 25.39 mg / 100g.

Average number of lobes

The results of Table (5) indicate that the interaction between the glutamic acid and sulfur fermentation factors has a significant effect. The highest number of lobes in the treatment of interference G_4 (spray of the Glutamic acid at the concentration of 200 mg.L^{-1}), with S_2 (sulfur spray at a concentration of 5 g.L^{-1}) of 44.0 (LF-1), which did not differ significantly from all interference factors, while S_0G_0 (distilled water spray) was 30.6.

Average weight of the lobe (gm. Head-1)

The results of Table (6) indicate that the interaction between the parameters of glutamic acid and sulfur fermentation has a significant effect. The highest weight in the treatment of interference was G_4 (spray of the Glutamic acid at the concentration of 200 mg.L^{-1}) with S_2 (sulfur spray at a concentration of 5 g.L^{-1}) (3.45 g), which differed significantly from the lowest rate of S_0G_0 (distilled water) at 1.92 g)

Table (2) Effect of Glutamic acid and Sulfur in the area of the paper area (cm2 and paper $^{-1}$)

Sulfur rate	G4	G3	G2	G1	G0	Glutamic / Sulfur
163.6 c	160.4 g	227.6 d	150.3 h	141.3 i	138.7 i	**S0**
186.9 b	225.9 d	193.2 e	180.8 f	150.2 h	184.6 f	**S1**
241.4 a	249.4 b	258.2 a	236.4 c	239.4 c	223.7 d	**S2**
	211.9 b	226.3 a	189.1 c	176.9 e	182.3 d	**Glutamic rate**

The common rates in the same letters within each transaction and their interactions are not significantly different from each other.
The Duncan Multiplicity test is at a probability level of 0.05

Table (3) Effect of Glutamic acid and Sulfur in the dry weight of the vegetative group (g)

Sulfur rate	G4	G3	G2	G1	G0	Glutamic / Sulfur
7.9 b	8.2 efg	9.0c def	8.1 efg	7.1 g	6.9 g	**S0**
9.0 b	11.2 def	7.9 fg	8.8 def	9.1 def	8.1 efg	**S1**
11.8 a	15.3 a	13.1 b	9.6 cde	11.1 bc	10.1 cd	**S2**
	11.6 a	10.0 b	8.8 bc	9.1 c	8.4 c	**Glutamic rate**

The common rates in the same letters within each transaction and their interactions are not significantly different from each other
The Duncan Multiplicity test is at a probability level of 0.05

Table (4) Effect of Glutamic acid and Sulfur in leaf content of total chlorophyll (mg / 100 g)

Sulfur rate	G4	G3	G2	G1	G0	Glutamic / Sulfur
30.93 c	32.97 f	33.08 f	32.64 f	30.59 f g	25.39 g	**S0**
43.06 b	50.69 bc	41.46 d	48.44 c	39.73 de	35.01 ef	**S1**
57.98 a	70.14 a	65.10 a	55.63 b	51.04 bc	48.03 c	**S2**
	51.26 a	46.54 b	45.57 b	40.45 c	36.14 d	**Glutamic rate**

The common rates in the same letters within each transaction and their interactions are not significantly different from each other
The Duncan Multiplicity test is at a probability level of 0.05

Table (5) Effect of Glutamic acid and Sulfur in the number of lobes

Sulfur rate	G4	G3	G2	G1	G0	Glutamic / Sulfur
33.3 c	35.6 ef	35.0 f	31.6 gh	34.0 fg	30.6 h	**S0**
39.4 b	41.0 abc	37.3 def	40.3 bcd	37.3 def	41.3 abc	**S1**
41.5 a	44.0 a	42.3 ab	43.0 ab	38.6 cde	40.0 abc	**S2**
	40.2 a	38.2 b	38.3 b	36.6 b	37.3 b	**Glutamic rate**

The common rates in the same letters within each transaction and their interactions are not significantly different from each other
The Duncan Multiplicity test is at a probability level of 0.05

Table (6) Effect of Glutamic acid and Sulfur in the average weight of the lobe (g)

Sulfur rate	G4	G3	G2	G1	G0	Glutamic / Sulfur
2.37 b	2.43 de	2.89 abcd	2.35 bcd	2.28 de	1.92 e	**S0**
2.61 b	3.22 abc	2.42 de	2.51 bcd	2.49 de	2.42 cd	**S1**
3.01 a	3.45 a	3.15 abc	3.25 a	2.52 ab	2.71 abcd	**S2**
	3.02 a	2.82 a	2.71 a	2.43 ab	2.35 b	**Glutamic rate**

The common rates in the same letters within each transaction and their interactions are not significantly different from each other
The Duncan Multiplicity test is at a probability level of 0.05

Allicin content of the lobes (µg.mL-1)

The results of Table (7) that the interaction between the acid and amino acid spraying treatments had a significant effect. The highest ratio of the substance in the treatment of interference was G_4 (spray of the Glutamic acid at the concentration of 200 mg.L^{-1}) with S_2(sulfur spray at a concentration of 5 g.L^{-1}) at 238.05 (µg.mL^{-1}), which differed significantly from all the interference factors, while the lowest rate was in the treatment of the comparison S_0G_0 (distilled water spray) at 37.10 (µg.mL^{-1}).

The content of the lobes of the amino acid Glutamic acid (µg.mL $^{-1}$)

The results in Table (8) indicate that glutamic acid splits had a significant effect on glutamic acid content (µg.mL $^{-1}$). The treatment was superior to G_4 (spray of the Glutamic acid at the concentration of 200 mg.L^{-1}) Was significantly higher than the other treatments, with the highest rate of amino acid Glutamic acid 661.4 (µg.mL^{-1}). While the lowest ratio was treated with G_0 (distilled water), which was 301.1 (µg.m-1), and the acid increase was 119.6%.

Glutamic acid and sulfur have a significant effect. The highest rate of amino acid glutamic acid was in the treatment of G_4 (spray of the Glutamic acid at the concentration of 200 mg.L^{-1}) with S_2(sulfur spray at a concentration of 5 g.L^{-1}) 1209.7 µg.mL^{-1}, which differed significantly from all the interference parameters, while the lowest was in the treatment of the comparison S_0G_0 (distilled water spray) which reached 220.5 µg.mL^{-1}.

Test the biological efficacy of the extract of garlic cloves and the antibiotic Vancomycin in inhibiting the growth of Gram positive bacteria:

The results in Table (9) indicate that the interaction between the glutamic acid and sulfur fermentation agents has a significant effect. The largest area of inhibition of 27.0 mm in the treatment of interference was G_4 (spray of the Glutamic acid at the concentration of 200 mg.L^{-1}) with S_2 (Sulfur spray at a concentration of5 g.L^{-1}), while the lowest 21.0 mm inhibition was observed in S_0G_0 (sprayed distilled water). It is also noted from Figure (1) that all the extracts of the water extract of the garlic cloves had a greater inhibition of the effect of the rate of antibody Vancomycin, which amounted to 14 mm against the positive bacteria of the color of Kram.

Table (7) Effect of Glutamic acid and Sulfur in the Allicin Lobe Content (µg.ml^{-1})

Sulfur rate	G4	G3	G2	G1	G0	Glutamic / Sulfur
47.54 c	59.60 g	53.25 h	46.74 i	41.03 j	37.10 j	**S0**
82.58 b	93.96 d	91.12 d	79.40 e	71.39 f	77.03 e	**S1**
145.08 a	238.05 a	115.6 c	165.61 b	111.61 c	94.56 d	**S2**
	130.53 a	86.65 c	97.25 b	74.67 d	69.56 e	**Glutamic rate**

The common rates in the same letters within each transaction and their interactions are not significantly different from each other
The Duncan Multiplicity test is at a probability level of 0.05

Table (8) Effect of Glutamic acid and Sulfur in the Content of the Lobe of Glutamic Acid (µg.mL $^{-1}$)

Sulfur rate	G4	G3	G2	G1	G0	Glutamic / Sulfur
297.5 c	382.8 cd	317.9 de	308.5 def	257.9 ef	220.5 f	**S0**
361.6 b	391.7 cd	426.6 bc	316.7 de	379.8 cd	293.0 def	**S1**
597.1 a	1209.7 a	496.1 b	474.4 bc	415.3 bc	389.9 cd	**S2**
	661.4 a	413.5 b	366.5 bc	351.0 c	301.1 d	**Glutamic rate**

The common rates in the same letters within each transaction and their interactions are not significantly different from each other
The Duncan Multiplicity test is at a probability level of 0.05

Table (9) Effect of Glutamic acid and Sulfur in inhibition Growth of Gram positive bacteria

Sulfur rate	G4	G3	G2	G1	G0	Glutamic / Sulfur
21.7 c	22.6 cd	22.0 cd	21.6 cd	21.6 cd	21.0 d	**S0**
23.4 b	23.3 cd	22.6 cd	26.3 ab	22.6 cd	22.3 cd	**S1**
25.3 a	27.0 a	26.3 abc	25.0 abc	24.6 abc	23.6 abcd	**S2**
	24.3 a	23.6 ab	24.3 a	22.9 ab	22.3 b	**Glutamic rate**

The common rates in the same letters within each transaction and their interactions are not significantly different from each other
The Duncan Multiplicity test is at a probability level of 0.05

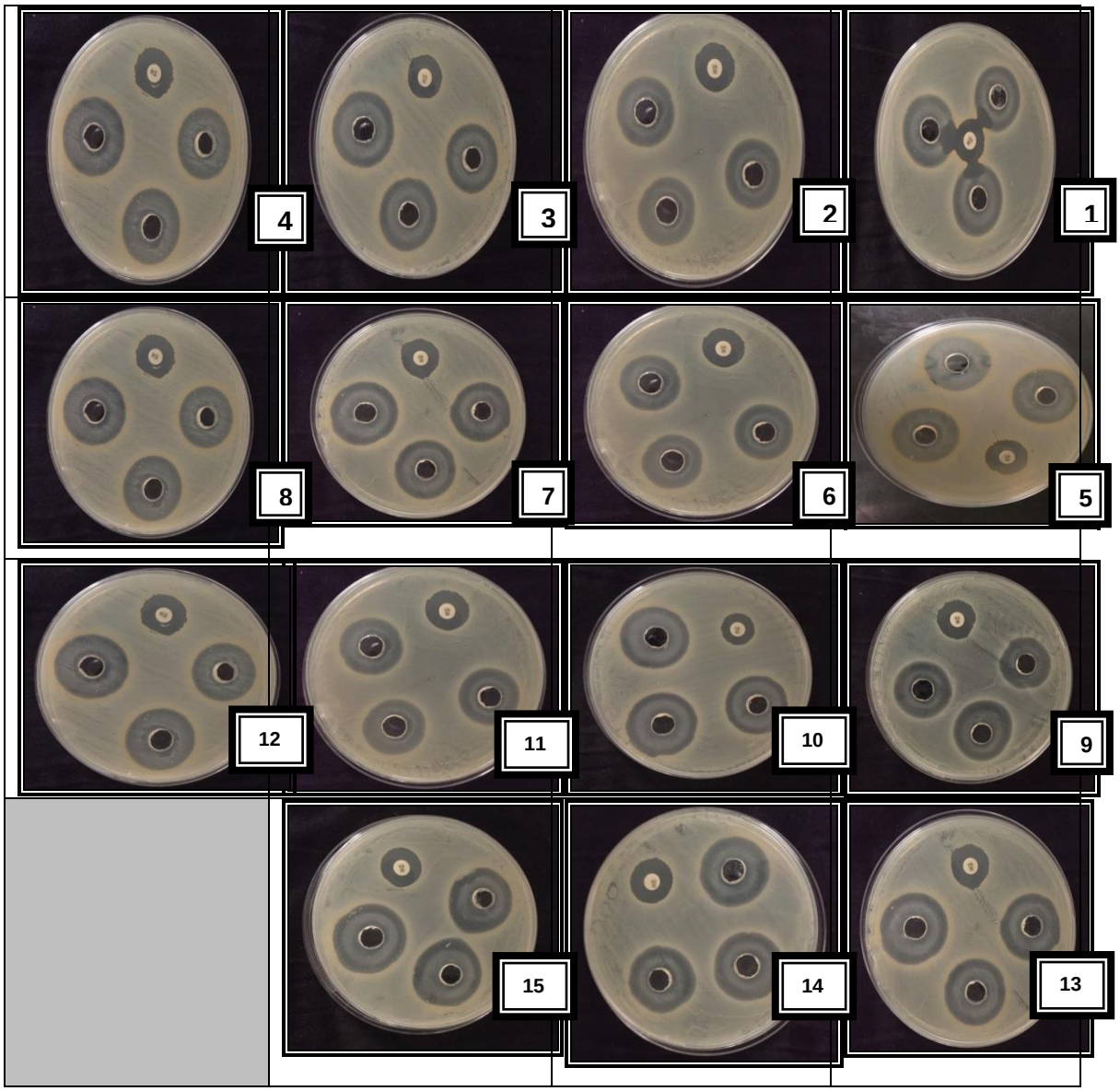

Figure (1) Effect of garlic clove extract for experimental treatments in inhibiting the growth of Bacteria Aureus. Staph is positive and compared with the Vancomycin antagonist

1. Effect of G0S0 interaction with addition of Vancomycin in Staphylococcus aureus positive bacteria
2. Effect of G1S0 interaction with addition of Vancomycin in Staphylococcus aureus positive bacteria
3. Effect of G2S0 interaction with addition of Vancomycin in Staphylococcus aureus positive bacteria
4. Effect of G3S0 interaction with addition of Vancomycin in Staphylococcus aureus positive bacteria
5. Effect of G4S0 interactionwith addition of Vancomycin in Staphylococcus aureus positive bacteria
6. Effect of G0S1 interaction with addition of Vancomycin in Staphylococcus aureus positive bacteria
7. Effect of G1S1 interaction with addition of Vancomycin in Staphylococcus aureus positive bacteria
8. Effect of G2S1 interaction with addition of Vancomycin in Staphylococcus aureus positive bacteria
9. Effect of G3S1 interaction with addition of Vancomycin in Staphylococcus aureus positive bacteria
10 .Effect of G4S1 interaction with addition of Vancomycin in Staphylococcus aureus positive bacteria
11. Effect of G0S2 interaction with addition of Vancomycin in Staphylococcus aureus positive bacteria
12. Effect of G1S2 interaction with addition of Vancomycin in Staphylococcus aureus positive bacteria
13. Effect of G2S2 interaction with addition of Vancomycin in Staphylococcus aureus positive bacteria
14. Effect of G3S2 interaction with addition of Vancomycin in Staphylococcus aureus positive bacteria
15. Effectof G4S2 interaction with addition of Vancomycin in Staphylococcus aureus positive bacteria

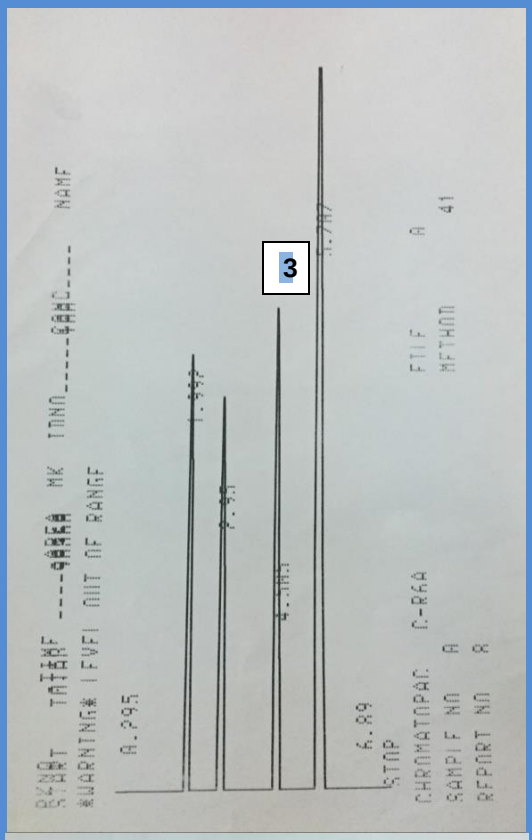

Figure (2) The time of detention for Allicin

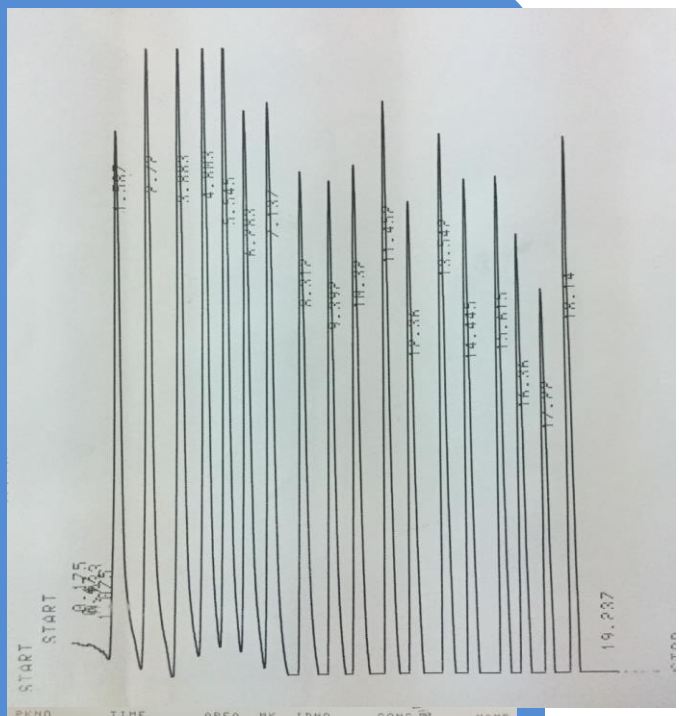

Figure (3) The retention time of amino acids
2-The time of the retention of Glutamic acid

CONCLUSION

. From this study, we conclude the following:

The treatment of G_4 overlap (spray of amino acid Glutamic acid at a concentration of 200 mg /L^{-1}) with S_2 (sulfur spraying at a concentration of 5 g. L^{-1}) has clearly contributed to improving the vegetative and quantitative characteristics and the specific characteristics of the garlic clove content by increasing the amino acid and total chlorophyll and also increased the proportion of medical substance Allicin in lobes.

2. The test of the water extract of the garlic cloves at the treatment of G_4 (spray of the amino acid glutamic acid at a concentration of 200 mg /L^{-1}) with S_2 (sulfur spraying at a concentration of 5 g L^{-1}) showed a clear effect compared to control treatment S_0G_0 (distilled water spray) As well as compared to the use of antibiotics (Vancomycin) in inhibiting the growth of bacteria and positive chromium dye.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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