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DIAGNOSIS OF ACTIVE COMPOUNDS OF ETHANOL AND HEXANE EXTRACT OF *AZADIRACHTA INDICA* BY GAS CHROMATOGRAPH MASS SPECTROMETER TECHNIQUE (GC-MS) AND EVALUATION OF ITS ANTAGONISM EFFICIENCY ON THE *FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI* GROWTH *IN VITRO*

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Abstract

This study was conducted to detect the active chemical compounds found in the ethanol and hexane extract of the Neem plant *Azadirachta indica* by conducting preliminary disclosures that established the presence of alkaloids, resins, flavonoids and tannins except for the phenols that gave a negative detection. Analysis results: The emergence of more than 40 active chemical compounds in the ethanol neem extract was the most common n-Hexadecanoic acid ratio at 8.27%, followed by compound 1-Methoxy 2, 3, cis-dimethylaziridine at 6.45% and other important compounds such as Neophytadiene and Ethyl orthoformate either. Hexane showed that it contains more than 50 active chemical compounds. The most effective was the ratio of Phytol with 18.21% and Hexadecane 1-iodo with 8.21%, followed by the n-Hexadecanoic acid with 5.71%. The results of the analysis also showed the presence of Stigmasterol and gamma.-sitosteral compounds in the ethanol and hexane extracts of neem plants and the efficiency of the extracts was tested in Inhibition of the diagonal growth of the four isolates of the fungus *Fusarium oxysporum* f. sp. *lycopersici* The results showed that the efficiency of ethanol and hexane extracts with concentrations of 1.25%, 2.5% and 5% showed that the concentration showed 5% superiority in inhibiting the growth of isolates. The highest in ethanol extract was above 96%, while 100% was recorded in the hexane extract.

Key words : Ethanol, Hexane Extract, *Azadirachta indica*, Gas Chromatograph Mass Spectrometer Technique (GC-MS).

Introduction

Nature is an enormous source and a store of many natural chemicals that have the ability and efficiency to be used as pesticides (Nair and Chanda 2007). Besides, plants produce various substances from chemical compounds, including primary metabolites that include carbohydrates, fats, and proteins, and secondary metabolites such as alkaloids, flavonoids, steroids, saponins phenols, and others (Ketkar and Kethar 1995). However, the Azadrachta indica tree is an evergreen tree belonged to the Milaceae family, which planting in the tropics and subtropics regions and its original territory India and Burma, and it is a fast-growing tree with an average length of 15-20 m (Ahana, 2005). The use of chemical pesticides is very expensive and causes real problems in pollution of the ecosystem; therefore, a strategy for plant disease control is the change towards environmentally friendly natural products (Mamdouh and Eweis, 2007). The oil extracted from neem leaves is highly active against many microbes (Asif 2012), and the efficacy of neem oil is attributed to its containment of alkaloids, glycosides, and fatty acids (Marukawa *et al.*, 1975). In addition, the neem leaf extract was able to inhibit the aflatoxins produced by the fungus Aspergillus parasiticus and antifungal extracted from the neem leaves, was also recorded a significant effect in the southern United States against the fungus Crinipellis perniciosa. As well as, Phytophthora sp. that causes witches' broom

disease and pot not a disease of cocoa (Gorbamiam, 2007; Ramos *et al.,* 2007). Neem leaf extract also proved effective in inhibiting the radial growth of the fungus Rhizopus sp. and Aspregillus sp. in the culture medium PDA (Mondali *et al.,* 2009), as (Siva *et al.,* 2008) recorded that neem leaf extract inhibited the growth of the fungus *F.oxysporum* by 98%. In addition, the efficiency of neem extract from leaves, bark, oil and fruits was recorded on soil pathogens, including Rhizoctonia solani and *F. oxysporum* and Sclerotium sp. Consequently, the most important goal of this study is to identify and diagnose the chemical compounds present in the leaves of the neem tree planted in Basra, which they are new to the environment. Besides, identify the effect of its cultivation in a new environment on the effective compounds in it and the possibility of using neem leaves extract as an antifungal against isolates of *Fusarium oxysporum* f. sp. Lycopersici that causes fusarium wilt of tomato in vitro.

Materials and Methods

The Isolates of fungus *F. oxysporum* f.sp. lycopersici

Four isolates were obtained for *F.oxysporum* f.sp. Lycopersici are diagnosed and purified from the advanced biology control laboratory in the College of Science-Department of Biology, in order to use it in the experiment of the efficient evaluation of the ethanol and hexane extract of neem A.indica. The isolates were taken from four regions in Basra Governorate are Al-Zubair F_1, Al-Bergussia F_2, Karma University F_3, and Department of Plant Protection in Karma F_4.

The Crushing of the *Azadirachta indica* Leaves

A. indica leaves were collected from a farm in the Abu- alkhasib region in Basra Governorate and were diagnosed by Professor Dr. Taha Al-Aidani, College of Agriculture- Plant Protection Department. Furthermore, the leaves washed from the remaining soil, then dried at room temperature by putting them on clean papers, and crushed by a mill type Philips to obtain a powder that used in the subsequent experiments.

The performing of preliminary chemical tests

1. Determination of pH

A10 g of plant powder was mixed with 50 ml of distilled water, and then were filtered using filter paper, and the filtrate was tested using a Johnson-made filter paper of size 5 m × 7mm.

2 Resin test

A 5 g of dry plant powder added to 50 ml of ethyl alcohol 95% and left for one minute in a water bath at 100 ° C, and then the solution was filtered by Wat.No.1 filter paper. As well as, 100 ml of water and 4% HCL was added to it, where the appearance of turbidity is an indication of the presence of resin materials (Shihata, 1951).

3. Saponins test

A 3 ml of plant extract was added to 2 ml of mercuric chloride, where the formation of a white precipitate is an indication of the presence of Saponins (Harborn, 1984).

4. Coumarins Test

A 1 g of plant powder was mixed with 10 ml of ethyl alcohol 95% in a test tube covered by wet filter paper with a sodium hydroxide solution 5%. Then, the tube was placed in a boiling water bath for a few minutes, where the appearance of greenish-yellow color is an indication of the presence of Coumarins (Geissman, 1962).

5. Alkaloids Test

Dragendorff reagent was used by putting 0.5 ml of plant aqueous extract in a test tube and an equal volume of the reagent was added, where the appearance of an orange precipitate is an indication of the presence of the alkaloid compounds (Harborn, 1984).

6. Tannins Test

An equal amount of ferric chloride $FeCl_3$ was added to the amount of extract in a test tube containing the plant aqueous extract, where the appearance of bluish-green color is an indication of the presence of tannins (Harborn, 1984).

7. Phenols Test

A 1 ml of plant aqueous extract was mixed with 1 ml ferric chloride, where the appearance of a bluish-green color is an indication of the presence of phenols (Shihata, 1951).

8. Flavonoids Test

A 2 ml of plant aqueous extract was mixed with 1 ml of alcoholic potassium hydroxide 0.5 M, where the appearance of yellow color is an indication of the presence of Flavonoids (Harborn, 1984).

Preparation of alcoholic extracts

The organic solvent Ethanol 95% was used, which is a polar solvent, and the hexane solvent, which is a non-polar solvent to prepare plant extracts. A 20 g of dry powder for the neem was taken and put it in a Thumbless and placed in the Soxhlet extractor using 200 ml of the organic solvents above separately for six hours, and then the ethanol extract was dried with a rotary evaporator at 45°C. As for hexane extract, it was left to volatilize at the laboratory temperature, after which the dry active substance was weighed for each extract to prepare the

concentrations used in subsequent experiments (Mansour, 1995). Finally, the extraction process was repeated for each solvent to obtain sufficient quantities of the active substance.

Diagnosis of chemical compounds in plant extracts using the GC-MS technique

All plant extracts, ethanol, and hexane extract were tested by the Gas Chromatography-Mass Spectrograph (GC-MS) Japanese-made that located in the Basra Oil Company, Nahir bin Omar site, type GC Shimadzu 2010, which its specification is shown in table 1. This device equipped with a capillary column phenyl-methyl siloxane HP-5MS 5%, as a stationary phase with dimensions of 30 m in length and 0.25 mm in diameter and its thickness is 250 µm using helium gas as a carrier gas. The separation process carried out according to the thermal program of the GC at a temperature of 40°C for 5 minutes and then increased to 280°C for a minute at a rate of 10°C per minute.

Table 1: GC-MS Specifications and working conditions.

No.	Gas Chromatography	Mass Spectrometer
1	Column Oven Temp.:40°C	Ion Source Temp.:230°C
2	Injection Temp.:290°C	Interface Temp.:290°C
3	Injection Mode:pulsed flow	Solvent Cut Time:4.00min
4	Pressure:7.0699 psi	Start Time:4.00 min
5	Total Flow :19ml/min	End Time: 35-40 min
6	Column Flow:1ml/min	ACQ Mode: Scan
7	Purge Flow:3ml/min	Event Time:0.50 sec.
8	Split Ratio 60	Scane Speed:1562(N_2)
9		Start m/z 35
10		End m/z 650

The injection was performed at 290°C under pressure 7.0699 psi and total flow 19 ml/min, column flow 3 ml/min, and the curves spectra were diagnosed according to the spectral library (NIST, 2005).

Antagonism test for plant extracts against the *F.oxysporum* isolates

Concentrations 1.25%, 2.5%, and 5% of the active substance were prepared for each plant extract (ethanol and hexane) and added it to 100 ml of sterile nutrient media PDA before it solidified. Besides, each concentration was mixed separately, and the media were poured according to each concentration at a rate of three dishes per isolate and for each concentration separately with the presence of comparable treatment in the nutrient media alone. Finally, the dishes incubated at a temperature of 26 ± 2 ° C for 7 days, after which the percentage of inhibition the radial growth of the fungus was calculated according to the following equation:

Percentage of inhibition =

$$\frac{\text{growth rate of control - growth rate of treated sample}}{\text{growth rate of control}} \times 100$$

Results and Discussion

Results of preliminary tests

Table 2 showed that the neem leaves extract contains secondary and active metabolism compounds, as all the tests represented by alkaloids, resins, saponins, coumarins, tannins, and flavonoids were given a positive result except the phenol test was negative. This is due to the fact that the extract is biologically active and used as an antifungal and antibacterial against many plant pathogens as well as bacteria. These results are consistent with (Enenche *et al.*, 2019) findings, except for the difference in the negativity of the phenol test, which may be due to the incompatibility of the test used.

The analysis results by the GC-MS technique

1. Ethanol extract

It was observed that the ethanol extract of neem was green when it is liquid and similarly after the extract was dried, 1.4 g of the active substance were obtained from extracting every 20 g of powder, where the extracted active substance was used to conduct chemical analysis by the GC-MS device. The analysis showed the presence of 43 chemical compounds and that the compound n-Hexadecanoic acid ranked the highest percentage exceeded other compounds by 8.27%. Followed by the compound 1Methoxy2, 3–cis-dimethylaziridine of 6.45%, followed by Cyclodecasiloxane, eicosamethyl of 5.45 %, then N-Benzyl-2-phenethylamine of 3.96, and Ethyl orthoformate of 3.79. It was also recorded the presence of Stigmasteral, Gamma. Sitosterol and Oleic Acid of fatty acids that their percentages reached (0.56, 1.20, and 2.89%), respectively, as shown in Table 3 and Fig. 1.

These results were agreed with (Enenche *et al.*, 2019), which indicated the presence of Phytol in the ethanol extract of neem and the compound Hexadecanoic acid, which is also a fatty acid and is an antifungal and antibacterial (Ogunlesi *et al.*, 2009). However, (Prashanth and Krishnaiah, 2014) pointed out the presence of Phytol,

Table 2: Results of preliminary tests of *A.indica* plant extract.

Plant	Phenols	Alkaloids	Resins	Saponins	Coumarins	Tannins	Flavonoids	PH
A.indica	-	+	+	+	+	+	+	5

Table 3: Chemical compounds in the ethanol extract of A.indica using GC-MS technique.

Seq.	Compound	Chemical formula	Retention time	Area %
1	Thioacetic acid	$C_20H_{25}N_3O$	5.48	0.52
2	Ethyl orthoformate	$C_3H_{10}OSi$	5.845	3.79
3	dl-Glyceraldehyde diethyl acetal	$C_7H_{16}O_4$	5.845	3.79
4	Furfural	$C_6H_9N_3S$	6.310	0.85
5	Aziridine, 2,2,3,3-tetramethyl-	$C_8H_{14}O_3$	8.228	0.65
6	2-Hexene, (Z)-	C_6H_{12}	8.228	0.65
7	3-Butenoic acid	$C_4H_8O_2$	8.228	0.65
8	2-Furancarboxaldehyde, 5-methyl-	$C_6H_6O_2$	8.98	2.28
9	2,4-Dihydroxy-2,5-dimethyl-3(2H)-f uran-3-one	$C_5H_8N_2O_3$	9.312	0.87
10	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-metyl-	$C_6H_8O_4$	9.13	0.87
11	Thiazole, 2-ethyl-	$C_{13}H_{26}O$	10.556	1.91
12	2-Hexanone, 4-methyl-	$C_7H_{14}O$	10.556	1.91
13	Methylamine, N, N-dimethyl-	$C_{19}H_{41}N$	10.556	1.91
14	Furaneol		11.34	1.50
15	1-Methoxy-2,3-cis-dimethylaziridin e (sin)	$C_6H_{12}N_2O_2$	11.814	6.45
16	Benzofuran, 2,3-dihydro-	C_8H_8O	13.662	3.96
17	N-Benzyl-2-phenethylamine	$C_{15}H_{17}N$	13.662	3.96
18	5-Hydroxymethylfurfural	$C_7H_{10}S$	13.926	2.20
19	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	14.885	2.36
20	3-Morpholinopropyl 2,4-dihydroxybenzoat–no)methyl]morpholin-4-ylmethyl	$C_{12}H_{24}O_3$	14.996	2.13
21	Phosphinic acid, [(formylm ethylami	H_3O_2P	14.996	2.13
22	Pentanoic acid, 4-methyl-, pentyl ester	$C_{12}H_{24}O_3$	17.706	0.26
23	2-Hydroxy-1-(1'-pyrrolidiyl)-1-buten-3-one	$C_6H_{11}NO_2$	17.48	0.89
24	Tetrafluoroanisol-2,3,5,6	$C_{12}H_{24}O_3$	18.088	1.46
25	Neophytadiene	$C_{12}H_{24}O_3$	21.111	3.47
26	Octadecyne-3	$C_{18}H_{34}$	21.111	3.47
27	13-Methyltetradecanal	$C_{19}H_{36}O_2$	21.535	2.04
28	trans-2-Dodecen-1-ol	$C_{12}H_{24}O$	21.535	2.04
29	1-Hexadecyne	$C_{16}H_{30}$	21.535	2.04
30	n-Hexadecanoic acid	$_2C_{16}H_{32}O$	22.424	8.27
31	Eicosane	$C_{20}H_{42}$	22.68	0.35
32	Lactose	$C_{18}H_{34}O_2$	23.613	3.77
33	1-Trimethylsilyl-3-(dimethyl-n-pen tylsilyl)but-1-ene	$C_{12}H_{16}$	23.613	3.77
34	Phytol	$C_{20}H_{40}O$	23.73	0.83
35	Cyclodecasiloxane, eicosamethyl	$C_{18}H_{34}O_2$	24.002	5.45
36	Octadecanoic acid	$C_{18}H_{36}O_2$	24.245	3.68
37	Cyclododecanone	$C_{18}H_{34}O_2$	25.767	2.89
38	Oleic Acid	$C_{18}H_{34}O_2$	25.767	2.89
39	9-Octadecenamide, (Z)-	$C_{18}H_{34}O_2$	25.899	1.54
40	Hexadecanoic acid, 2-hydroxy-1—(hydroxymethyl)ethyl ester	$C_{35}H_{68}O_5$	27.031	1.82
41	Glycerol 1-palmitate -	$C_{35}H_{68}O_5$	27.031	1.82
42	Stigmasterol	$C_{26}H_{44}O_5$	33.973	0.56
43	Gamma.-Sitosterol	$C_{29}H_{50}O$	34.876	1.20
	.			100. Σ

Table 3: Chemical compounds in the hexane extract of A.indica using GC-MS technique.

Seq.	Compound	Chemical formula	Retention time	Area %
1	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	$C_{11}H_{16}O_2$	17.692	0.60
2	Dodecanoic acid	$C_{12}H_{24}O_2$	18.137	0.58
3	Hexadecane	$C_{16}H_{34}$	18.498	0.66
4	Heptadecane	$C_{17}H_{36}$	19.631	0.77
5	Tetradecanoic acid	$C_{14}H_{28}O_2$	20.354	1.00
6	Octadecane	$C_{18}H_{38}$	20.701	0.82
7	Hexadecane, 2,6,10,14-tetramethyl-	$C_{20}H_{40}$	20.805	0.59
8	Hexacosane	$C_{26}H_{54}$	20.805	0.59
9	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9$	21.069	1.74
10	Neophytadiene	$C_{17}H_{36}$	21.125	1.92
11	2-Pentadecanone, 6,10,14-trimethyl	$C_{18}H_{36}O$	21.160	0.66
12	9-Octadecyne	$C_{10}H_{18}$	21.354	0.62
13	Phytol	C_6H_6O	21.542	0.85
14	Nonadecane	$C_{19}H_{40}$	21.715	0.78
15	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	22.556	5.71
16	Eicosane	$C_{20}H_{42}$	22.695	0.79
17	Heneicosane	$C_{21}H_{44}$	23.619	0.67
18	Ethyl 9,12,15-octadecatrienoate 9,12,15-Octadecatrienoic acid, ethylester (Z,Z,Z)	$C_{20}H_{34}O_2$	24.259	2.35
19	Hexadecane, 2,6,10,14-tetramethyl-	$C_{20}H_{42}$	24.530	0.65
20	Docosane	$C_{22}H_{46}$	24.530	0.65
21	Tetrapentacontane, 1,54-dibromo-	$C_{25}H_{50}$	26.190	0.90
22	Ethyl 9-hexadecenoate	$C_{17}H_{32}O_2$	26.190	0.90
23	Cyclopentane, (4-octyldodecyl) -	$C_{25}H_{50}$	26.190	0.90
24	Hexadecanoic acid, 2-hydroxy-1	$C_6H_{10}O_5$	27.52	1.33
25	droxymethyl)ethyl ester25	$C_{18}H_{36}O_2$	27.52	1.33
26	Octadecanedioic acid	$C_{20}H_{25}N_3O$	27.52	1.33
27	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	27.337	0.58
28	Phthalic acid, di(2-propylpentyl) ester	$C_{24}H_{38}O_4$	27.337	0.58
29	9-Octadecenoic acid (Z)-, 2,3-dihy droxypropylester	$C_{18}H_{34}O_2$	28.407	0.79
30	Heptacosane	$C_{27}H_{56}$	28.470	0.83
31	Nonacosane	$C_{29}H_{60}$	30.019	5.35
32	Heptadecane, 3-methyl	$C_{18}H_{36}$	30.019	5.35
33	Hexadecane, 1-iodo-	$C_{12}H_{26}$	32.027	8.21
34	Triacontane, 1-iodo	$C_2H_4Cl_2$	32.027	8.21
35	Nonadecane, 9-methyl-	$C_{20}H_{42}$	32.027	8.21
36	Vitamin E	$C_{29}H_{50}O_2$	32.368	3.22
37	Tricosane	$C_{23}H_{48}$	33.174	0.61
38	Pentacosane	$C_{25}H_{52}$	33.174	0.61
39	Campesterol	$C_{28}H_{48}O$	33.556	0.70
40	Cholestene-3-ol, 24-methyl-5-	$C_{28}H_{47}Cl$	33.556	0.70
41	Stigmasterol	$C_{29}H_{48}O$	34.056	1.85
42	Tritriacontane	$C_{33}H_{68}$	34.772	2.62

Table 3 contd....

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Seq.	Compound	Chemical formula	Retention time	Area%
43	Octacosane	$C_{28}H_{58}$	34.772	2.62
44	.gamma.-Sitosterol	$C_{29}H_{52}O_2$	35.001	2.89
45	Lanosterol	$C_{30}H_{50}O$	36.391	0.61
46	Hentriacontane	$C_{31}H_{64}$	38.816	0.57
47	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	19.346	1.87
48	Bicyclo[3.1.1]heptane, 2,6,6-trime thyl-, [1R-(1.alpha.,2.beta.,5.alp ha).]-	$C_{10}H_{18}$	21.125	1.92
49	Phytol	$C_{20}H_{40}O$	23.877	18.30
50	Benzeneethanamine, N-[(pentafluoro phenyl)methylene]-.beta.,3,4-tris [(trimethylsilyl)oxy]-	$C_{20}H_{60}O_{10}Si_{10}$	29.658	2.28
				100Σ

and other diagnosed compounds agreed with (Oladotun *et al.*, 2018) findings by diagnosing the same compounds in the ethanol extract of the neem plant.

2. The results of active chemical compounds in the hexane extract of A.indica leaves

From the study results, it was observed that the color of the hexane extract of A.indica leaves was blackish dark green with a viscosity. Furthermore, the analysis results that are shown in table 3 and Fig. 2, which carried out by the GC-MS device indicated that the extract contains 50 chemical compounds in which the compound Phytol ranked the highest percentage of the compounds and reached 18.30%. The percentage of 8.21% was recorded for Hexadecane, 1-iodo, Triacontane, 1-iodo and Nonadecane, 9-methyl, and the compound Hexadecanoic acid recorded a percentage of 5.71%, whereas Stigmasterol recorded a percentage of 1.85%, as well as Gamma.-sitosterol by 2.89% and Octacosane by 2.62%. These results were agreed with (Babatunde *et al.*, 2019) study, that recorded Hexadecane and Tetradecanoic acid, N-Hexadecanoic acid, Phytol, Eicosane, Tetracosane, Octadecane, Vitamine E, Gamma-.sitosterol, 9,12,15-Octadecatrienoic acid (Z, Z, Z,), which is an antifungal, antibacterial, antioxidant and anti-cancer (Iheagwam *et al.*, 2018)

3. Effect of ethanol extract on the radial growth of fungus *F. oxysporum* f.sp. lycopersici

The results shown in Fig. 3 indicated that the ethanol extract of A.indica has affected the radial growth of *F. oxysporum* f.sp. lycopersici in the culture media PDA. The concentration 1.25% g / L reached 64.7% for isolating number 4 with a significant difference from the inhibition percentage for F_1, F_2, and F_3 isolates that reached 51.8% and without significant differences between them. In contrast, the concentration 2.5% g / L has more affected to reduce the radial growth of fungus isolates, where the highest effect was in isolate F_2 by 79.2%, while it reached 53.6% in isolate F_3 and 75.5% in isolate F_1, and reached 77.4% for isolate F_4. Additionally, the concentration 5% exceeded in reducing the radial growth for all isolates, the highest were in isolate F_2 with 96%, while in isolates F_1 and F_3 reached 82.6%, while the radial growth recorded 88% in isolate F_4. It is evident that the ethanol extract can reduce the radial growth of *F.oxysporum* f.sp. lycopersici isolates particularly at 5% concentration. This may be due to the active chemical compounds that were detected by the GC-MS analyze, including Eicosane Hexadecanoic acid, which are antifungal. These results are consistent with (Kumar and Garampalli, 2015) study by using neem extract against fungus *F.oxysporum* f.sp. lycopersici and the percentage of inhibition reached 75%. As well as, the result agreed with (Siva *et al.*, 2008), that the ethanol neem extract reduced the radial growth of the fungus *F.oxysporum* f.sp. Melongena by 96%.

4. Effect of hexane extract for A. indica plant on the radial growth of fungus F.O.L

(Fig. 4) showed that the effect of the hexane neem extract has affected the radial growth of the fungus *F.oxysporum* f.sp. lycopersici. It recorded the highest percentage at concentration 5% for isolate F_1 by 100% with a significant difference from its effect percentage on other isolates reached 77.7% for isolate F_3 and 77.2 for F_4 and 73.5 for F_2. The concentration 2.5% was less effective in inhibiting the radial growth of fungus *F. oxysporum*, as recorded the lowest on isolate F_4 reached 44.2%, and the highest on isolate F_2 reached 66.2%. The concentration of 1.25% also had a lower effect on the radial growth of fungus reached 25.6% for the isolate F_4 and 33.2% for F_3, while the effect on the growth of F_1 and F_2 isolates reached 55.5%. The results indicate the possibility of using neem extract in the biological control

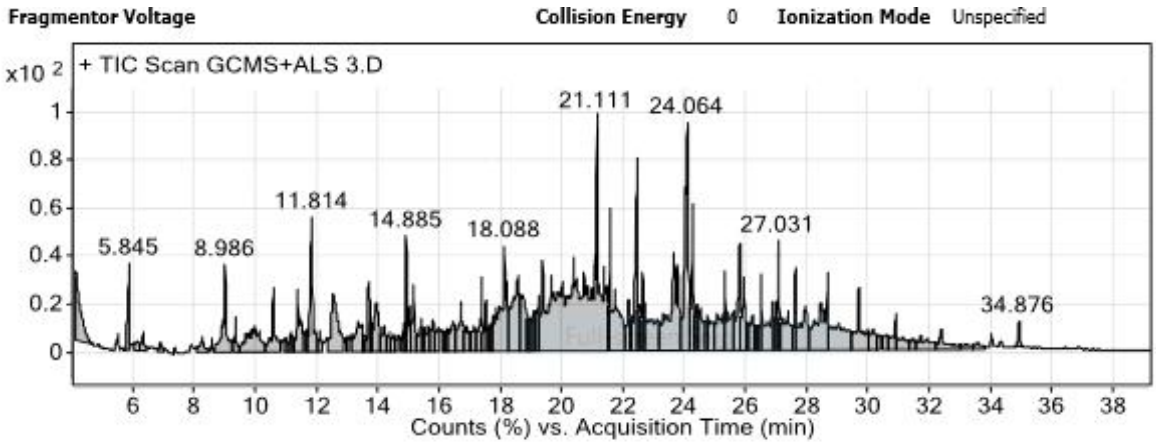


Fig. 1: Spectrum of active chemical compounds in the ethanol extract of A.indica according to the GC-MS device.

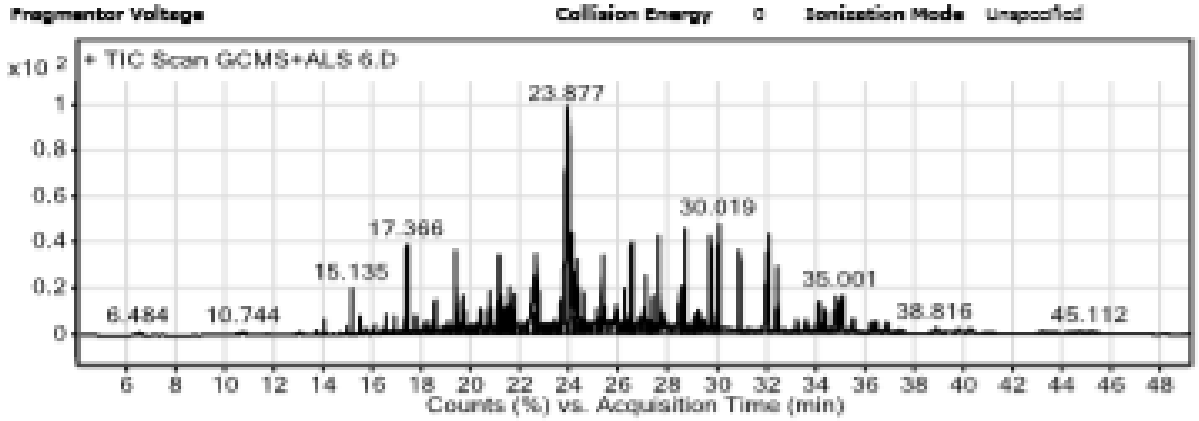
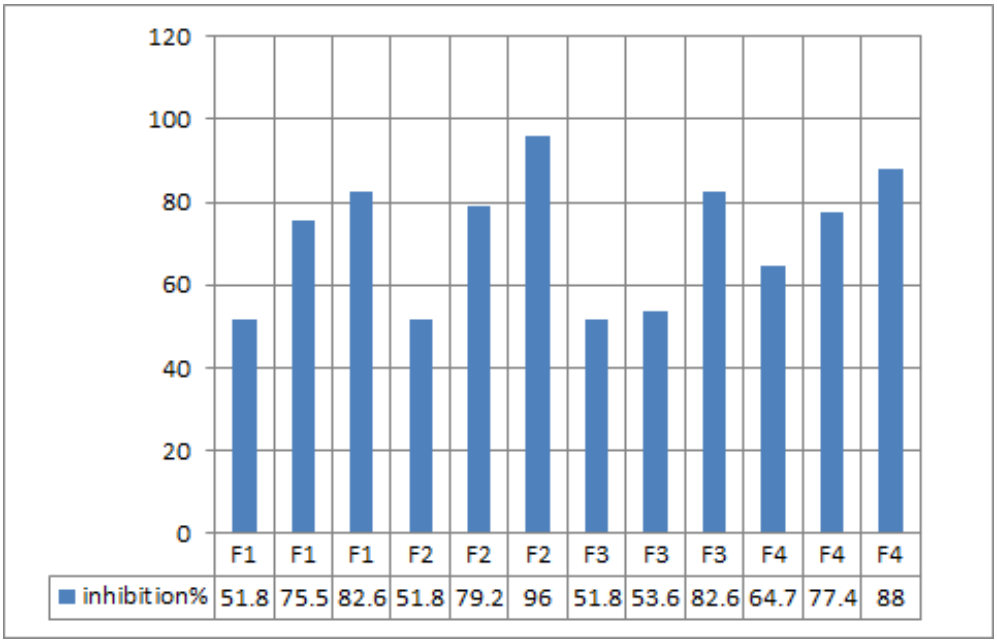
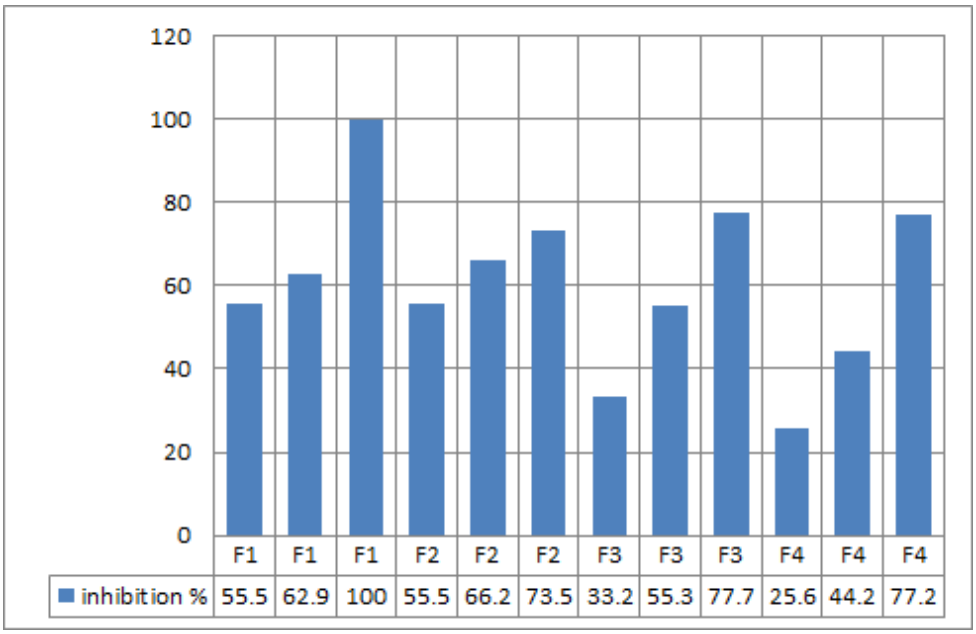


Fig. 2: Spectrum of active chemical compounds in the hexane extract of A.indica according to the GC-MS device.



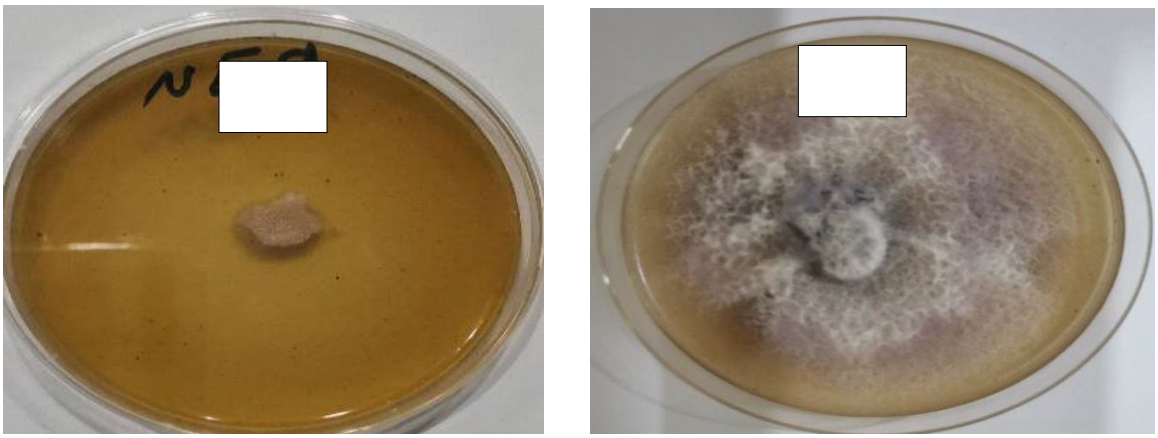
L.S.D = 4 for concentration L.S.D = 4.6 for fungus

Fig. 3: The effect of ethanol extract for A.indica on the radial growth of F.O.L. isolates in the culture media.



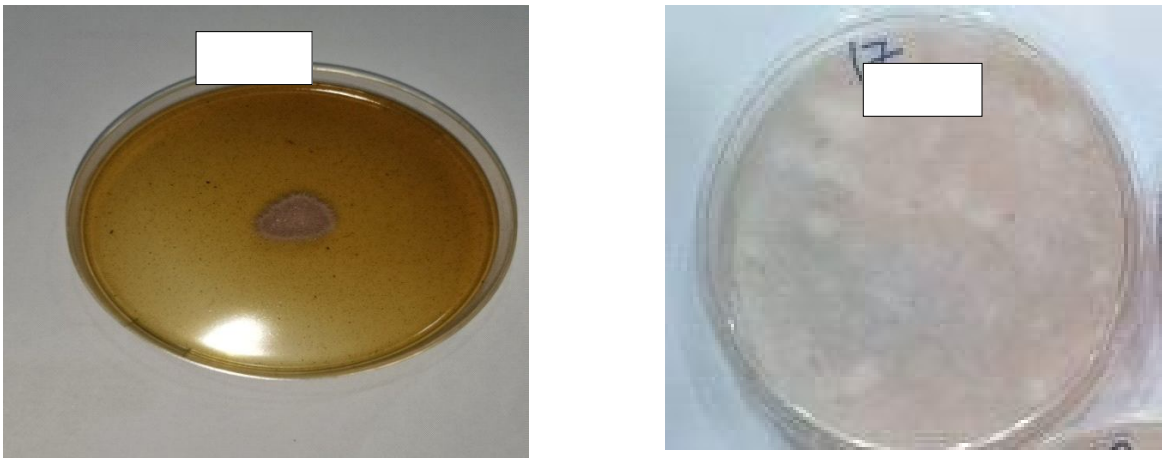
L.S.D = 4.5 for concentration L.S.D = 5.3 for fungus

Fig. 4: The effect of hexane extract for A.indica on the radial growth of *F.oxysporum* f.sp.lycopersici isolates in the culture media PDA.

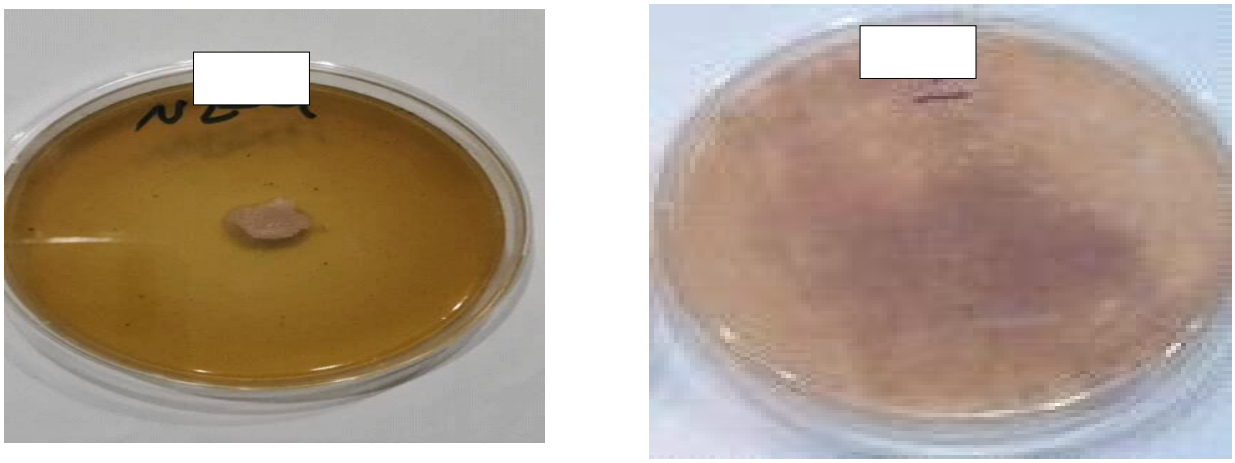


Picture 1: Effect of alcoholic extract for neem plant on an isolate of Fusarium oxysporum f.sp.lyco. F1.

Picture 2: Effect of alcoholic extract for neem plant on the growth of Fusarium oxysporum f.sp.lyco. isolate F2.



Picture 3: Effect of alcoholic extract for neem plant on isolate of Fusarium oxysporum f.sp. lycopersic F3.



Picture 4: Effect of ethanol extract for neem plant on the growth of *F.oxysporum* f.sp. lycopersici isolate F4.

of the fungus f.sp. lycopersici for its efficiency in reducing the radial growth of the fungus in the culture media PDA; also, the neem extract contains a high percentage of protein and carbohydrates, which are the best antimicrobials (Asif, 2012). These results are consistent with (Moslem and Al-kholie, 2009) which pointed out that the hexane extract of neem leaves gave an inhibition of the *F.oxysporum* growth in the culture media by 100%, and A. solani reached 80%. (Geraldo *et al*., 2010) mentioned the ability of neem oil to affect the radial growth, sporulation and dry weight of fungus *F.oxysporum* f.sp. Medicageni and is considered one of the means of the crop management program.

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