EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

TOXICOLOGY & PEST CONTROL

F

ISSN
2090-0791

WWW.EAJBS.EG.NET

Vol. 13 No. 2 (2021)

www.eajbs.eg.net

Citation: *Egypt. Acad. J. Biolog. Sci.* (F.Toxicology& Pest control) *Vol.13(2)pp1-15(2021)*
DOI: 10.21608/EAJBSF.2021.181340

Egypt. Acad. J. Biolog. Sci., 13(2):1- 15(2021)

Egyptian Academic Journal of Biological Sciences
F. Toxicology & Pest Control
ISSN: 2090 - 0791
http://eajbsf.journals.ekb.eg/

Prospects of Neem Essential Oil as Bio-Pesticide and Determination of Its Residues in Eggplant Plants During Crop Production Cycle

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ARTICLEINFO
Article History
Received: 8/5/2021
Accepted: 2/7/2021

***Keywords*:**
Neem essential oil, bioinsecticides, residue, GC/MS, eggplant, acaracide.

ABSTRACT

Essential oils and their derivatives are considered alternative means for controlling many harmful insects. As well, their rapid degradation in the environment and increased specificity do not harm beneficial insects. A method for the determination of Neem oil residues in eggplant (*Solanum melongena*L.) by GC/Ms Mass is described. In this study, the effect of Neem essential oil (*Azadirachta indica* Juss) as biopesticide on the economical production of eggplant (*Solanum melongena* L.) was investigated. Prior to use, the volatile and organic constituents of commercial applied Neem essential oil were identified using FTIR analysis. After application, Azadirachtin is an active ingredient in neem oil which appears to cause 90% of the effect on most sucking pests. Among them, the major constituents are triterpenes known as limonoids, Nitrogen, Oxygen, Floride and Carbon atoms.

Neem oil residues were detected in all samples from eggplant which were collected from the field after 12, 24 days of spraying. It appears that no effective material residue present.

INTRODUCTION

Neem oil is extracted from the Neem tree (*Azadirachta indica* Juss*)* a member of the Meliaceae family that originates in the Indian subcontinent and is now valued worldwide as an important source of phytochemicals for use in human health and pest control (Norten and Pütz, 1999; Forim *et al.*, 2014). The main neem product is the oil extracted from its seeds by different techniques. The other parts of the neem tree contain less azadirachtin but are also used for oil extraction (Nicoletti *et al*., 2012).

Neem oil contains at least 100 biologically active compounds. Among them, the major constituents are triterpenes known as limonoids; the most important being azadirachtin, which appears to cause 90% of the effect on most insect pests. However, these effects are probably secondary to the action of azadirachtin in blocking microtubule formation in actively dividing cells (Morgan, 2009).

Lucantoni *et al.* (2006)indicate that among the botanical insecticides currently marketed, Neem oil is one of the least toxic to humans and shows very low toxicity to beneficial organisms, so it is, therefore, very promising for the control of many pests.

Azadiractin ($C_{35}H_{44}O_{16)}$ is a ctetranoritepeniod (limonoids) extractable from Azadirchta plant species. This compound in neem have insecticidal properties as antifeedant, repellence ovipositor determent molting inhibition and growth retardant for a variety of insects and arthropod (Lalea and Abdulrahman, 1999; Mala and Muthalagi,2008). The azadirichtin, neem contains more than 20 compounds that responsible for characterizes small crushed seeds and neem oil. Because of this selectivity and its rapid degradation, azadirachtin is considered to be less damaging than synthetic insecticides to the environment and to pose a much smaller threat to non-target organisms, including humans *via* food residues, surface and groundwater contamination, or accidental exposure (Koul, *et al.*, 1990; Quarles, 1994).

Thus, this study aims to:

- Identify the chemical constituents of Neem essential oil, using GC/Ms,
- To determine and measurement its residues in eggplant (leaves and fruits),
- As well, to determine the efficiency of the biological activity on these plant-sucking pests.

MATERIALS AND METHODS

Materials:

Commercial essential oil of Neem (*Azadirachta indica)* oil was obtained from EL-HAWAG Company for Extracted Oils –Badr City, Egypt. It was manufactured according to Egyptian Standard Specifications No: 150/2/159 for 2009. This essential oil was kept in a refrigerator until used without any modifications. The chemical structure of the oil is shown in Figure 1.

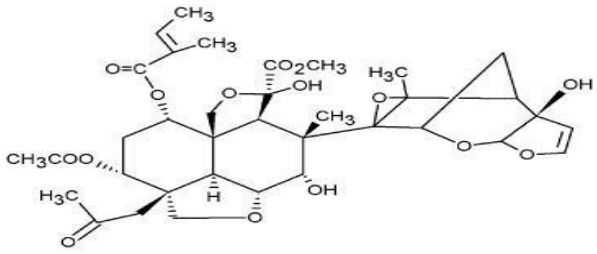

Fig.1. Chemical structure of Neem essential oil

Methods:

a) Field Experiments (Experimental design):

Experiments were carried out during winter and summer seasons of (November 2017, 2018) at crop fields near Benha, Qalubiya Governorate. Eggplant (*Solanum melongena L.)* was planted in an area of (189m^2). The area was divided into two equal plots. The experiments plots were cleared, prepared before asuitable seedling of Eggplant was transplanted. Each block was separated from the other by a50 cm blank area.

Treatments were done in completely randomized blocks design and replicated four times. The growing plants were sprayed with the essential oils (Neem oil) at the recommended dose of (1 Liter /100-liter water) and the control crop was sprayed with water using a plastic drum sprayer (25-liter capacity). Plants from each treatment were combined and placed in individual plastic bags.

Besides the determination of essential oil residues, a sample of the crops and leaves from each treatment were taken before and after to indicate the pest count.

b) Method Validation for Neem Essential Oils Residues Determination:

Before the determination of Neem essential oil residue in eggplant, the analytical method was validated in terms of linearity (instrument response), accuracy (spike and recovery), the limit of detection (LOD), the limit of quantification (LOQ), inter and intra-day precision and stability studies as per ICH guidelines (2005).

Linearity of the instrument response and the concentration range limits were determined by preparing a working standard solution with a six-point concentration of the oil-soluble compounds for injection in GC and UV-Vis. Calibration curves were plotted and the linearity was determined by the linear regression equation between standard concentration and the corresponding peak area and using Coefficient of determination (R^2). The LODs and LOQs of the proposed method were calculated on the basis of $3.3\sigma/S$and as $10\sigma/S$, respectively, (σ: the standard deviation of *y*-intercepts of regression analysis; *S*: the slope of the calibration curve)**.**

Accuracy -Spike and Recovery of Neem essential oil was determined from untreated eggplant using HPLC. For recovery and repeatability parameters, representative water matrix was spiked by essential oil at 10 ng/ml to50 ng/ml and was repeated for10 during three days (EEC Drinking Water Guidelines, 91/692/EEC, COM 789 Final 2016/0394 (COD), 2016). Samples of untreated eggplant were fortified with Neem oil standard solutions to reach concentrations of 5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm of standard (97%) Neem oil. Prior to extraction, the fortified samples were allowed to settle for 30 min. Three replicates for each concentration were analyzed to validate and evaluate the accuracy of the method.

C) Identification of Chemical Components of The UsedNeem Essential Oil Using GC/MS:

GC/MS analysis was performed using a Thermo scientific, Trace GC Ultra/ISQ single Quadruple MS.TG.5MS fused silica capillary column. All the identified components were identified based on a comparison of their relative retention time and mass spectra with those of the NIST, WI4rew2432LLY library data of GC/MS system (Table 1) (Adams, 2007).

Table1. Standard conditions for GC/MS for the determination of essential oil and its residues on eggplant**.**

Column Type	Ionization energy	Carrier Gas	Flow rate mL/min	Initial temperature	Final temperature
Capillary column (30m,0.250mm) Film thickness	70 eV	Helium	1 mL/min	40°C (Held for 3 min)	280 °C increasing at a rate of 5° C/min (Hold 2 min) then to 310°C (Hold 10 min)

D) Effect of Neem Oil on Pest Count in Sampled Crops and Leaves Sampling Before and After Treatment:

To study the effect of essential oil (Neem oil) on controlling sucking pests attacking eggplant plants, samples (40 leaves) were taken randomly before and after treatments, at1, 3, 7, 14 and 21 days after treatment application and from control. Samples of plants from each treatment were combined placed individually in plastic bags. All sucking pests were counted per 11-inch² area for incidence of spider mites *(Tetranycus urticae),* egg stage of spider mites *(Tetranycus urticae),* nymph of whitefly *(Bemisia tabaic),* Thrips (*Thrips tabaci*) *and* Leafhoppers (*Jassid*). The initial effect of the different spray methods was estimated one day after the application. The accumulated general reduction was also estimated for a counting carried out after 21 days from each application. Percentage of

population reduction for each pest species/each treatment was calculated according to Henderson's formula **(Henderson and Tilton, 1955)** as follows:

$$\textbf{Percentage of reduction} = \left[1 - \frac{Ta \times Cb}{Tb \times Ca}\right] \times 100$$

Where,
Ta: number after treatment in the treated plot.
Tb: number before treatment in the treated plot.
Ca: number after treatment in check plot.
Cb: number before treatment in check plot

RESULTS AND DISCUSSION

1- Method Validation for The Determination of Neem Essential Oil: Determination Linearity, LOD and LOQ on eggplant:

From Table (2) good linearity was observed in the range of 0.5–1000 ng /ml for essential oil. Coefficients of determination (R^2)was 0.9997 in the linearity experiments. In addition, Relative Standard Deviation (RSD) or (back-calculated concentration) has not deviated more than ±20% from the true (nominal) concentration.

Table2. Linearity parameters for the analytical determination of Neem essential oil

Parameters	Value
Linearity range	0.5 –1000 ng /ml
coefficient of determination (R^2)	0.9997
Slope	17×10^{-2}
Intercept	0.9
Linear Regression equation **Y**: Area, **X: concentration**, m: Slope, **b**: intercept	**Y= mx+b** $Y = (17 \times 10^{-2} \times 1000) + 0.9$
LOD	0.5 ng/ml
LOQ	1000 ng/ml

2- Spike and Recovery Accuracy Test of Neem essential oil:

Samples of untreated eggplant were fortified with Neem oil standard solutions to reach concentrations of 5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm of standard (97%) Neem oil. Prior to extraction, the fortified samples were allowed to settle for 30 min. Three replicates for each concentration were analyzed to validate and evaluate the accuracy of the method**.** Also, the results obtained from the accuracy of the analytical method employed in this study are summarized in Table (3) and Figure (2). The % recovery experiments with recoveries ranged between 70 and 120% (SANTE, 2015).

Table3. Accuracy (Spike and Recovery %) for HPLC method for Neem essential oil determination in eggplant blank:

Concentration of Neem oil applied	Recovery amount of Neem oil (ppm)	Recovery %
5 ppm	1.9856	99.280
10 ppm	1.99718	99.859
15 ppm	1.93774	96.887
20 ppm	1.96393	98.466
25 ppm	1.99244	99.622

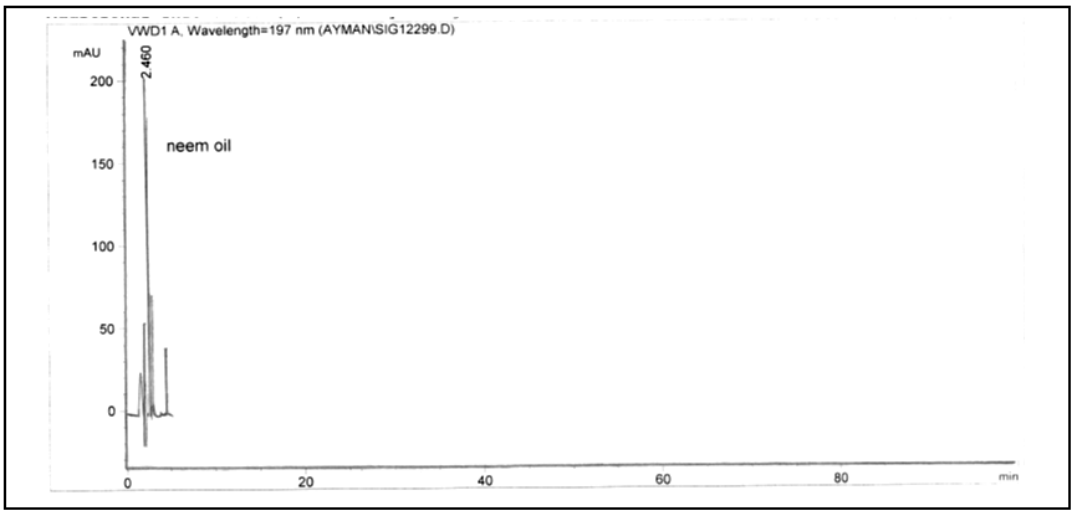

Fig.2. Standard curve of Neem essential oil by HPLC.

3- Analysis and Identification of Neem Oil Active and Functional Groups Using (FTIR):

The biochemical content of Neem oil was investigated using FT-IR. Table (4) and Figure (3) show the representative FTIR spectra obtained for Neem oil in the (3008.4 to 586.82 cm^{-1}) region. The frequency ranges from 1160.42 cm^{-1} peaks are represent the C=S in thiocarbonyl compounds. Compound stretching vibration, the presence of SO_2 in Sulfonyl chlorides and– (CH_2) n- in hydrocarbons.

Table (4) show the presence of a phytochemical compound in the extract of Neem oil revealed the presence of phytochemicals such =CH in aromatic and unsaturated hydrocarbons at 3008.4 cm^{-1}, -CH_3 attached to O or N at 2853.72 cm^{-1}, C=O in ketones at 1743.88 cm^{-1}, C-N in aromatic amines at 1238.28 cm^{-1}, C=S in thiocarbonyl compounds at 1160.42 cm^{-1} and C-I in iodo compounds at 586.82 cm^{-1}. The presence of a phytochemical compound in Neem oil extract agrees with other investigators (Mahmoud 2004; Tanwar *et al.*, 2013 and Sharma *et al*., 2017).

Table 4. Identified Functional groups in Neem oil analyzed by FT-IR

Peak Number	**X (cm^{-1})**
1)=CH in aromatic and unsaturated hydrocarbons	3008.4
2) –$CH_3$and –CH_2- in aliphatic compounds	2923.16
3) -CH_3 attached to O or N	2853.72
4) C=O in ketones	1743.88
5) CH_3 in aliphatic compounds	1463.94
6) C-N in primary amides	1417.97
7) SO_2 in Sulfonyl chlorides	1377.59
8) C-N in aromatic amines	1238.28
9) C=S in thiocarbonyl compounds	1160.42
10) C=S in thiocarbonyl compounds	1119.37
11) CH=CH – in trans disubstitued Alkenes	975.33
12) CH=CH – in trans disubstitued Alkenes	967.84
13) –(CH_2)n- in hydrocarbons	722.10
14) C-I in iodo compounds	586.82

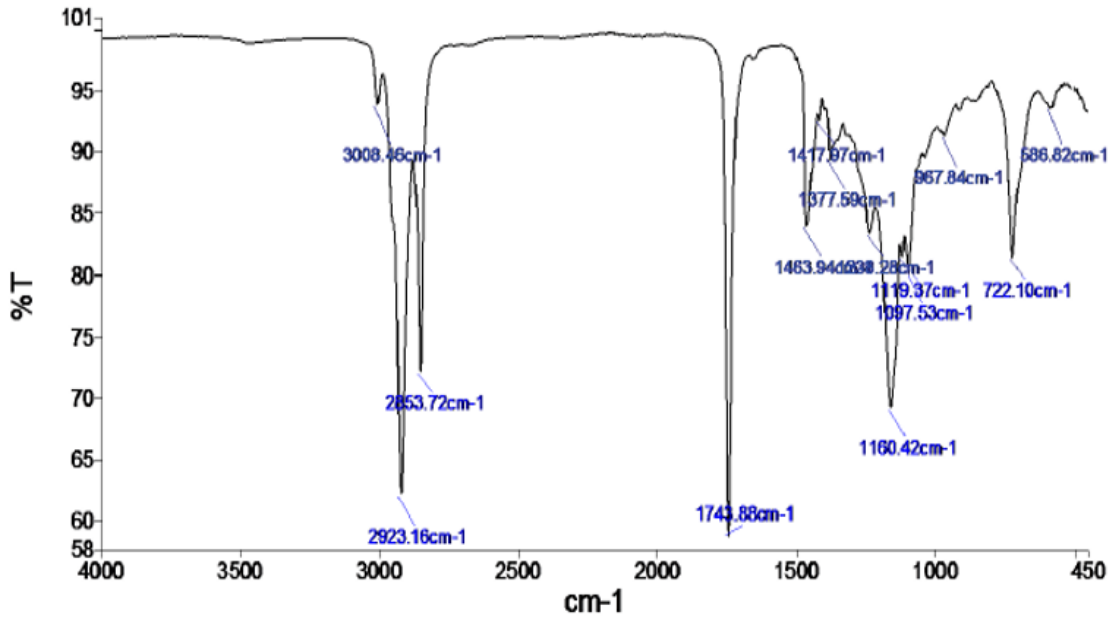

Fig. 3: FT-IR Spectrum of Neem oil.

4- Degradation of Neem oil in eggplant using the proposed GC/MS Mass:

a- Neem oil residue on eggplant after direct spray.

Table (5) indicates that **{**Benzene, (1butylheptyl) ($C_{17}H_{28}$)} and {Benzene, (1pentylheptyl) ($C_{18}H_{30}$)} are the major compounds (14.19% and 9.02%) respectively.

Table5: GC-MS analysis of Neem essential oil.

No	Compound	Rt(min)	(Peak area) %
1	Methyl2,12dibromo7phenyl5,6,7,8tetrahydro1,13 diazadibenz [a, j] anthracene14carboxylata ($C_{28}H_{20}Br_2N_2O_2$)	28.28	0.14
2	1Hentetracontanol($C_{41}H_{84}O$)	28.43	0.20
3	Benzene, (1methylnonyl) (CAS) ($C_{16}H_{26}$)	28.76	2.74
4	7(SecButyl)2,2dimethylchromene ($C_{15}H_{20}O_2$)	29.35	5.56
5	Benzene, (1butylheptyl) ($C_{17}H_{28}$)	29.46	14.19
6	BENZENE, (1PROPYLOCTYL) ($C_{17}H_{28}$)	29.70	7.7
7	Pyrido[2,1a] isoindolium Chloride ($C_{12}H_{10}C_1N$)	29.98	0.12
8	BENZENE, (1ETHYLNONYL) ($C_{17}H_{28}$)	30.21	5.90
9	cis13Eicosenoicacid 501($C_{20}H_{38}O_2$)	30.67	1.00
10	Aceticacid,17(4chloro5methoxy1,5dimethylhexyl) 4,4,10,13,14Pentamethyl2,3,4,5,6,7,10,11,12,13,14,15,16,17tetradeca hydro1phenanthry ($C_{33}H_{55}ClO_3$)	30.86	0.15
11	3Phenylpropionicacid,2,2,2trifluoroethylester ($C_{11}H_{11}F_3O_2$)	31.09	5.90
12	Benzene, (1pentylheptyl) ($C_{18}H_{30}$)	31.56	9.02
13	8Hydroxy2,2a,3,4tetrahydro1Hazeto[1,2a] quinolin1on($C_{11}H_{11}NO_2$)	31.68	7.43
14	Benzene, (1propylnonyl) ($C_{18}H_{30}$)	31.95	4.23
15	2Furyl4methoxy6nbutylphenol ($C_{15}H_{18}O_3$)	32.45	3.24
16	Cyclohexane, decyl (CAS)($C_{16}H_{32}$)	33.32	3.28

Figure (4) shows that Neem oil residues were detected in all samples after direct spraying. It appears that the most effective material that contains Oxygen, Nitrogen, Florid elements.

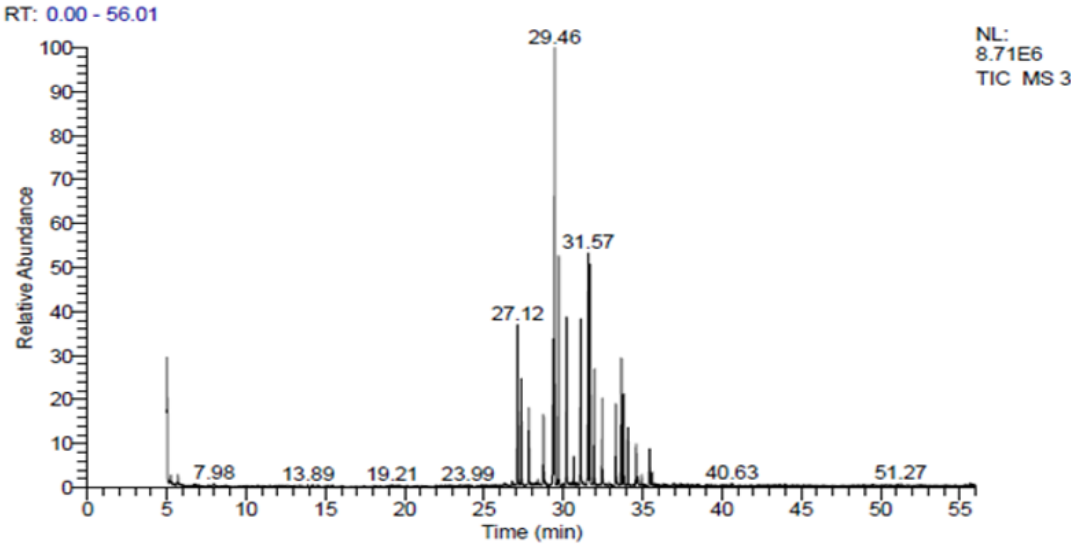

Fig. 4. GC/MS of Neem oil (ppm) on eggplant after direct spray.

Figure (5) shows the Neem oil residues detected in all eggplant samples after direct spraying were {Benzene(1-butylHeptyl) ($C_{17}H_{28}$)}, {Benzene-1-butylHeptyl ($C_{17}H_{28}$)} and {1-n-butyl-1-phenyl epoxide $C1_2H_{16}O$)}.

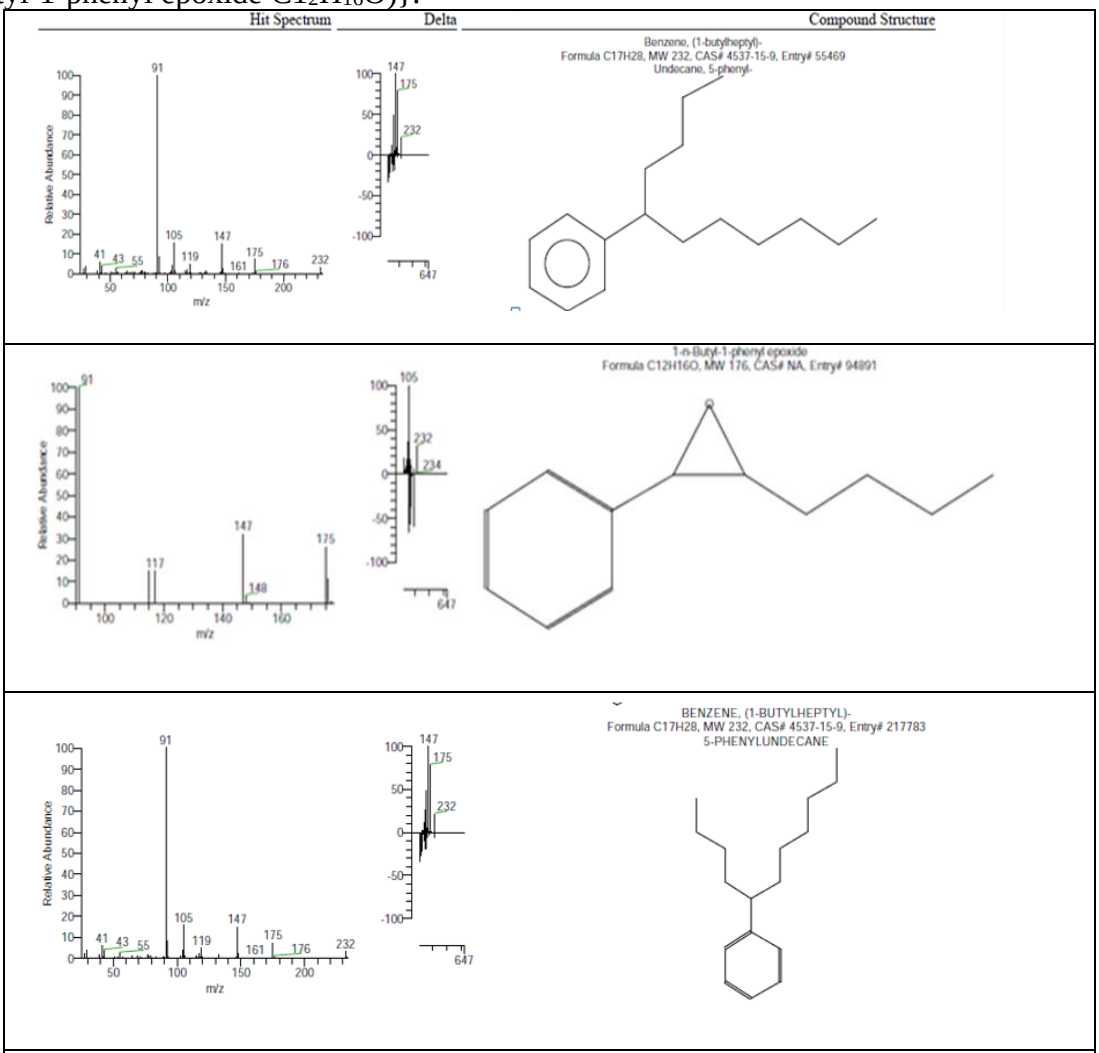

Fig. 5. Mass spectrum of Neem residues (ppm) by GC/MS.

B-Neem Oil Residue on Eggplant After One Day.

Table (6) indicate the main Neem essential oil residues (ppm) by GC/MS after one day contained {5[O (tButyl)diphenylsilyl] 1'(phenylsulfonyl) methylene]2',3'O [isopropylidene] àDribofuranos($C_{31}H_{38}O_7SSi$)}and {3Acetoy8deacetoxyunaconitine ($C_{35}H_{49}NO_{10}$)} as the major compounds (3.42% and 2.97%) respectively.

Table6. GC-MS analysis of Neem essential oil on eggplant after one day.

No	Compound	Rt(min)	(Peak area) %
1	5[O(tButyl)diphenylsilyl]1'(phenylsulfonyl)methylen]2',3'O [isopropylidene]àDribofuranose ($C_{31}H_{38}O_7SSi$)	24.51	3.42
2	5Nitro1Hindole3carboxamide($C_9H_7N_3O_3$)	26.53	2.84
3	Penitrema($C_{37}H_{44}ClNO_6$)	33.88	2.49
4	(2S,2'R,3R/S)2[2'Hydroxyperoxy3(phenylthiol)propyl] 5àcholestan3one2',3peroxyhemiacetal($C_{36}H_{56}O_3S$)	36.96	1.93
5	3Acetoy8deacetoxyyunaconitine($C_{35}H_{49}NO_{10}$)	39.11	2.97
6	tetratertbutyl2,6di(3propenyl)3,7dimethoxybicyclo [3.3.0] octa3,7diene2,4,6,8dicarboxylate ($C_{36}H_{54}O_{10}$)	44.97	2.13
7	2[3,4Bis(tetradecyloxy)phenyl]4,4,5,5tetramethy l1,3,2dioxaborolane ($C_{40}H_{73}BO_4$)	45.03	2.38
8	(R, S) {5[4(e)(2(1,4,5,8,9,10Hexahydro1,4,5,8 tetraoxo9,10(obenzeno)anthracenyl)cyclohex(e)yl] 10,15,20triptolylporphyrinato} zinc (II)($C_{67}H_{48}N_4O_4Zn$)	46.57	2.54
9	(2Methoxyethoxy) methyl2,12Dibromo7phenyl5,6,8, [a, j] anthracene14carboxylate ($C_{33}H_{28}Br_2O_4$)	48.36	1.97
10	Methyl13hydroxyphaeophorbideb($C_{36}H_{36}N_4O_7$)	49.00	2.59
11	2,2Bis[4[[4chloro6(3ethynylphenoxy)1,3,5triazin2yl] oxy] phenyl] propane ($C_{37}H_{24}Cl_2N_6O_4$)	49.31	2.42
12	[Tri {Titanium pentamethylcyclopentadienyl(oxa)} (ethyl) {N, Ndiphenlmethyleneimino)} ($C_{45}H_{59}NO_3Ti_3$)	49.95	1.89
13	(4Bromophenyl) bis(2,4dibromophenyl) amine ($C_{18}H_{10}Br_5N$)	51.04	2.74
14	2,2Bis[4[(4,6dichloro1,3,5triazin2yl) oxyphenyl] 1,1,1,3,3,3hexafluoropropane ($C_{21}H_8Cl_4F_6N_6O_2$)	51.57	2.62
15	4,4',4",4'"Tetrabromotetraphenylmethane($C_{25}H_{16}Br_4$)	53.12	2.87
16	Hexadecanamide, N, Nbis{[2(2butoxyethoxy) ethoxy] carbonylmethyl} ($C_{36}H_{69}NO_9$)	54.27	1.89

Figures (6&7) shows that Neem oil residues detected in all sample from eggplant after one day as {[2,2Bis[4[(4,6dichloro1,3,5triazin2yl) oxy] phenyl] 1,1,1,3,3,3 hexafluoropropane ($C_{21}H_8C_{14}F_6N_6O_2$), {4,6bis (heptafluoroisopropyl) 5hexafluoroisopropylidene 2phenyl1, 2,3triazacyclohexa3,6diene($C_{18}H_5F_{20}N_3$)} and {DSHAKRHHGYKRKFHEKHHSHRGY/7 (N/A)}.

RT	Probability	Compound Name	SI	Area %	Area	Molecular Weight	Molecular Formula	Library
51.57	14.62	2,2-Bis[4-[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]phenyl]-1,1,1,3,3,3-hexafluoropropane	311	2.62	1082 12.11	630	C21H8Cl4F6N6O2	mainlib
51.57	12.92	4,6-bis-(heptafluoroisopropyl)-5-hexafluoroisopropylidene-2-phenyl-1,2,3-triazacyclohexa-3,6-diene	308	2.62	1082 12.11	643	C18H5F20N3	Wiley9
51.57	7.05	DSHAKRHHGYKRKFHEKHHSHRGY/7	291	2.62	1082 12.11	3041	N/A	nist_ms ms2

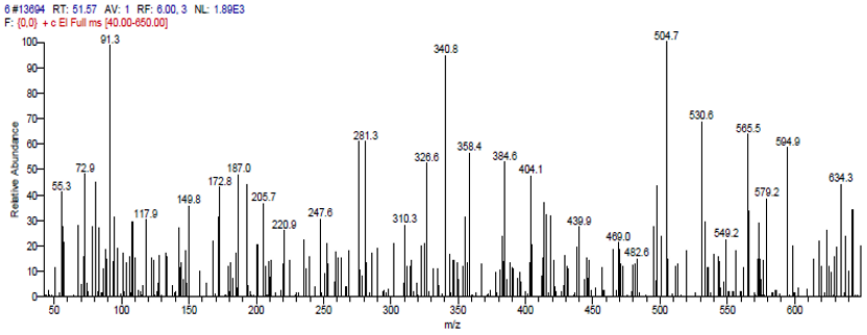

Fig.6. GC/MS of Neem oil on eggplant after one day.

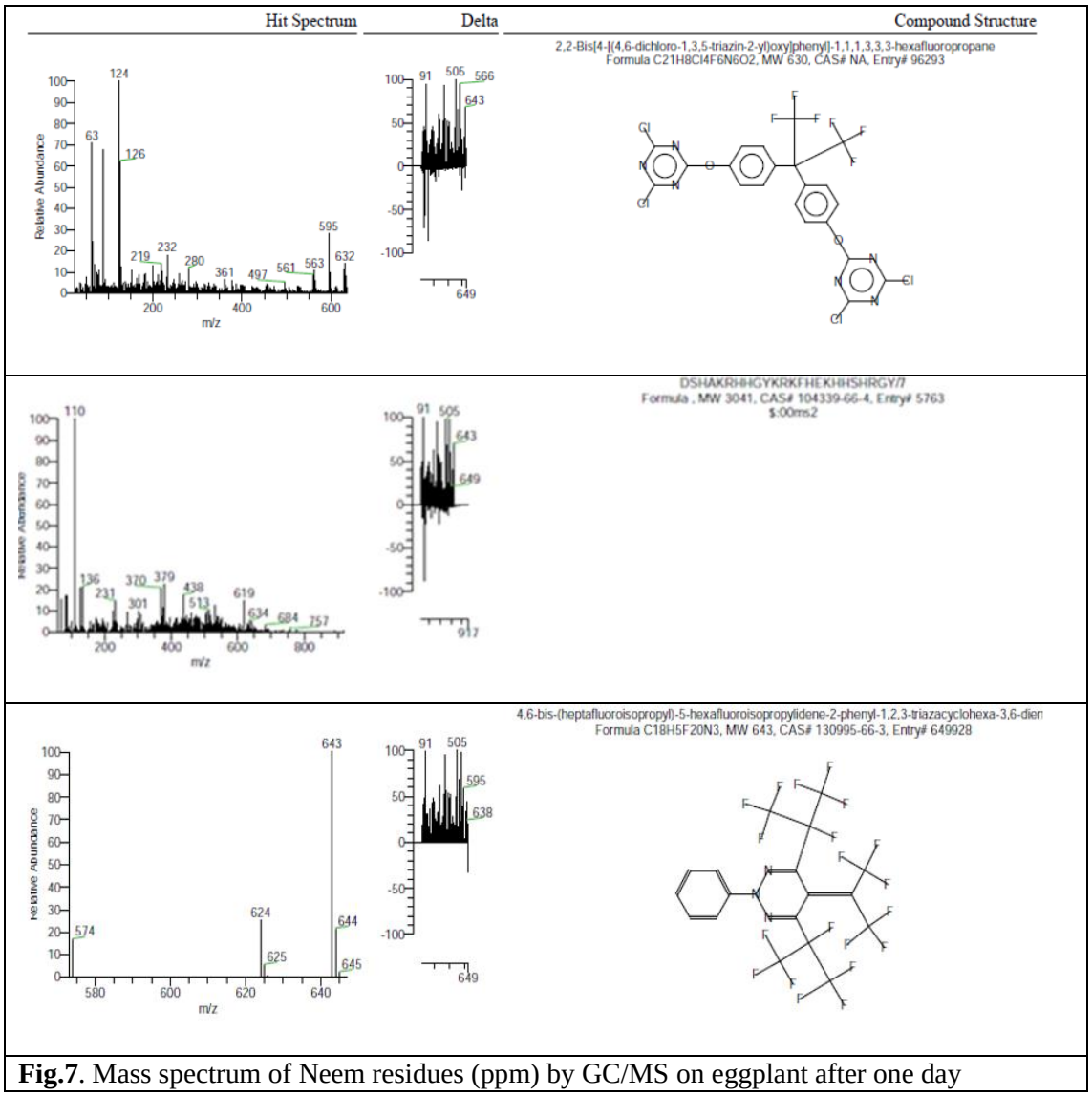

Fig.7. Mass spectrum of Neem residues (ppm) by GC/MS on eggplant after one day

C-Neem Oil Residue on Eggplant After12, 24 Day:

Figure (8) show that Neem oil residues were detected in all sample from eggplant which was collected from the field after 12, 24 days of spraying. It appears that no effective material residue present.

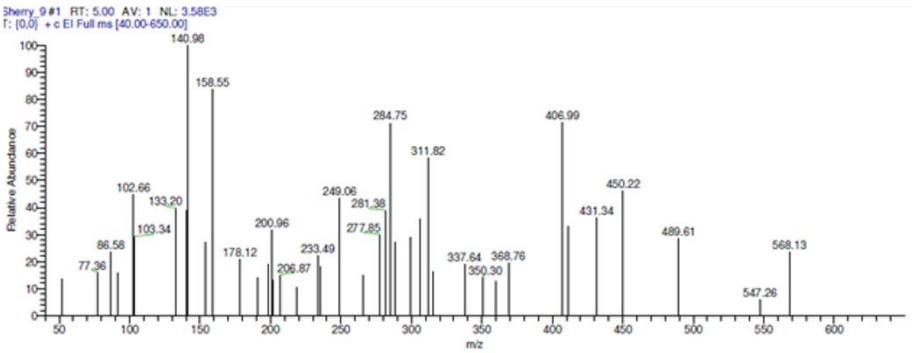

Fig. 8. GC/MS of Neem oil on eggplant after 12, 24day.

The results are in agreement with (Fernandes *et al.* 2007) who found that the quality of volatile oil Neem flower powder yielded volatile oil to an extent of 0.08% on a dry weight basis. The GC and GC-MS results showed that the volatile oil is rich in sesquit repines caryophyllene (56.03%), caryophyllene oxide (17.41%) and α- Caryophyllene (12.10%). α-Caryophyllene (monocyclic sesquiterpne) and caryophyllene (bicyclic sesquiterpene) are isomers. α- Caryophyllene (humulene) was reported to be effective against inflammatory diseases.

Also (Stokes and Redfern, 1982; Hull, *et al*. 1993) stated that maybe due to the presence of C–C p-bonds. The strained molecular structure consisting of epoxide rings and ester groups may make azadirachtin prone to undergo addition, ring cleavage, *etc*. Further, its instability to UV radiation may also affect the percentage of *azadirachtin* present in Neem seed kernels. In general, azadirachtinia very labile when exposed to air, moisture and sunlight.

5- Biological Activity of Neem Essential Oil:

Field observation showed that five insect pest species were found in eggplant plots throughout the growing season; i.e., Spider mites (*Tetranychus urticae)*; Whitefly (*Bemisia tabaci)*; Thrips (*Thrips tabaci*) and Leafhopper (*JassidEmposca. Sp*). The use of essential oils as an alternative in insect pest management programmers is a sustainable alternative as they can be obtained from nature (Chaubey, 2017).

Pest Count in Sampled Crops and Leaves Sampling Before and After Treatment.

Data in tables (7 & 8) show the effect of Neem essential oil on controlling pests infested eggplant during winter and summer (2017,2018) season. The compounds tested significantly reduced spider mite (*Tetranychus urtica*) population on eggplant compared with the check. Regarding the initial effect (one day after spraying), Neem essential oil-controlling the spider mite mobile stages in winter more than in summer which reduced the population of mites adult stage (74.93%,42.31%) respectively.

After 3, 7, 14, 21 days of spray, the reduction percentages of spider mite increased with time elapsed after treatment in two seasons. The result obtained that reduction in summer more than in winter, (84.62% and 80.73%) respectively. Kheradmand *et al*. (2015), conclude that essential oil extracted from aromatic plants has considerable potential for pest control. Oils indicated toxicity and repellency effects as a fumigant on *T. urticae*. Essential oils consist of the terponoid component mixture and, thus, rapid resistance development in spider mites will be compared to insecticides consisting of one active substance.

Tables (7 & 8) show the effect of Neem essential oil on the two-spotted spider mite (*Tetranychus urtica*) egg stage, on eggplant crops (2017 and 2018) season. The compounds tested significantly reduced egg of spider mite on eggplant compared with the check.

Regarding the initial effect (one day after spraying), the reduction percentage on Egg of spider mite recorded (89.37%, 36.3%) by Neem essential oil in winter and summer respectively.

Table7. Effect of Neem essential oil on pests invested eggplant crops in winter 2017**.**

pests	No. of pests before treatment/leaf	Initial Effect after1 day	R%	3 day	7 day	14 day	21 day	Total	mean	R%
spider mite adult stage	71	20	**74.93%**	3	15	5	60	83	20.75	**80.73%**
Control	89	100	---------	120	130	140	150	540	135	---------
spider mite egg stage	112	10	**89.37%**	20	10	10	30	70	17.5	**86.98%**
Control	125	105	---------	135	160	185	120	600	150	---------
Whitefly	102	5	**95.51%**	5	5	10	30	50	12.5	**91.51%**
Control	110	120	-------	135	150	170	180	635	158.75	------
Jassid	242	40	**84.11%**	20	40	10	50	120	30	**90.00%**
Control	250	260	--------	280	300	320	340	1240	310	----------
Thrips	82	5	**94.51%**	40	20	20	20	100	25	**80.40%**
Control	90	100	---------	120	130	150	160	560	140	--------

Table 8. Effect of Neem essential oil on pests infested eggplant crops in (June) summer 2018

pests	No. of pests before treatment/leaf	Initial Effect after 1 day	R%	3 day	7 day	14 day	21 day	Total	mean	R%
Spider mite adult stage	52	40	**42.31%**	10	0	0	10	20	5	**84.62%**
Control	60	80	----------	30	40	40	40	150	37.5	----------
spidermite egg stage	55	30	**36.36%**	30	20	0	0	50	12.5	**69.69%**
Control	70	60	----------	60	50	60	40	210	52.5	---------
Whitefly	40	10	**66.67%**	2	0	0	1	3	0.75	**94.00%**
Control	80	60	----------	40	40	20	0	100	25	----------
Jassid	82	80	**62.60%**	40	30	0	0	70	17.5	**49.78%**
Control	100	60	----------	60	60	50	0	170	42.5	----------
Thrips	212	70	**65.41%**	30	30	10	20	90	22.5	**48.11%**
Control	220	210	----------	100	20	10	50	180	45	---------

After 3, 7, 14, 21 days of spray, the reduction percentages on the egg of spider mite increased to 69.69%in summer but decreased in winter to 86.98%. Al-mazra'awi, (2014) indicated that, all the tested plant extracts were ineffective against the egg stage as the percentage of unhitched eggs was less than 30%. Eggs of many mites and insect pests are generally regarded asless susceptible to adverse effects such as chemicals or unfavorable weather conditions.

Data in Tables (7 & 8) show Neem essential oil tested significantly reduced whitefly population on eggplant compared with the check. Regarding the initial effect (one day after spraying), the Neem essential oil reduction percentage on whitefly mobile stag recorded (95.51, 66.67%in winter and summer respectively.

After 3, 7, 14, 21 days of spray, the reduction percentages on whitefly *(Bemisia tabaci*) affected with time elapsed after treatment. The mean content of reduction percentages of the tested Neem essential oil (91.51%, 94.0%) in winter and summer respectively.

Many studies confirmed the effects of plant oils against the whitefly (*Bemisia tabaci*) immature stage. Nzanza and Mashela(2012) showed that fermented plant extracts of Neem and wild garlic alone in combination, have insecticidal properties to maintain lower population densities of whitefly and aphid. Himat, (2004) concluded that Neem formulation

proved to be effective in reducing the hatchability of the egg's whitefly (*Bemisia tabaci*). The high pesticide activity of the Neem essential oil is perhaps attributable to the high level of hexadeconic (52.2%) and fatty acids (52.6%-72.3%) olec acid (15.7%) and tricsone (10.5%) which the other tested plant oil or contain in lower amounts.

Little is known about the mode of action of azadirachtin Neem essential oil as a feeding inhibitor, although it is possible that it stimulates cells involved in feeding inhibition, causing weaknesses and pest death (Brahmachari, 2004).

Also, Tables (7& 8) show the effect of Neem essential oil significantly reduced Leafhoppers population on eggplant compared with the check. Regarding the initial effect (one day after spraying), the reduction by the Neem essential oil was (84.11%) in winter reduced to (62.6%) in summer. After 3,7,14,21 days of spray, the reduction percentages of the tested Neem essential oil increased with time to (90.00%) in winter and decreased to (49.78%) in summer.

This study found that a chemical compound contained in Neem essential oil a repellent action relation to leafhoppers is exhibited by such compounds that posse's insecticide effect. Essential oils act at multiple levels in the insects affected by different seasons, so the possibility of generating resistance is little probable (Bakkali *et al.*, 2008).

Data in Tables (7 &8) show the effect of Neem essential oil on Thrips (*Thrips tabaci*) populations in winter and summer (2017, 2018). Neem essential oil significantly reduced the Thrips population on eggplant compared with the check. Regarding the initial effect (one day after spraying), the Neem essential more effective in controlling the Thrips mobile stages in winter 94.51% while reduced to 65.41% in summer.

The mean content of reduction percentages of the Neem essential oil 3,7,14 and21 days recorded that Neem essential oil reduction (80.40%, 48.11%) in winter and summer respectively. Neem essential oil was succeeded against Thrips (*Thrips tabaci*). These results may be correlated to differences in the chemical structure of these oils based on GC/MS analysis.

The oil is considered a contact insecticide, presenting systemic and translaminar activity (Cox, 2002).

Conclusions

The presented results of this study showed strong adverse effects of Neem essential oil in the mortality rate of different eggplant pests affected by the structure of Neem essential oil, time of a spray and season.

The biological activities of essentials oils against insect pests, and their potential as a bio rational alternative to control pests. The present results concluded that the repellent effect and toxicity of the tested oils make them potential materials for use in a comprehensive integrated pest management program for the subject pest.

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ARABIC SUMMARY

استخدام زيت النيم (*Azadirachtaindica*Juss) كمبيد حيوى حشرى وتعيين مخلفاته فى نبات الباذنجان خلال دوره انتاج المحصول.

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2- قسم الكمياء - كلية العلوم - جامعة حلوان
3- رئيس قسم الكمياء- كلية العلوم - جامعة بنها

لقد استخدمت الكثير من الزيوت العطريه ومنها زيت النيم كبدائل لمكافحة العديد من الحشرات الضارهونتيجه لزيادة خصوصيتها لا تضر بالحشرات النافعة في البيئة.استخدام النيم كمبيد حيوى حشرى يحافظ على الانتاج الاقتصادى لمنتجات الباذنجان وتشمل الدراسة تاثير زيت النيم على انتاج الباذنحان قبل الاستخدام وتحديد مكوناته المتطايره والعضوية للزيت وذلك باستخدام جهاز. FT-IR

وكذلك بعد التطبيق ثم تحديد بقايا زيت النيم داخل النبات اثناء وانتاج المحصول باستخدام جهاز GC-MS /Mass .

ولقد اوضحت النتائج ان مركب النيم يتكون من أكثر من مائة عنصر من العناصر الفعالة مثل النيتروجين والاكسجين والفلوريد ليظهر تاثيره كمبيد حيوى حشرى.

تضمنت خطة الدراسة النقاط الاتية:

1.تحديد الزيت المستخدم والمحصول.

2.دراسة الطرق العملية التحليلة المناسبة لاستخراج بقايا الزيت من المنتجات الزراعية.

3تقيم بقايا الزيت فى العينات المجمعة بطرق الفصل عن طريق جهاز.GC-MS/ Mass.

ويمكن تلخيص النتائج المتحصل عليها فيما يلي:

اولا: تحليل متبقيات زيت النيم حيث اوضحت النتائج ان المواد المتطايره تختفي قبل فترة 12 يوم.

ثانيا: زيت النيم حيث وجد ان المادة الفعالة تحتوي كلا من العناصر الاتية (النيتروجين والاكسجين والفلوريد) والروابط بين ذرتى الكربون وتتمثل فى

{Benzene (1-butylHeptyl) ($C_{17}H_{28}$)}, {Benzene -1-butylHeptyl ($C_{17}H_{28}$)} and **{1-n-butyl-1-phenyl epoxide $C_{12}H_{16}O$)}.**

ثالثا: اوضحت النتائج ان زيت النيم يتحكم في تعداد الافات. حيث يستخدم فى الحد من الاصابة بالافات الثاقبة الماصة فيؤثرفى خفض تعداد الأفات الأتيه:خفض اعداد العنكبوت الاحمر بنسبة 80.73% فى الشتاء و 84.62%فى الصيف، وبالنسبة لبيض العنكبوت الاحمر 86.98% فى الشتاء و69.69% في الصيف،واما الذبابة البيضاء 91.51% فى الشتاء و94% في الصيف،بينما الجاسيد 90 % فى الشتاء و 49.78%فى الصيف، وسجل التربس 80.40% فى الشتاء و 48.11% فى الصيف.