Al-Qadisiyah Journal of Pure Science

Volume 25 | Number 4 Article 9

10-7-2020

Antibacterial Activities For Root Extracts Of (goldenrods) Solidago Canadensis L Treated by Nano And Bio Fertilizer

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Recommended Citation

Al-Rekaby, Layth Sareea and Atiyah, Khalid Mohsein (2020) "Antibacterial Activities For Root Extracts Of (goldenrods) Solidago Canadensis L Treated by Nano And Bio Fertilizer," *Al-Qadisiyah Journal of Pure Science*: Vol. 25: No. 4, Article 9.
DOI: 10.29350/qjps.2020.25.4.1201
Available at: https://qjps.researchcommons.org/home/vol25/iss4/9

Al-Qadisiyah Journal of Pure Science
ISSN(Printed): 1997-2490 ISSN(Online): 2411-3514
DOI: /10.29350/jops.

Antibacterial activities for root extracts of (goldenrods) *Solidago canadensis* L treated by Nano and bio fertilizer

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Article History
Received on:20/9/2020
Revised on:15/10/2020
Accepted on:19/10/2020

Keywords:
goldenrods , *Solidago Canadensis* , Bioactive constituents, Antibacterial activities, Root extracts, GC-MS , nanofertilizer and biofertilizer

DOI:
https://doi.org/10.29350/jops.2020.25.4.1201

ABSTRACT

This experiment include the effect of three concentration of Nano fertilizer 0, 1 and 2 gm./L , and bio fertilizer two concentration , 0 and 20 gm./L and their interactions on production of active ingredient in root of goldenrods. This compounds were measured by GC-Mass. Antimicrobial activities were measured by using the agar well diffusion method (Muller Hinton Agar) on some bacteria . Factors include type of extract for each plant treatment and two concentrations extract (100 and 200 mg / ml). The results show higher level increased of concentration Benzofuran after treatment by 2 gm./L Nano fertilizer without Bio fertilizer, The higher level increased of concentration Stigmasta-7,16-dien-3-ol, (3.beta).after treatment by treatment 1 gm./L Nano fertilizer and 20 gm. /L Bio fertilizer , The higher level increased of concentration Phthalic acid after treatment by treatment 1 gm./L Nano fertilizer and 20 gm. /L Bio fertilizer , The higher inhibition diameter of *Staphylococcus aureus* after treatment by treatment 1 gm./L Nano fertilizer and 20 gm. /L Bio fertilizer , Which is Achieve 15.00 mm and The higher inhibition diameter of *Pseudomonas aeruginosa* after treatment by treatment 2 gm./L Nano fertilizer and 20 gm. /L Bio fertilizer , Which is Achieve 15.00 mm.

Key wards : goldenrods , *Solidago Canadensis* , Bioactive constituents, Antibacterial activities, Root extracts, GC-MS , nanofertilizer and biofertilizer

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1-Introduction

Medicinal plants play an fundamental role in the development of civilizations, as medicinal plants are a major resource for traditional medicines many modern medicines are produced [5] . belongs *Solidago canadensis* L to Asteraceae family, a medicinal plant that has been used in traditional medicine around the world for distant time [17]. It has been used in Traditional medicine in Europe to treat chronic nephritis, , cystitis , rheumatism, and as anti-inflammatory drug [4]. It is also an antioxidant activity, for other diseases such as arthritis, eczema, and other skin diseases, antibacterial activities, anti-cough, analgesic, antispasmodic, sedative and antihypertensive [13].

Since nano Particles can act as stimuli for defense in plants that are often escort by the production of secondary metabolites [9]. It possesses Nano fertilizer great potentials as new effective Abiotic elicitors in plant biotechnology to encourage the synthesis of secondary metabolism products [7] . The Nanotubes treatment caused a significant variations in all measured parameters. GC-MS analysis shows quantitative and qualitative change of bioactive compounds[2]. also bio-fertilizers are also utilized as inducers to improve the synthesis of secondary metabolites in medicinal plants [16]. They consist of living microorganisms, when utilized colonizing the root or inner part of the plant and encourage growth by rising the availability of the primary nutrients of the plant , uses of nitrogen fixation and phosphorus dissolution, and stimulating plant growth through the synthesis of growth-enhancing substances [12]. *Staphylococcus aureus* is a human pathogen that causes infectious diseases in both hospitals and medical facilities. The gram-positive pathogen is armed with a host of virulence factors that facilitate the onset of infection in hosts. Resistant to losing it and minimizing it from antibiotics [8]. bacteria *Pseudomonas aeruginosa* is a common bacterium, Gram-negative opportunistic pathogen able of infecting humans . It is one of the major pathogen associated with nosocomial infections, *P. aeruginosa* is inherently resistant to a number of antibiotics and can develop resistance to many other antibiotics, effect treatment hard . *P. aeruginosa* educe a strong inflammatory response during the infectious , may temporarily suppress symptoms by Antibiotic treatment *P. aeruginosa* of chronic infections ; however, they do not eradicate the pathogen [3]. Therefore, the aim of the research determine the quantitative and

qualitative changes of the medically active substances in root extract of the treated plants, as well as to determine the biological activity.

2-Materials and methods

Experiment was conducted in University of Al-Qadisiyah Biology College of Science Department which included planting of goldenrods in 18 plastic pots (plant per pot) on 2/10/2019. Treatments were add out with sprayed on the plant on 2/11/2019, as Nano fertilizer was sprayed on the plant shoot until the complete wetness at three levels 0, 2 and 4 gm/L , while bio fertilizer was added on soil at two levels 0 and 20 gm/L. The addition of two factors was repeated after one month, the content of bioactive compounds per plant measured After finishing the experiment (01/5/2020), Then preparation of alcohol extract of roots of plants according to the method of [11], by GC-MS technique (Manufacturer: Agilent 5977 A MSD , USA.

The efficacy test experiment included knowing the effect of the alcoholic extract of root of the goldenrods plant on the bacterial genera using the agar well diffusion method, if the process of preparing the media of the Petri dishes account the sterilization conditions and then adding the bacterial suspension by wiping the surface of the middle with a cotton ball. Sterile and then a drill with a diameter of 6 mm was made through a sterile cork punch. After that, the concentrations of the extract prepared in advance in an amount of 0.1 ml were added to each hole with a hole left for the distilled water and a drill for the antibiotic Amoxicillin at a concentration of 250 mg/ml at three replicates for each of the concentrations In which the bacterial species were treated for the extracts, the dishes were transferred to the incubator at a temperature of 37°C for a period of 24 hours and then the plates were extracted to determine and measure the diameter of the inhibition zone around each hole using a Vernier Digital with the measurement being outside the diameter of the drill, the results were compared with the comparison factors and analyzed [17].

3-Results

GC-Mass analysis of root extracts goldenrods

The fertilizers treatment of the goldenrods root plant showed a difference in the numbers and quantity of compounds detected by the GC-MS device among 40 compounds identified in the control sample according to the retention time of

detention and the relative area of the compounds, The obvious differences showed the effect of treatments with nano fertilizers and bio fertilizers on the content of the plant root. The goldenrods was represented by an increase in the proportions of some compounds over others, the emergence of new compounds, and the disappearance of others, according to the statistical analysis data presented in Table (1) for the results of alcohol analysis. Extract from the root of the Goldenrods plant showing the sharing of transactions in a group of compounds that are : Benzofuran ,alpha.- Amyrin, C-Friedours-7-en-3-ol,(3.beta , (Stigmasta-7,16-dien-3-ol, (3.beta ,Kolavenol, Hexadecanoic acid, ethyl ester, -2Isopropylamino-4-methylbenzonitr ile, 2-Methoxy-4-vinylphenol, Phthalic acid, Bis(2-ethylhexyl) phthalate .

Table (1): shows the compounds involved in the treatments in the results of the alcohol extract analysis of the root of the Goldenrods plant.

Compound	RT	Nano fertilizer (gm/L)					
		0		1		2	
		Bio fertilizer (gm/L)					
		0	20	0	20	0	20
Benzofuran	13.593	0.81	0.66	0.22	0.12	9.72	0.15
alpha.-Amyrin	35.375	5.42	4.23	4.92	3.55	1.59	2.32
C-Friedours-7-en-3-ol,(3.beta)(Baurenol)	36.744	9.52	6.37	14.77	1.87	7.38	2.00
Stigmasta-7,16-dien-3-ol, (3.beta).	34.151	7.21	4.90	4.09	9.56	4.91	3.55
Kolavenol	25.012	5.16	6.75	13.14	1.44	3.40	2.85
Hexadecanoic acid, ethyl ester	22.350	0.38	0.38	0.93	0.85	0.38	0.45
2-Isopropylamino-4-methylbenzonitr ile	17.736	3.31	1.53	0.21	0.38	2.80	2.95
beta-copaene	--------	------	-----	1.05	0.23	----	----
Propanoic acid	29.584	----	------	1.39	------	1.38	------
-2Methoxy-4-vinylphenol	14.659	3.59	1.70	0.67	2.97	1.88	0.77

Phthalic acid	21.111	0.65	3.96	0.62	1.09	0.53	0.30
Bis(2-ethylhexyl) phthalate	27.093	17.52	0.46	0.62	0.70	0.16	0.41

Effect of addition of nan and bio fertilizers and their interactions in the amount of Stigmasta-7,16-dien-3-ol, (3.beta)) in the root of the Goldenrods plant

The results of the statistical analysis presented in Table (2) for the compound Stigmasta-7,16-dien-3-ol, (3.beta)) were found in the root of the Goldenrods plant for the compounds that were detected in the data of the GC Mas device, which was the retention time (34.151), as the addition of the nano fertilizer at a concentration of 1 gm/ L showed an increase in the percentage of the compound, but it was not significant, as it was recorded at 6.82, and when the concentration of the nano fertilizer was increased to 2 gm/ L, the percentage of the compound decreased until it reached a ratio of 4.23 compared to the comparison plants. It scored 6.05, as for the use of bio fertilizer, it led to an increase in the compound percentage, but it was not significant, as it achieved a percentage of 6.00 compared to the comparison plants, which recorded 5.40.

The results of the statistical analysis of the same table also indicate that the treatment of the nano fertilizer with a concentration of 1 gm/ L and the bio fertilizer of 20 gm/ L recorded a significant increase in the percentage of the compound, as it achieved 9.56, and the treatment of the nano fertilizer with a concentration of 2 gm/ L and the bio fertilizer 20 gm/ L did not An increase in the compound percentage was achieved, as it decreased to 3.55 compared to the comparison plants, which recorded 7.21.

Table (2): shows the effect of addition of nan and bio fertilizers and their interactions in the amount of Stigmasta-7,16-dien-3-ol, (3.beta)) in the root of the Goldenrods plant

Concentrate Nano fertilizer gm/ L	Concentrate Bio fertilizer gm/ L		Average Nano fertilizer
	0	20	
0	7.21	4.90	6.05
1	4.09	9.56	6.82
2	4.91	3.55	4.23

Average Bio fertilizer	**5.40**	**6.00**	
Values RLSD At a significant level 0.05	**Nano fertilizer**	**Bio fertilizer**	intervention
	1.25	1.02	1.77

Effect of addition of nan and bio fertilizers and their interactions in the amount of Phthalic acid in the root of the Goldenrods plant

The results of the statistical analysis presented in Table (3) of the Phthalic acid compound in the root of the gold stick plant for the compounds that were revealed in the data of the G-C Mas device, which had a holding time (21.111) .

The percentage of the compound decreased by adding the nano fertilizer when increasing its concentration from 1 to 2 gm / L , as it reached from 0.85 to 0.41, respectively, compared to the comparison treatment, which amounted to 2.30. As for the use of the bio fertilizer, it led to a significant increase in the percentage of the compound, which reached 1.81 compared to a treatment. The comparison, which was 2.05, as for the use of biological fertilizer, it achieved a significant increase in the percentage of the compound, reaching 1.78, compared to the comparison treatment, which amounted to 0.60.

As indicated by the results of the statistical analysis of the same table, the combination of the nanofertilizer with a concentration of 1 gm / L and the bio fertilizer of 20 gm / L showed an increase in the percentage of the compound, but it was not significant if the ratio was 1.09, while the combination of the nanoconstrictor with a concentration of 2 gm / L and the biological fertilizer 20 gm / L did not achieve any increase in the percentage of a compound if it decreased when the treatment showed a ratio of 0.30, while the percentage of comparison plants was 0.65.

Table (3): shows the effect of addition of nan and bio fertilizers and their interactions in the amount of Phthalic acid in the root of the Goldenrods plant

Concentrate Nano fertilizer gm/ L	**Concentrate Bio fertilizer gm/ L**		**Average Nano fertilizer**
	0	**20**	
0	**0.65**	**3.96**	**2.30**
1	**0.62**	**1.09**	**0.85**

2	**0.53**	**0.30**	**0.41**
Average Bio fertilizer	**0.60**	**1.78**	
Values RLSD	**Nano fertilizer**	**Bio fertilizer**	intervention
At a significant level 0.05	**0.77**	**0.63**	**1.09**

Effect of the alcoholic extract of the root of Goldenrods plant treated by nano and bio fertilizers and their interaction of inhibiting the growth of *Staphylococcus aureus*

Table (4) indicated for the results mentioned in the statistical analysis table a significant increase in inhibition of growth of *Staphylococcus aureus* bacteria, reaching 10.66 and 15.83 mm when increasing the concentration of the extract from 100 to 200 mg / ml, respectively. The results in the same table showed that the effect of average extracts of the shoots treatments when treatment A2B2 achieved the highest bacterial inhibition diameter as it reached 15.00 mm compared to plants of treatment A1B1 that achieved 12.50 mm.

Table (4): shows the effect of the alcoholic extract of the root of the Goldenrods plant when adding nano and bio fertilizers and their intervention of inhibiting the growth of bacteria *Staphylococcus aureus*

Treatments	**Concentrations mg/ml**		**effect of the rate of extracts the treatments of root**	**Comparison of treatments**	
	100	**200**		**Amoxicillin**	**DMSO**
A1B1	**10.00**	**15.00**	**12.50**	**25**	**0**
A1B2	**10.00**	**14.00**	**12.00**	Values RRLSD 0.01 extracts of root 1.92 For extract concentrations 1.57 intervention 3.06	
A2B1	**11.00**	**15.00**	**12.50**		
A2B2	**12.00**	**18.00**	**15.00**		
A3B1	**11.00**	**17.00**	**14.00**		
A3B2	**10.00**	**15.00**	**12.50**		
effect of average concentrations of extract	**10.66**	**15.83**			

A: Nano fertilizer (A1 : 0 gm/L , A2: 1 gm/L , A3: 2 gm/L)

B: Bio fertilizer (B1 : 0 gm/L , B2 : 20 gm/L)

The effect of interaction between the treatments and their concentration showed a significant effect in increasing the diameter of inhibition of bacterial growth, as the highest average inhibition diameter was 18.00 mm at the A2B2 combination at a concentration of 200 mg / ml compared to the lowest average diameter of inhibition of 10.00 mm at the combination A1B1 at a concentration of 100 mg / ml.

Effect of the alcoholic extract of the root of Goldenrods plant treated by nano and bio fertilizers and their interaction of inhibiting the growth of *Pseudomonas aeruginosa*

Table (5) indicated for the results mentioned in the statistical analysis table a significant increase in inhibition of growth of *Staphylococcus aureus* bacteria, reaching 10.50 and 15.16 mm when increasing the concentration of the extract from 100 to 200 mg / ml, respectively. The results in the same table showed that the effect of average extracts of the shoots treatments when treatment A3B1achieved the highest bacterial inhibition diameter as it reached 15.00 mm compared to plants of treatment A2B2 that achieved 14.00 mm.

Table (5): shows the effect of the alcoholic extract of the root of the Goldenrods plant when adding nano and bio fertilizers and their intervention of inhibiting the growth of bacteria *Pseudomonas aeruginosa*

Treatments	Concentrations mg/ml		effect of the rate of extracts the treatments of root	Comparison of treatments	
	100	200		Amoxicillin	DMSO
A1B1	9.00	12.00	11.50	23	0
A1B2	11.00	12.00	11.50	Values RRLSD 0.01 extracts of root 1.87 For extract concentrations 1.46 intervention 3.72	
A2B1	10.00	16.00	13.00		
A2B2	11.00	17.00	14.00		
A3B1	12.00	18.00	15.00		
A3B2	10.00	14.00	12.00		

effect of average concentrations of extract	**10.50**	**15.16**		
A: Nano fertilizer (A1 : 0 gm/L , A2: 1 gm/L , A3: 2 gm/L) B: Bio fertilizer (B1 : 0 gm/L , B2 : 20 gm/L)				

The effect of the bilateral interaction between the treatments and their concentration showed a significant effect in increasing the diameter of inhibition of bacterial growth, as the highest average inhibition diameter was 18.00 mm at the A3B1combination at a concentration of 200 mg / ml compared to the lowest average diameter of inhibition of 9.00 mm at the combination A1B1 at a concentration of 100 mg / ml.

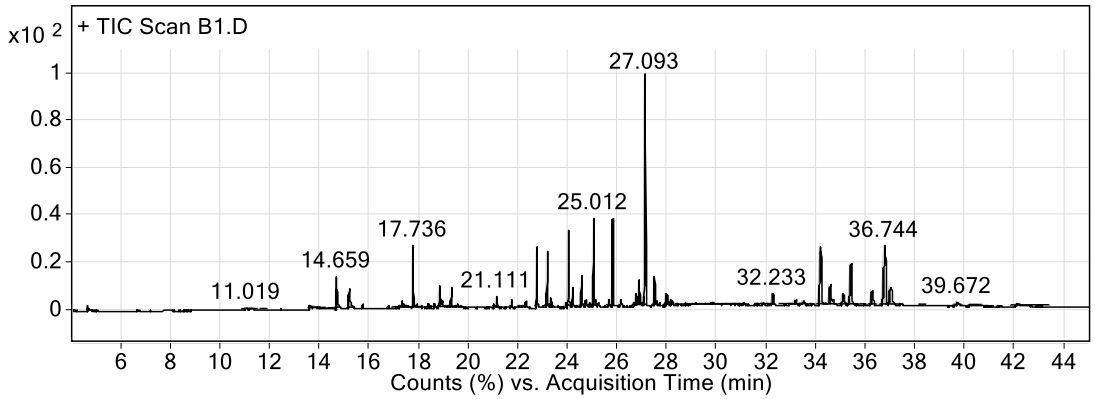


Figure1

GC-MS chromatogram of alcohol extract from root of Solidago canadensis L. from combination treatment of 0 gm./L Nano fertilizer and 0 gm./L bio fertilizer.

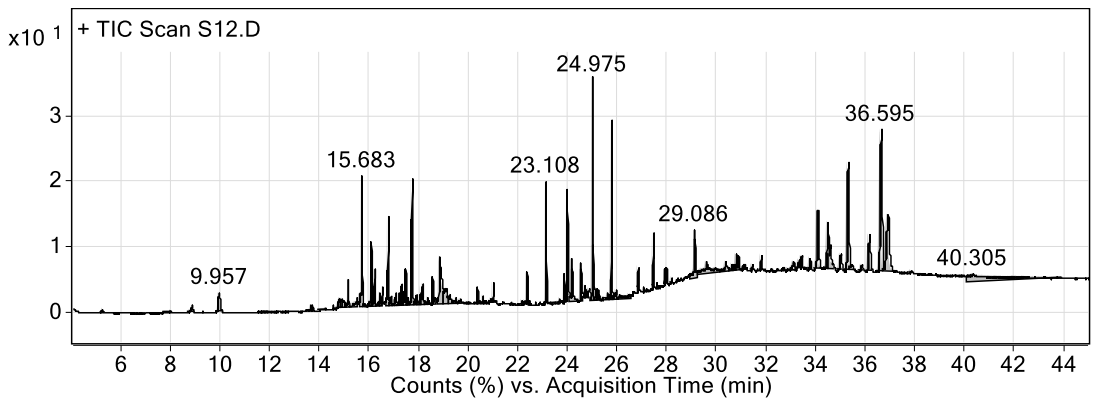


Figure 2

GC-MS chromatogram of alcohol extract from root of Solidago canadensis L. from combination treatment of 2 gm./L Nano fertilizer and 0 gm./L bio fertilizer.

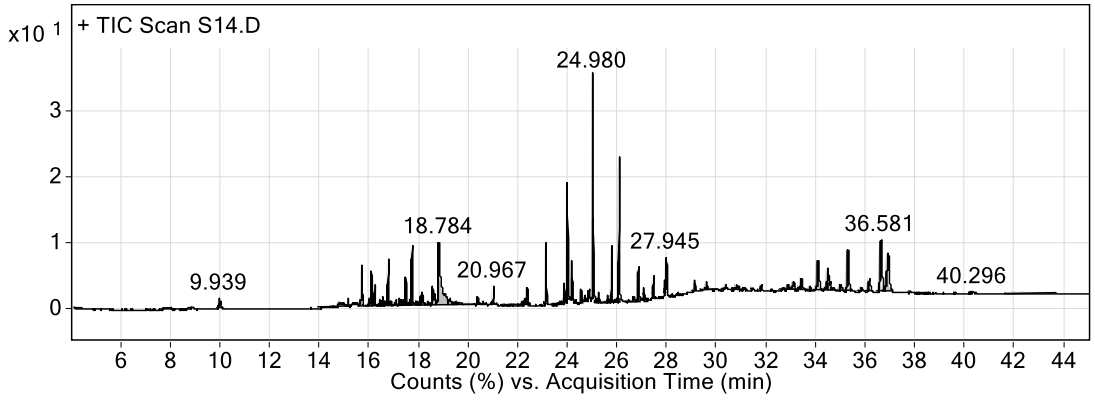


Figure 3

GC-MS chromatogram of alcohol extract from root of Solidago canadensis L. from combination treatment of 2 gm./L Nano fertilizer and 20 gm./L bio fertilizer.

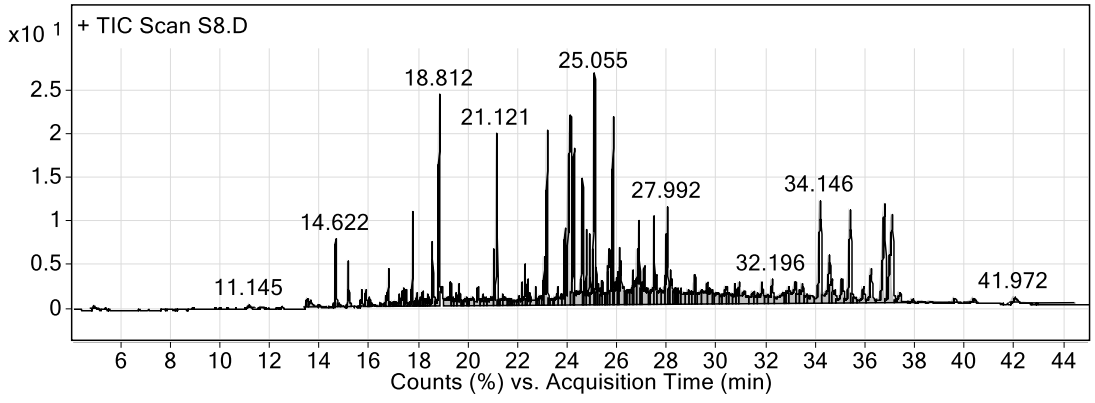


Figure 4

GC-MS chromatogram of alcohol extract from root of Solidago canadensis L. from combination treatment of 0 gm./L Nano fertilizer and 20 gm./L bio fertilizer.

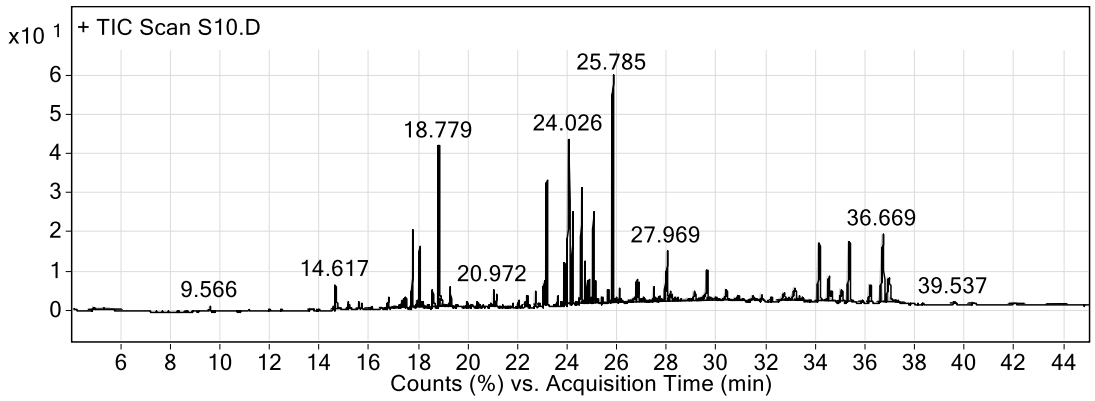


Figure 5

GC-MS chromatogram of alcohol extract from root of Solidago canadensis L. from combination treatment of 4 gm./L Nano fertilizer and 0 gm./L bio fertilizer.

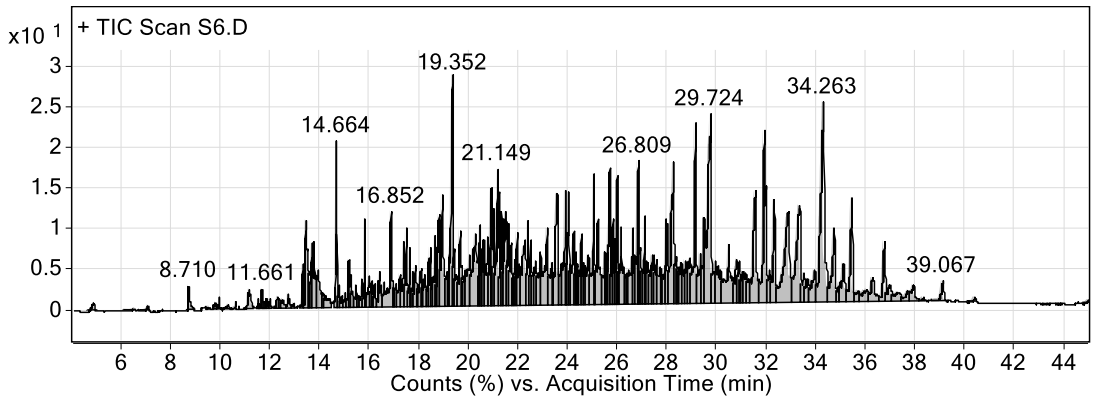


Figure 6

GC-MS chromatogram of alcohol extract from root of Solidago canadensis L. from combination treatment of 4 gm./L Nano fertilizer and 20 gm./L bio fertilizer

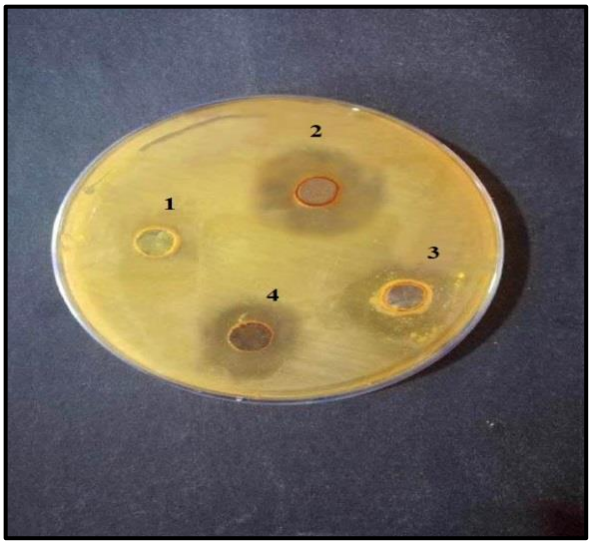
Picture (4-2): shows the effectiveness of the alcoholic root extracts of the gold stick plant, with the combination of A1B2, in inhibiting *Staphylococcus aureus.*

1-Represents the addition of the solvent DMSO

2- The antibiotic Amoxicillin

3- The addition of the extract concentrations 100

4: The addition of the extracted concentration 200



Picture (4-2): shows the effectiveness of the alcoholic root extracts of the gold stick plant, with the combination of A1B2, in inhibiting *Pseudomonas aeruginosa*

1-Represents the addition of the solvent DMSO

2- The antibiotic Amoxicillin

3- The addition of the extract concentrations 100

4: The addition of the extracted concentration 200

4-Discussion

The study showed that the alcohol extracts of plants treated with nano and bio-fertilizers and their interactions had an effect on inhibiting the growth of bacteria positive for *Staphylococcus aureus* and Gram negative bacteria for *Pseudomonas aeruginosa* in varying proportions, and the reason for the effect of the extract in inhibiting some bacterial activity may be The same anti-bacterial activity that appeared in the GC-MS gas chromatography examination, whose ratios differed according to the different treatments, as the detection of the compounds showed a

high percentage of **Beta-Copaene** which has anti-bacterial activity, as indicated by studies on essential oils containing Beta-Copaene- Possesses anticancer, antioxidant, liver protective and anti-inflammatory activities, [10], The test also revealed the presence of **Alpha-Amyrin** which has anti-inflammatory properties [15], Also Benzofuran which has anti-bacterial properties [11], aside from compound **Phthalic acid** Which has high effectiveness in inhibiting the growth of bacteria [1], and compound **Bis(2-ethylhexyl) phthalate** It also has anti-bacterial properties [14].

Phenolic such as Phthalic acid act to cause damage to the cell membranes of bacteria if they have the ability to change the nature of cell membrane proteins by binding to the active site of cell enzymes and inhibiting action, which works to change the permeability of the membranes and inhibit the basic enzymes, which leads to imbalance in cellular functions and their inability To survive by forming hydrogen bonds between phenolic hydroxyl groups and proteins, which causes damage to the function of the necessary enzymes in the bodies of the bacteria[6].

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