

IV. RESULTS AND DISCUSSION

PART 1

Larvicidal Activities of Prepared Plant Crude Extracts
Against the 3rd Larval Instar of Culex pipiens in Laboratory

1.1. Preliminary evaluation of larvicidal activities of prepared plant crude extracts in organic solvents

Three plants were selected in the present study namely: Withania somnifora, Pinus halepensis and Carpobrotus edulis. Crude extracts of each plant were prepared into three organic solvents separately, i.e. ethanol, chloroform and petroleum ether preliminary bioassay tests to clarify the relative larvicidal activities of each crude exctract were carried out in laboratory against 3rd larval instars of Culex pipiens mosquitoes. Concentration of 1000 ppm was tested based on the previous preliminary bioassay data of each extract. Examination of the obtained results, it could be concluded the important role of plant type and solvent of extraction in determining the larvicidal activities of the tested extracts (Table-7).

Chloroform extract of *Withania somnifera* showed the highest mortality of exposed larvae (100%), followed by ethanol extract (80%), while petroleum ether extract was the least effective in this respect (20%). The relative potencies based on petroleum ether extract (1.0) were 5.0 and 4.0 times more effective than the least effective extract. A different trend of

efficiency was obtained with *Pinus halepensis* extracts. In general these extracts gave less larvicidal activities than those obtained with *Withania somnifera* extracts. Again, chloroform extract gave the highest mortality on culex larvae (60%), while ethanol and petroleum ether extracts showed inferior efficacy in this respect. Taking ther least effective extract as a base line for comperison (1.0), the relative potencies were 1.5 and 3.0 times more affectives in case of ethanol and chloroform extracts, respectively.

On the other hand, *Carpobrotes edulis* extracts showed inferior larvicidal activities against *culex* larvae. The corrected mortality percent reached 30.0, 20.0 and 10.0% in case of ethanol, chloroform and petroleum ether extracts, respectively.

As conclusion, this preliminary evaluation showed the superior larvicidal activity of chloroform and ethanol extracts of *Withania somnifera* compared with the other two plant extracts. Accordingly, it was decided to continue trials and experimentations on these two potent extracts (chloroform & ethanol) of *Withania somnifera* only.

1.2. Larvicidal activities of chloroform and ethanol crude extracts of *Withania somnifera* against 3rd instar larvae of *Culex pipiens* in laboratory:

Serial concentrations of chloroform extract (60, 100, 200, 500 and 730 ppm) and ethanol extract (254, 356, 588, 670 and 1000 ppm) were tested against 3rd instar larvae of culex mosquitoes in laboratory. The aim was to establish the mortality

Table (7): Preliminary evaluation of larvicidal activities of crude extract of the three tested plants against 3rd instar larvae of *C.pipiens*.

Extracts tested	Concentrations p.p.m	% corrected mortality after 24 h from treatment	Relative potencies
Withania somnifera Ethanol	1000	80%	4.0
Withonia somnifera chloroform	1000	100%	5.0
Whithonia somnifera petroleum ether	1000	20%	1.0
Pinus halepensis	1000	30%	1.5
ethanol			
Pinus halepensis chloroform	1000	60%	3.0
Pinus halepensis petroleum ether	1000	20%	1.0
Carpobrotus edulis ethanol	1000	30%	3.0
Carpobrotus edulis chloroform	1000	20%	2.0
Carpobrotus edulis petroleum ether	1000	10%	1.0

regression lines of each extract and obtain the LC50's as well as the slope values of the established lines.

Examination of the obtained results, it could be concluded the important role of solvent of extraction and tested concentration in determining the larvicidal activities of the evaluated crude plant extract. The high values of slopes of the established regression lines indicated the good homogeneity of the tested larvae population, i.e. 4.2 and 2.9 in case of chloroform and ethanol extracts, respectively. Data in Table (8) indicate the superior activity of chloroform extract than ethanol extract; the LC50's reached 244.7 and 514.7 ppm, respectively.

It is clearly evident to notice the great influence of extract concentration in this respect. The higher the concentration was the higher the larvae mortality and vice versa. In terms of figures, the corrected mortality percent reached 10, 30, 40, 70 and 100% with chloroform extract of *Withania somnifera* when tested at 60, 100, 200, 500 and 750 ppm, respectively. The corresponding mortality percent of ethanol extract of the same plant reached 20, 30, 50, 70 and 80% at 254, 356, 588, 670 and 1000 ppm, respectively.

Such data indicate that chloroform extract of *Withania* somnifera proved much more efficiency than ethanol extract. The relative potency of chloroform extract reached 2.10 times more effective than ethanol extract. This finding may be attributed to the polarity of chloroform (3.84x10⁻³⁰) compared with ethanol

(5.67x10⁻³⁰). Such results are in agreement with that obtained by several researchers.

Massoud and Labib, (2000) found that the stem of *C.noimol* proved to have insecticidal activity against culicidae larvae. The oil extract of Myrrh Possesses median lethal activity against 2nd, 3rd and 4th instar larvae of *Culex pipiens*. Moh-Aspollah-Sukari (1992) stated that toxicity studies of the volatile fraction of some of plant extracts was isolated and the major components were characterized. The contact insecticidal activity and the toxicity test of the plant extract on fishes were studied.

Gebre-Amlak and Azerefegne(1999) studied the insecticidal activity of chinaberry, endod and pepper tree against the maize stalk borer (Lepidoptera: Noctuidae) in Southern Ethiopia. A preliminary field test carried out in maize in southern Ethiopia during 1993-94 showed that application of extracts of fruits of chinaberry (Melia azedarach), endod (Phytolacca dodecandra) and pepper tree (Schinus molle) significantly reduced the levels of leaf infestation and deadheart injury due to larvae of the maize stalk borer, Busseola fusca, and resulted in increases in crop yield. The effects of extracts of leaves and fruits of each plant extract on B. fusca larvae were separately evaluated at the following season. Extracts of both leaves and fruits of chinaberry (either fresh or dried) were effective in reducing the number of larvae. All the rates used significantly reduced the number of larvae below those in the untreated controls. Fresh leaves and fruits of endod were also effective

against *B. fusca*. Fruits of pepper tree were superior to leaves. Fresh leaves of this plant did not reduce the number of larvae. Two applications of any of the three botanicals were not sufficient to provide complete protection of maize against second generation larvae. This suggests that these botanicals have only short persistence, and more than two applications of the extracts would be necessary to reduce pest numbers.

Table (8): Larvicidal activities of tested Withania somnifera ethanol and chloroform extracts a gainst 3rd instar larvae of C.pipiens.

Ethanol	extract	Chlorofor	m extract	
Concentration ppm	% corrected mortality	Concentration ppm	% corrected mortality	
1000	80%	730	100%	
670	70%	500	70%	
588	50%	200	40%	
356	30%	100	30%	
254	20%	60	10%	
LC50 = 514.9		LC50 =	244.7	
r = 0.98		r = 0	.95	
b = 2.9		b = 1	.83	

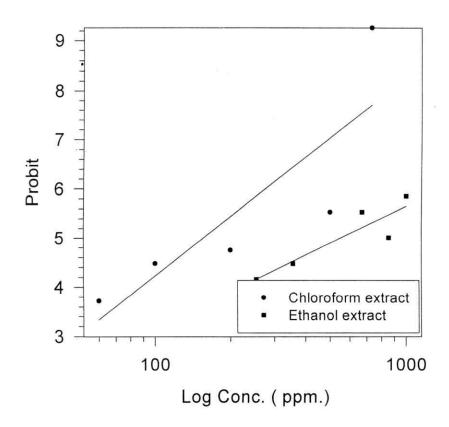


Fig. (4): LC-P regression mortality lines of *W.somnifera* crude extracts in chloroform and ethanol extracts against 3rd instar larvae of *Cluex pipiens*.

1.3. Larvicidal activities of prepared EC formulation of Withania somnifera against 3rd instar larvae of Culex pipiens:

Different concentrations of prepared EC (Emulsifiable concentrate were bioassayed in laboratory against 3rd instar larvae of *culex pipiens*. The mortality-regression lines were established and the LC50's of chloroform and ethanol EC extracts were obtained. The slope values of the established regression lines confirmed the homogeneity of the studied strain of mosquito larvae. Examination the obtained results in Table (9) indicated the superior larvicidal activity of EC formulation of chloroform extract compared with EC/ethanol extract. The LC50's reached 10.5 and 21.4 ppm, respectively. The relative potency of EC/chloroform extract being 2.0 times as effective as EC/ ethanol extract.

Data in the same table indicate the great role of EC concentration on insecticidal activity of tested preparation. The high the EC concentration was the higher the mortality percent and vice versa. In terms of figures, the corrected mortality percent were 8.0, 16.10, 27.65, 49.7, 72.,06 and 88.82 in case of EC/chloroform extract at 16, 25, 33 and 41 ppm, respectively. The mortality values of EC/ethanol extracts reached 16.18, 27.35, 38.53, 60.88 and 94.41 at 8, 16, 25, 33 and 41 ppm, respectively.

Reviewing the above mentioned results, it could be concluded the great insecticidal activities of prepared EC

formulation of *Withania somnifera* in chloroforom or ethanol against 3rd instar larvae of *culex* mosquitoes compared with the crude extracts with the same solvents. The LC50's of EC extracts reached 10.5 and 21.4 ppm with chloroform and ethanol extracts, respectively. The corresponding LC50's of crude extracts reached 244.7 and 514.9 ppm with the same organic solvents, respectively. The relative potencies of EC formulations reached 10.5 and 21.4 of chloroform and ethanol/EC extracts, respectively. Data, also indicate that the higher the EC concentration and/or crude extracts was the higher the mortality percent on *culex* larvae and vice versa.

Such results are in great agreements with the results obtained by many researchers. One can speculate that people controlled and killed mosquitoes and other domestic insect pests by physically removing them or by using plant parts and plant derivatives before the advent of synthetic chemicals. In all probability, these plants contained insecticidal phytochemicals that were predominantly secondary compounds produced by plants to protect themselves against herbivorous insects. However, there is a little other than anecdotal, traditional or cultural evidence on this topic (Grodner, 1997; Casida and Quistad, 1998). In the first century AD, Pliny the Elder, the Greek philosopher, wrote "Natural History" in which he recorded all the known pest control methods. At the same time the Chinese recorded their use of powdered chrysanthemum as an insecticide. Pyrethrum, derris, quassia, nicotine, hellebore, anabasine, azadirachtin, d-limonene camphor and turpentine were some of

the more important phytochemical insecticides widely used in developed countries before the introduction of synthetic organic insecticides (Wood, 2003).

Table (9): Larvicidal activities of prepared EC formulation of *Withania somnifera* against 3rd instar larvae of *C.pipiens* laboratory.

EC formulation in Ethanol (50%)			EC form	ulation in (5%)	Chloroform
Conc. Ppm	Observed mortality	Corrected	Conc. Ppm	Observed	Corrected
8	25%	16.18%	8	25%	16.18%
16	35%	27.35%	16	35%	27.35%
25	45%	38.53%	25	55%	49.70%
33	65%	60.88%	33	75%	72.06%
41	95%	94.41%	41	90%	88.82%
LO	$c_{}^{C50} = 21.4 \text{ p}$	pm	LC	250 = 20.28	ppm
	r = 0.75			r = 0.9	
	b = 3.1			b = 2.99	

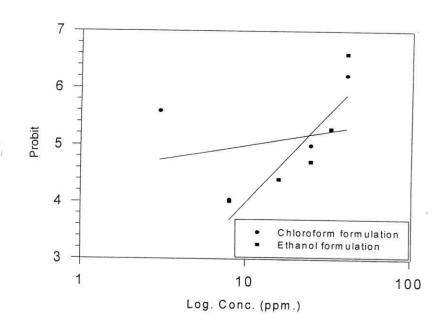


Fig. (5): LC-P line of EC formulation of W.somnifera against 3rd instar larvae of Cluex pipiens.

PART 2

Insecticidal Activities of Tested Crude and EC Formulations of Withania somnifera against larvae of the cotton leafworm, S.littoralis (Boisd) in laboratory

Data concerning the insecticidal activities of the tested crude extracts in chloroform and ethanol as well as their prepared EC formulations against 2nd and 4th instar larvae of the cotton leafworm, and after different post-treatment intervals are tabulated in Tables (10, 11) and illustrated as mortality regression lines in Fig. (6). The cumulative mortality percent were also calculated within 24-72 hrs, and tabulated in the same tables. Examination of the obtained results, it could be concluded the influencial role of the studied factors, i.e. nature of EC extract formulation, tested concentration and post-treatment interval in determining the insecticidal activities of the tested preparations. To facilitate the presentation of data, each preparation will be discussed separately in the followings:

2.1. Chloroform crude extracts of W.somnifera:

Data in Tables (10, 11) and in Fig. (6) indicated the important role of time after treatment, larval instar and crude extract concentration on the insecticidal activity of the tested extracts. The higher the concentration was the higher the larval mortality and vice versa. This was pronounced with both larval instars and with the considered post-treatment intervals. Also, the

same trend was noticed on the cumulative mortality. In terms of figures, the mortality percent of treated 2nd instars larvae reached 22.29, 30.45, 37.83, 45.60, 68.91 and 84.45% with 400, 1065, 1850, 2064, 2580 and 3095 ppm, respectively.

As for larval instar, data in the same tables indicated that 2nd instar larvae were more responsive to crude extract of *W.somnifera* in chloroform than 4th instar of the cotton leafworm. Again, the higher the extract concentration was the higher the mortality of larvae and vice versa. The cumulative mortality percent of 4th instar larvae within 24-72 hrs after treatment were 30.06, 37.83, 37.83, 68.12 and 84.45% at 800, 2000, 3300, 3700 and 4120 ppm, respectively. The LC50's of crude chloroform extracts reached 1594.6 and 1973.1 ppm in case of 2nd and 4th instar larvae.

Such results are in accordance with that obtained by **Asher** (1983), he reported that 8 withanolides and related compounds derived from solanaceae plant. *Physalis peruviana*, *W.somnifera* proved antifeedant activity against final-instar larvae of *S.littoralis* (Boisd). **Nelson and Venugopal**, (2006) studied the antifeedant and growth disruptive effects of various plant products on *S.littoralis*. Four of 38 plant products evaluated (i.e. *Azadirachta indica*, *Acorus calamus*, *W.somnifera* and *Gloriosa superba* showed significant antifeedant activity against *S.littoralis*.

Table (10): Larvicidal activities of different concentrations of

. Withania somnifera chloroform extract against

2nd instar larvae of Spodoptera littoratis in
laboratory.

Concent- ration	% corrected post-tre			
tested ppm	24 h	48 h	72 h	
400	5.5%	16.645%	23.29%	
1065	5.95%	14.95%	30.45%	
1850	10.0%	26%	37.83%	
2064	20.0%	30.1%	45.60%	
2580	25.0%	40.5%	68.91%	
3096	18.95%	53.95%	84.45%	
LC50 =	1594.6 ppm			
r =	0.68			
b =	1.7			

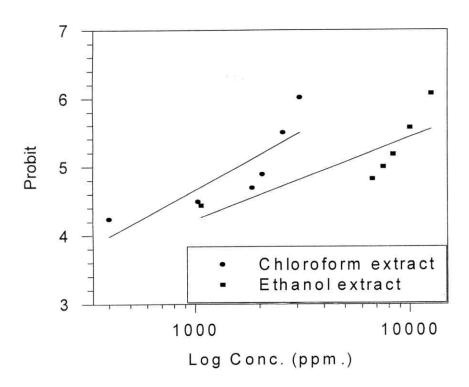


Fig. (6): LC-P lines of W.somnifera extracts against 2nd instar of Spodoptera litoralis.

Table (11): Larvicidal activities of different concentrations of Withania somnifera chloroform extract against 4th instar larvae of Spodoptera littoratis in laboratory.

Concent- ration	% corrected mortality at the indicated post-treatment intervals (hr)			
tested ppm	24 h	48 h	72 h	
800	7.56%	20.56%	30.06%	
2000	14.0%	24.6%	37.83%	
3300	10.0%	25.5%	37.83%	
3700	16.41%	46.41%	68.92%	
4120	25.0%	44.39%	84.45%	
LC50 =	1973.1 ppm			
r =	0.73			
b =	1.6			

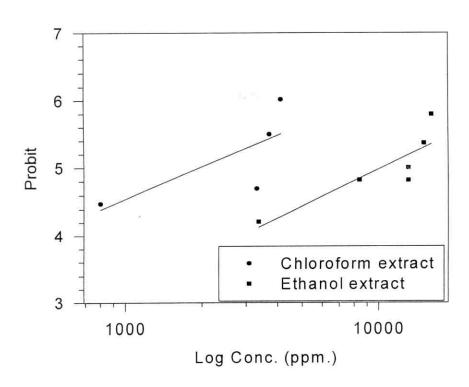


Fig. (7): LC-P lines of W.somnifera extracts against 4th instar of Spodoptera litoralis.

2.2. Ethanol crude extracts of W.somnifera:

Data in Tables (12, 13) and Fig. (7) indicate the superior insecticidal activities of crude ethanol extract of *W.somnifera* plant against 2nd instar larvae than 4th instar of the cotton leafworm, showing the LC50's of 6939.2 and 9387.6 ppm, respectively. The relative potency of this extract on 2nd larval instar being 1.35 times more than 4th instar.

The slope of the established mortality regression lines confirmed the homogeneity of the used larval population, i.e. 3.4 and 1.9 with 2nd and 4th larval instar, respectively.

Data in the same table indicate that the cumulative mortality percent were increased by raising of extract concentration. The 2nd instar larvae showed 28.57, 50.60, 42.85, 57.14, 71.42 and 85.7% mortality at 4200, 6720, 7560, 8400, 10500 and 12600 ppm, respectively. The 4th instar larvae showed 21.42, 42.66, 50.00, 64.28, and 78.57% with 3360, 8400, 13044, 15012 and 16080 mortality ppm, respectively.

Reviewing the above mentioned results, it could be concluded the more insecticidal activities of chloroform crude extract of *W.somnifera* against both 2nd and 4th instar larvae of the cotton leafworm than ethanol extract. Taking the LC50's of ethanol extract as a base line (1.0) for comparison, the relative potency of chloroform extract reached 5939.2, 1594.6 (2nd instar) and 9387.6, 1973.1, (4th instar), respectively. Such findings are in harmony with the results obtained by many researchers. **Ramesh Chandra** (2004) reported that medicinal plants in chitrakoot,

Madhya prodesh, India were studied for insect pest infestation. Of the 245 medicinal plants surveyed, 43 were infested by 70 insect pests. More than one pest fed on the same plant. W.somaifera, Tricentrus bicolor and Menitha sp. Spodoptera exiqua, S.littoralis and Microtermes sp. were among the highest number of insects attacked the plants.

Table (12): Larvicidal activities of different concentrations of Withania somnifera ethanol extract against 2nd instar larvae of Spodoptera littoratis in laboratory.

Concent- rations	% corrected mortality at the indicated post-treatment intervals (hr)			
tested ppm	24 hr	48 hr	72 hr	
4200	9.52%	21.52%	28.57%	
6720	20.0%	34.0%	50.0%	
8400	17.14%	37.14%	57.14%	
10050	25.0%	53.21%	71.42%	
12600	20.0%	60.3%	85.7%	
LC50 =	6939.2ppm	_		
$\mathbf{r} =$	0.97			
b =	3.4			

Table (13): Larvicidal activities of different concentrations of Withania somnifera ethanol extract against 4th instar larvae of Spodoptera littoratis in laboratory.

Concent- rations	% corrected post-trea	MI .		
tested ppm	24 hr	48 hr	72 hr	
3360	6.0%	16.0%	21.42%	
8400	8.66%	28.66%	42.66%	
13044	25.0%	35.0%	50.0%	
15012	14.28%	34.28%	64.28%	
16080	18.57%	58.57%	78.57%	
LC50 =	9387.6ppm			
r =	0.92			
b=	1.9			

2.3. EC Formulation from chloroform extract of W.somnifera:

Data in Table (14) and Figs. (8 & 9) indicate the great insecticidal activities of EC formulation which prepared from chloroform extract of W.somnifera plant against 2nd instar larvae of the cotton leafworm. This EC formulation showed moderate activity on 4th instar larvae. The LC50's were 93.8 and 455.7 ppm with 2nd and 4th instars, respectively. The relative potency of this extract on 2nd instar larvae reached 4.86 times more than 4th instar. The concentration of tested EC/formulation extract played important role on its insecticidal activity. The higher the concentration was the higher the mortality of leafworm larvae and vice versa. In terms of figures, the cumulative mortality percent reached 36.37, 42.70, 57.58, 68.00, 73.48 and 78.79% when used at 25, 100, 250, 400, 450 and 500 ppm, respectively. The corresponding values on the 4th instar larvae were 30.06, 41.72, 53.37, 76.68 and 84.45% at 200, 500, 800, 900 and 1000 ppm, respectively.

Again, the slope values of the established regression lines confirmed the homogeneity of the studied population of cotton leafworm larvae, i.e. 0.8 and 1.9 with 2nd and 4th instars, respectively. Data in the same tables indicated the great role of time elapsed from treatment. The longer after treatment period was the higher the mortality percent of leafworm and vice versa. This was pronounced with both instar larvae.

Reviewing the above mentioned results, it is clearly noticed the superior larvicidal activity of EC formulation from chloroform extract than the crude extract of the same solvent only. The LC50's reached 93 and 1594.6 ppm (2nd instar) and 455.7 and 1773.2 ppm (4th instar) in case of EC and crude extracts, respectively. Taking the LC50's of crude extract as a base line for comparison (1.0), the relative potency of EC formulation reached 17 and 4.33 on 2nd and 4th instar larvae, respectively.

Our findings are in harmony with that obtained by several investigators. Chitra-srivastava and Reddy (2006) carried the evaluation of antifeedant activity of neem formulation against *S.littoralis*. The antifeedant activities of various neem-based formulations (applied at 0.002, 0.004, 0.006, 0.008, 0.01, 0.04, 0.06, or 0.08%) which had Azadirachtin as the main active ingredient, were evaluated against *S.littoralis* larvae using the leaf disc no-choice test. The treatments were: Achook 0.15 or 0.30 EC and 5.0% azadirachtin resulted in 83.6 and 85.8% reduction in feeding, respectively when applied at 0.08% at the same application rate gronim 0.3% resulted in the greatest reduction in feeding (77.8%).

Rahman and Kanaujia (2003) studied the effect of sublethal dosages of neem formulations on the growth and development of *Spodoptera litura* (Fab.) The effects of sublethal doses (0.1, 0.05 and 0.25%) of neem formulations viz. Multineem, Nimbicidine and neem seed kernel extract on the growth and development of *S. litura* were determined. Treatment with NSKE at all the tested doses resulted in the lowest larvae length and weight, survival and pupation.

Table (14): Larvicidal activities of different concentrations of chloroform EC formulation (5%) of Withania somnifera crude extract against 2nd instar larvae of Spodoptera littoratis in laboratory.

Concent- rations	% corrected mortality at the indicated post-treatment intervals (hr)			
tested ppm	24 hr	48 hr	72 hr	
25	7.5%	27.5%	36.37%	
100	15.6%	32.2%	42.7%	
250	20.0%	35.5%	57.58%	
400	15.0%	45.12%	68.0%	
450	13.18%	38.18%	73.48%	
500	20.29%	48.79%	78.74%	
LC50 =	93.8			
$\mathbf{r} =$	0.87			
b =	0.84			

Table (15): Larvicidal activities of different concentrations of chloroform EC formulation (5%) of Withania somnifera crude extract against 4th instar larvae of Spodoptera littoratis in laboratory.

Concent- rations	% corrected post-tr	1		
tested ppm	24 hr	48 hr	72 hr	
200	8.3%	18.8%	30.06%	
500	7.72%	23.32%	41.72%	
800	12.4%	32.6%	53.37%	
900	15.88%	41.68%	76.68%	
1000	18.75%	48.75%	84.45%	
LC50 =	455.7	N		
r =	0.746			
b =	1.9			

2.4. EC Formulation from ethanol extract of W.somnifera:

Data in Tables (16 & 17) and Figs. (8 & 9) indicate the higher insecticidal activity of EC ethanol formulation of *W.somnifera* against the 2nd instar larvae of the cotton leafworm as shown by the LC50 (884.7 ppm). The corresponding LC50 on 4th instar larvae was 3434.3 ppm. The relative potency of this extract on 2nd instar larvae based on LC50 of 4th instar (1.0) reached 3.88 more efficacy than the older larvae. The cumulative mortality percent were steadily increased by increasing extract concentration. Mortality percent reached 34.42, 45.35, 61.79, 67.21, 72.67 and 78.14% when used at 250, 1000, 2500, 4000, 4500 and 5000 ppm, respectively. The same trend of results was obtained with the other post-treatment intervals. The longer the post-treatment interval was the higher the mortality percent and vice versa.

Reviewing the aforementioned results, it is clearly evident to notice the great insectcidal activities of EC formulation than the crude extract by the same solvent. The LC50's were 884.7 and 6939.2 ppm (2nd instar larvae) and 3434.3 and 9387.6 ppm (4th instar) in case of EC and crude extracts, respectively. The relative potency of EC formulation based on crude extracts (1.0) reached 7.84 and 2.73 times more than crude efficacy on 2nd and 4th instars, respectively.

As conclusion chloroform extracts as crude or in EC formulation proved more satisfactory insecticidal activities on the cotton leafworm larvae than ethanol extract. Also, both extracts and EC formulations showed higher insecticidal

activities on 2nd instar larvae compared with the 4th instar. Such findings are in accordance with that obtained by many researchers. **Sridhar, and Suganthy (2006)** reported that the feeding inhibition study revealed that the area fed by *S.littoralis* was minimum in 5% neemazal-f at 1 ml/litre (0.84 cm²) followed by 1.41 cm² in 1% neemazal-f at 5 ml/litre.

Table (16): Larvicidal activities of different concentrations of Ethanol EC formulation 50% of Withania somnifera crude extract against 2nd instar larvae of Spodoptera littoratis in laboratory.

Concent- rations	% corrected post-tr	Jan 1971		
tested ppm	24 hr	48 hr	72 hr	
250	9.21%	24.21%	34.42%	
1000	20.0%	32.9%	45.35%	
2500	10.82%	33.42%	61.74%	
4000	25.5%	46.3%	67.21%	
4500	30.0%	55.8%	72.67%	
5000	17.84%	53.14%	78.14%	
LC50 =	934			
r=	0.96			
b =	0.8			

Table (17): Larvicidal activities of different concentrations of Ethanol EC formulation 50% of Withania somnifera crude extract against 4th instar larvae of Spodoptera littoratis in laboratory.

Concent- rations	% corrected mortality at the indicated post-treatment intervals (hr)		
tested ppm	24 hr	48 hr	72 hr
2000 .	6.94%	23.24%	41.84%
5000	12.7%	28.3%	49.38%
8000	20.0%	42.45%	56.92%
9000	30.0%	55.5%	78.46%
10000	25.4%	60.4.0%	88.77%
LC50 =	3434.3		
r =	0.63		
b =	1.6		

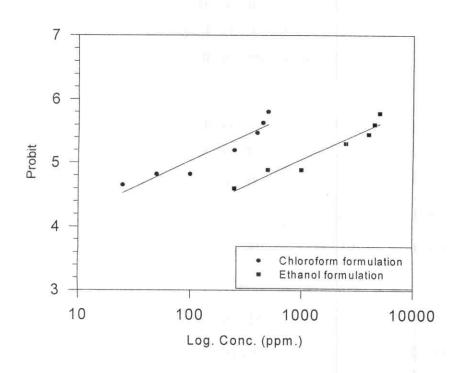


Fig. (8): LC-P line of W.somnifera formulation against 2nd instar of Spodoptera litoralis.

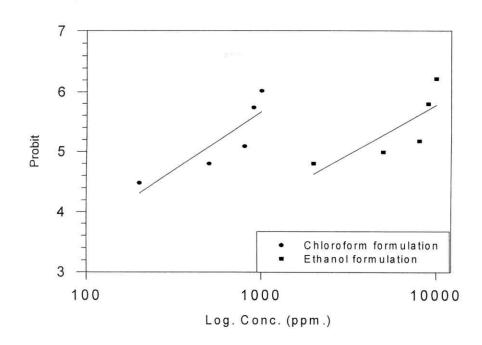


Fig. (9): LC-P line of W.somnifera formulation against 4th instar of Spodoptera litoralis.

Table (18): Comparative cumulative larvicidal activities of Withania somnifera extract against 2nd and 4th instar larvae of Spodoptera litoralis.

	LC5	Relative		
Larval instar	Ethanol	Chloroform	potency of chloroform extract	
2 nd instar larvae	6939.2	1594.6	4.35	
4 th instar larvae	9387.6	1973.1	4.76	

Table (19): Comparative cumulative larvicidal activities of EC formulation of *Withania somnifera* extract against 2nd and 4th instar larvae of *Spodoptera litoralis*.

Larval instar	LC50 p.p.m		Relative
	Ethanol 50% EC	Chloroform 5% EC	potency of EC chloroform formulation
2 nd instar larvae	884.7	93.8	9.43
4 th instar larvae	3434.3	455.7	7.54

PART 3

Isolation and Larvicidal Activities of TLC-Separated Chromatograms of Withania somnifera Extracts Against 3rd Instar Larvae of C.pipiens in Laboratory

3.1. TLC isolation of Withenia somnifera extract components

Data in Table (20) and Figs. (10 & 11) indicated the numbers of separated bands by thin layer chromatography technique, their relative flow rates (RF's) and their larvicidal activities expressed as corrected mortality percentage against the 3rd instar larvae of *culex pipiens* in the laboratory. Examination of the obtained results clearly indicated the separation of 13 bands with sequencial RF's value. In terms of figures, the RF's of the separated bands from ethanolic extract of *W.somnifera* were 0.04, 0.08, 0.12, 0.16, 0.24, 0.32, 0.40, 0.48, 0.60, 0.64, 0.72, 0.76 and 0.80, respectively. The same numbers of band, were separated from chloroform extract with RF's of 0.032, 0.064, 0.096, 0.120, 0.150, 0.220, 0.290, 0.520, 0.450, 0.580, 0.670, 0.740 and 0.830, respectively.

3.2. Insecticidal activity

Data in Table (20) clearly indicate the superior larvicidal activity of the band having RF of 0.76 from ethanol extract, followed by bands with RF's of 0.80, 0.08, 0.04 and 0.24; showing the mortality percent of 80.0, 66.66, 60.00, 60.00 and 53.00 respectively. The bands with RF's of 0.15 and 0.60 came next, showing 40% mortality on exposed larvae. The other bands treated showed inferior larvicidal activities on mosquits larvae.



Fig. (10): TLC separated bands of chloroform extracts of W.somnifera.

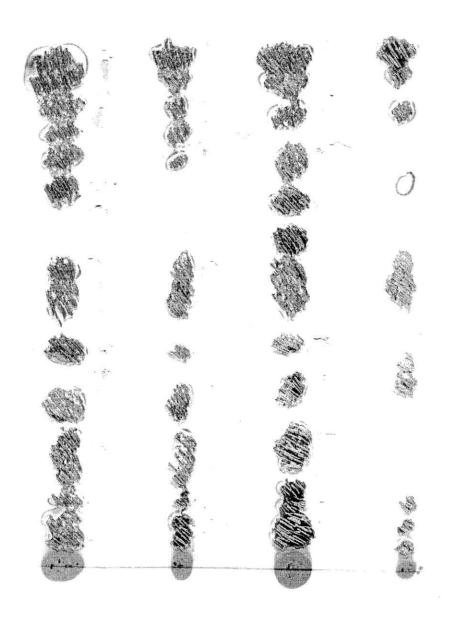


Fig. (11): TLC separated of ethanol extracts of W.somnifera.

On the other hand, 13 bands were also separated from chloroform extract of *W.somnifera*, having the RF's of 0.032, 0.064, 0.096, 0.120, 0.150, 0.220, 0.290, 0.520, 0.450, 0.580, 0.670, 0.740 and 0.830, respectively. It is clearly evident to notice that the band with RS 0.83 was the only band showed moderate larvicidal activity, reaching 60.0% kill on 3rd instar larvae of *C.pipiens* in laboratory. The other bands showed inferior mortality ranged from 10 to 40%.

The relative biological activities of TLC separated bands were studied and reported by several investigators. Ahmed et al., (1984) separated components of the petroleum ether extracts of seven plant species by using thin layer chromatography technique. The isolated components were tested for their insecticidal effect against the fourth instar larvae of *S.littoralis*.

Table (20):- Larvicidal activities of TLC separated chromatography of *Withania somnifera* ethanol and chloroform extract against 3rd instar larvae of *C.pipiens*.

Ethanol extracts		Chloroform extracts	
RF	% mortality	RF	% mortality
0.04	60.00%	0.032	25.00%
0.08	60.00%	0.064	10.00%
0.12	33.33%	0.096	35.00%
0.16	40.00%	0.120	30.00%
0.24	53.00%	0.160	30.00%
0.32	10.00%	0.220	40.00%
0.40	20.00%	0.290	10.00%
0.48	13.33%	0.520	15.00%
0.60	40.00%	0.450	15.00%
0.64	26.66%	0.580	20.00%
0.72	13.33%	0.670	10.00%
0.76	80.00%	0.740	20.00%
0.80	66.66%	0.830	60.00%

3.3. Confirmation analysis of the most active components of ethanol extract Withania somnifera by GC/MS

3.3.1. Ethanol extract:

Separated bands of ethanol extract No. (12 and 13) from T.L.C plates which showed high potential against 3rd instar larvae of *Culex pipiens* has been identificated by GC/MS using standard library (NIST version 3.0).

The result of identification by GC/MS suggest that the band No. (12) contain (6) compounds as follows:

- 1- Talbotine (CAS) SS TALBOTIN SS indolo [3,2,1-ij] pyrano [3,4-b] [1,5] naphthyridine-12 a (12 H) carboxylic acid 9-e thylidene –5,6,7, 7a, 8, 8a, (Fig. 12).
- 2- 8-Amino-3- (carbomethoxy) -1- methyl -5- (3,4,5-trimethoxyl phenyl) -1H-pyrimido [4,5-cl-1,2-diazepin -6-(7H) -one. (Fig. 13)
- 3- Methyl (23F) -3. beta. -acetoxy-12, 21-dioxo-23-phenylth-5. beta., 21E-cholan-24-oate SS Cholan-24-oic acid, acetyloxy) 12, 21-dioxo-2. (Fig. 14)
- 4- 4-Dibenzothienyl –2- (4,4-dimethyl –2- oxazolin –2- yl) phenylmethanol SS 4-Dibenzothiophenemethanol, alpha, [2-(4,5-dihydro-4, 4-dimethyl. (Fig. 15)
- 5- 26, 27-Dinorergoata-5, 22-dien-3-ol, (3, beta, 22E) (CAS) SS 24-NOR –CHOLESTA-5, 22 (TRANS) DIEN –3, BETA –OL SS No rcholestadienol SS 26, 27. (Fig. 16)
- 6- 6-t-Butyl 5-Iodo-4- (2-methoxycarbonylethyl) -3- methoxycarbonylmethyl pyrrole-2- carboxylate SS 1H-Pyrrole-3-propanoic acid, 5- [(1,1-dimet)]. (Fig. 17)

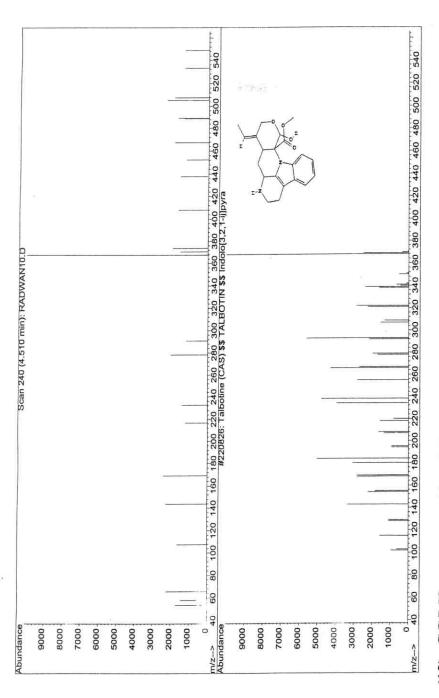


Fig. (12): GC/MS spectrum of band No. 12: compound.

No.1: Talbotine (CAS) SS TALBOTIN SS indolo [3,2,1-ij] pyrano [3,4-b] [1,5] naphthyridine-12 a (12 H) – carboxylic acid 9-e thylidene –5,6,7, 7a, 8, 8a.

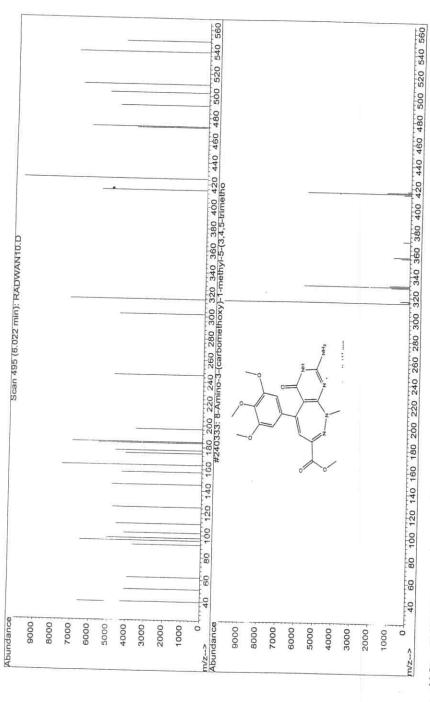


Fig. (13): GC/MS spectrum of band No. 12: compound

No.2: 8-Amino-3- (carbomethoxy) -1- methyl -5- (3,4,5-trimethoxyl phenyl) -1H-pyrimido [4,5-cl-1,2-diazepin -6- (7H) -one.

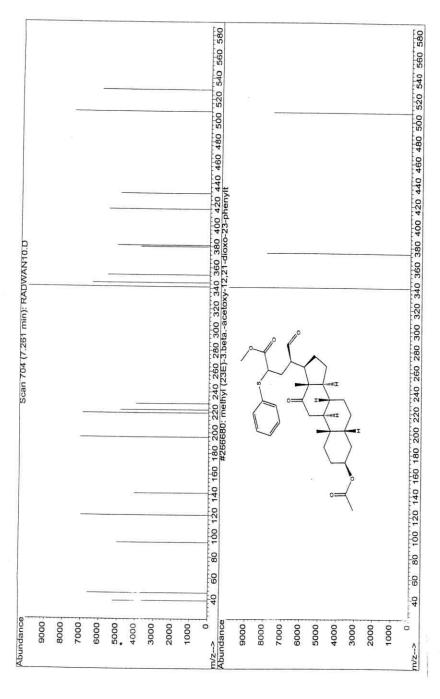
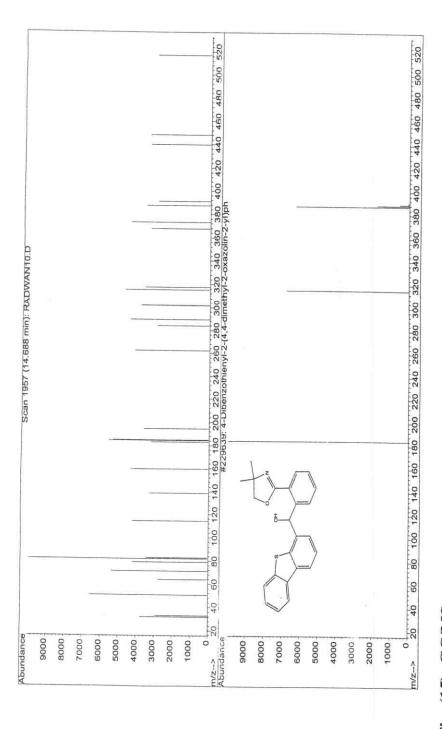


Fig. (14): GC/MS spectrum of band No. 12: compound.

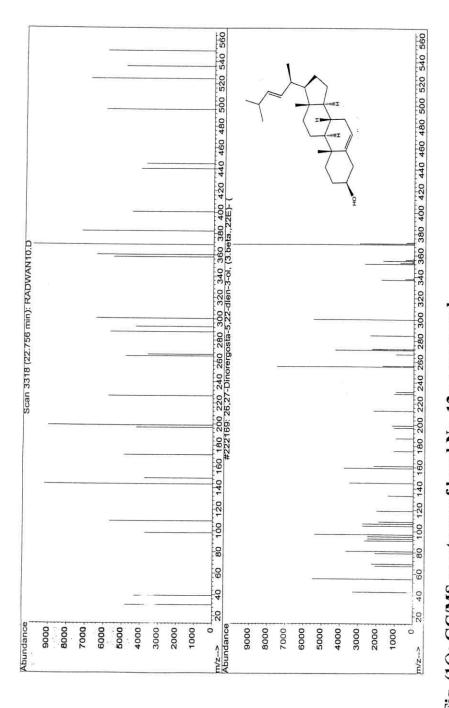
No. 3: Methyl (23F) -3. beta. -acetoxy-12, 21-dioxo-23-phenylth-5. beta., 21E-cholan-24-oate SS Cholan-24-oic acid, acetyloxy) 12, 21-dioxo-2.



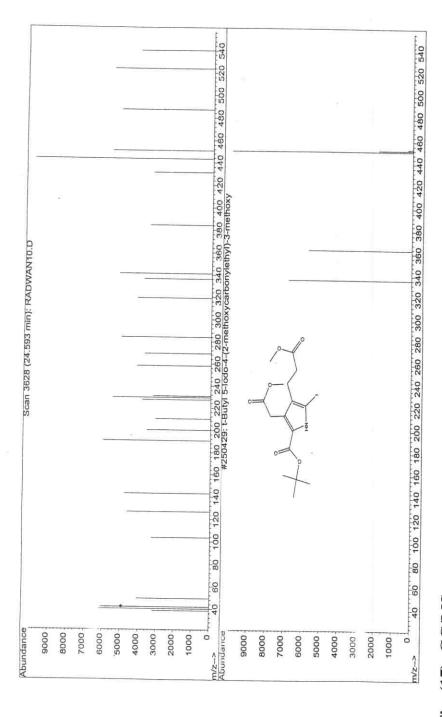
phenylmethanol -2- oxazolin -2- yl) Dibenzothiophenemethanol, alpha, -[2-(4,5-dihydro-4, 4-dimethyl. Fig. (15): GC/MS spectrum of band No. 12: compound. (4,4-dimethyl -2-4-Dibenzothienyl

4

SS



No.5: 26, 27-Dinorergoata-5, 22-dien-3-ol, (3, beta, 22E) - (CAS) SS 24-NOR -CHOLESTA-5, 22 (TRANS) - DIEN -3, BETA -OL SS No rcholestadienol SS 26, 27. Fig. (16): GC/MS spectrum of band No. 12: compound.



-3- methoxy-carbonylmethyl pyrrole-2carboxylate SS 1H-Pyrrole-3- propanoic acid, 5- [(1,1-dimet)]. Fig. (17): GC/MS spectrum of band No. 12: compound. (2-methoxycarbonylethyl) t-Butyl 5-Iodo-4-

The band No. (13) contains (2) compounds as follows:

- 1- Isocephalotaxinone. (Fig. 18).
- 2- N-(Methylsulfonyl)-O-(trimethylsilyl) hydroxylamine. (Fig. 19).

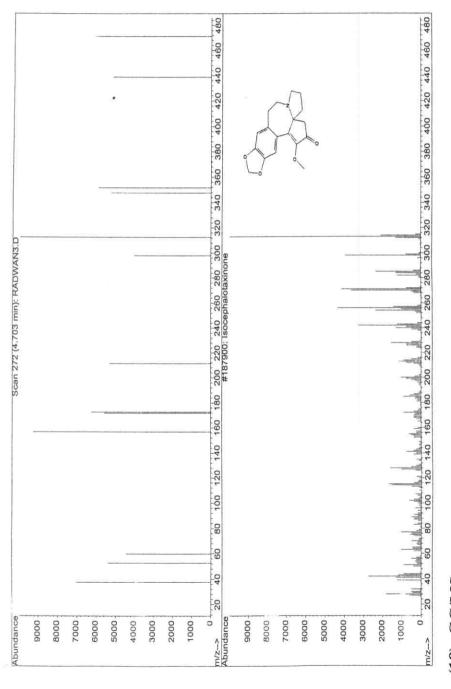


Fig. (18): GC/MS spectrum of band No. 13: compound. No.1: Isocephalotaxinone.

-101-

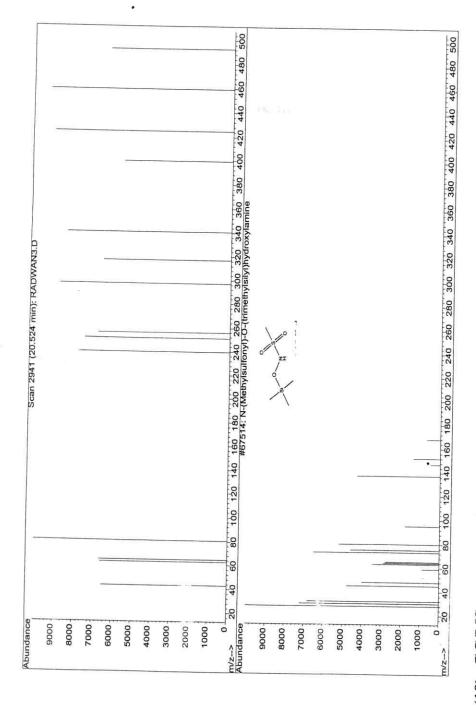


Fig. (19): GC/MS spectrum of band No. 13: compound. No.2: N-(Methylsulfonyl)-O-(trimethylsilyl) hydroxylamine.

3.3.2. Chloroform extract:

Separated bands of chloroform extract No. (6 and 13) from TLC plates which showed high activity against 3rd instar larvae of *Culex pipiens* has been identificated by GC/MS using standard library (NIST version 3.0).

The result of identification by GC/MS suggest that the band No. (6) contain (4) compounds as follows:

- 1- 1-Methylspiro [5,5] unec-2-ene-4, 7-dione SS spiro [5.5] u ndec-9-ene-1, 8-dione, 11-methyl-, cis-.(Fig. 20).
- 2- Thymol acetate. (Fig. 21).
- 3- Alpha, -Tumerone. (Fig. 22).
- 4- Phenanthrene, tetradecahydro- (CAS) SS perhydrophenant hrene SS Tetradecahydrophenanthrene. (Fig. 23).



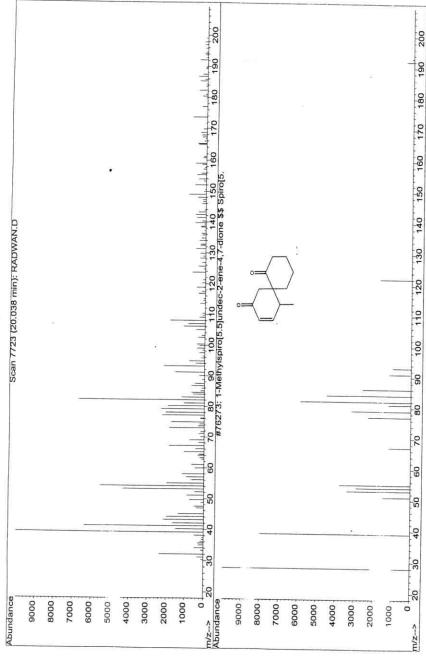


Fig. (20): GC/MS spectrum of band No. 6 compound.

No. 1: 1-Methylspiro [5,5] unec-2-ene-4, 7-dione SS spiro [5.5] u ndec-9-ene-1, 8-dione, 11methyl-, cis-

Results and Discussion

-104-

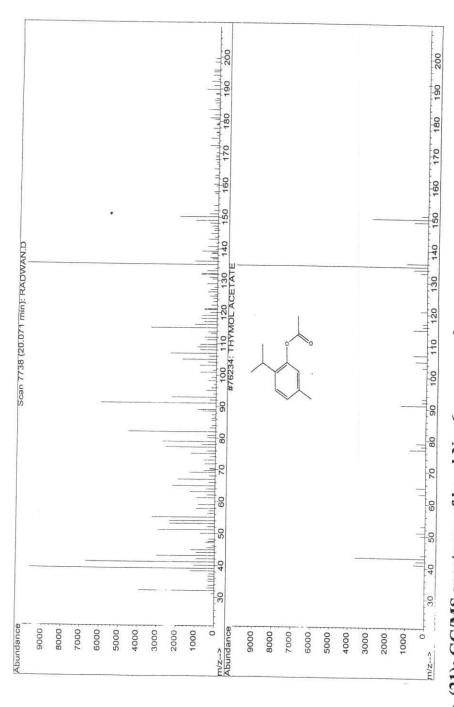


Fig. (21): GC/MS spectrum of band No. 6 compound. No. 2: Thymol acetate.

-105-

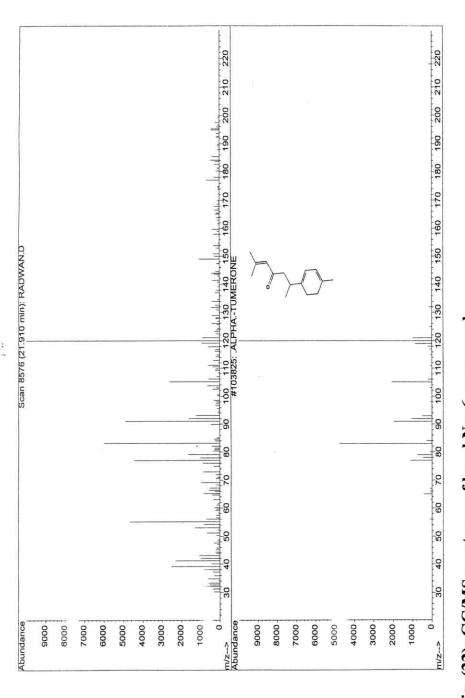
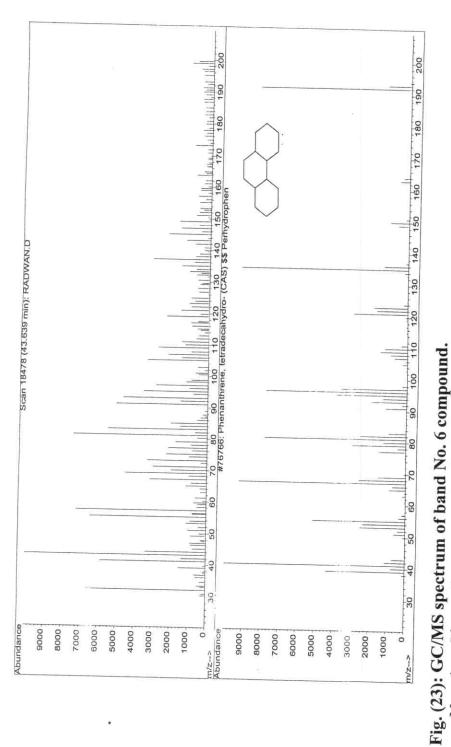


Fig. (22): GC/MS spectrum of band No. 6 compound. No. 3: Alpha, -Tumerone.

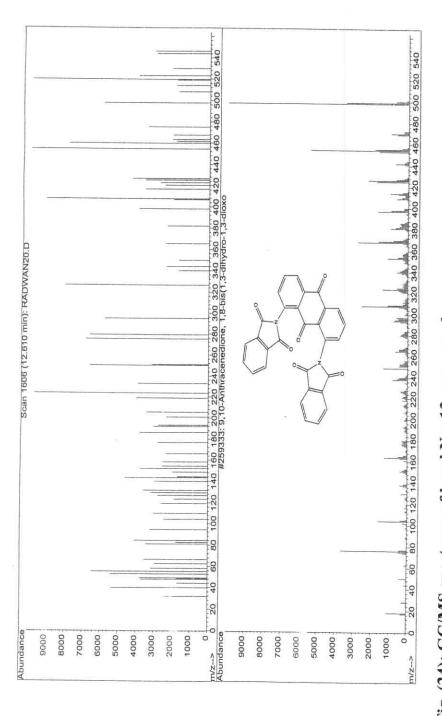
-106-



Phenanthrene, tetradecahydro- (CAS) SS perhydrophe-nant hrene SS Tetradecahydrophenanthrene.

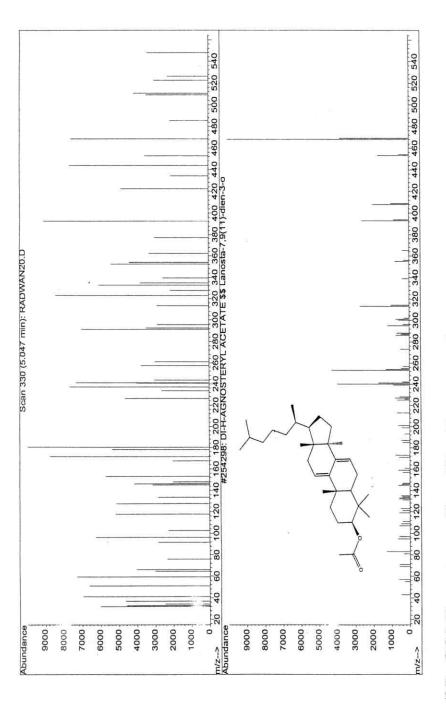
In case of band No. (13) contain (4) compounds as follows:

- 1- 9,10—Anthracenedione, 1,8-bis (1,3- dihydro-1,3- dioxo (2h) isoindol –2-yl). (Fig. (24)
- 2- DI-H-Agnosteryl Acetate SS Lanosta-7,9 (11)-dien-3-ol, acetate, (3,beta)- (CAS) SS 3-Beta, -Acetoxy-5- Alpha, -Lanosta-7,9 (11)-Diene S (Fig. (25).
- 3- Gradirubrine (CAS) SS 9H-Azuleno [1, 2, 3-ij] isoquinolin-9- one, 10-hydroxy-4, 5, 6-trimethoxy. (Fig. 26).
- 4- Dichlorofuroxan. (Fi.g 27).

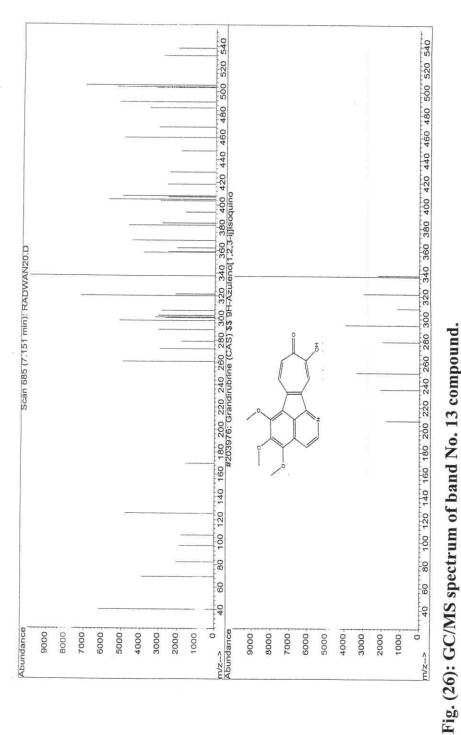


No.1: 9,10—Anthracenedione, 1,8-bis (1,3- dihydro-1,3- dioxo (2h) isoindol -2-yl). Fig. (24): GC/MS spectrum of band No. 13 compound.

-109-



No.2: DI-H-Agnosteryl Acetate SS Lanosta-7,9 (11)-dien-3-ol, acetate, (3,beta)- (CAS) SS 3-Beta, -Acetoxy-5- Alpha, -Lanosta-7,9 (11)-Diene S Fig. (25): GC/MS spectrum of band No. 13 compound.



No.3: Gradirubrine (CAS) SS 9H-Azuleno [1, 2, 3-ij] isoquinolin-9- one, 10-hydroxy-4, 5, 6trimethoxy.

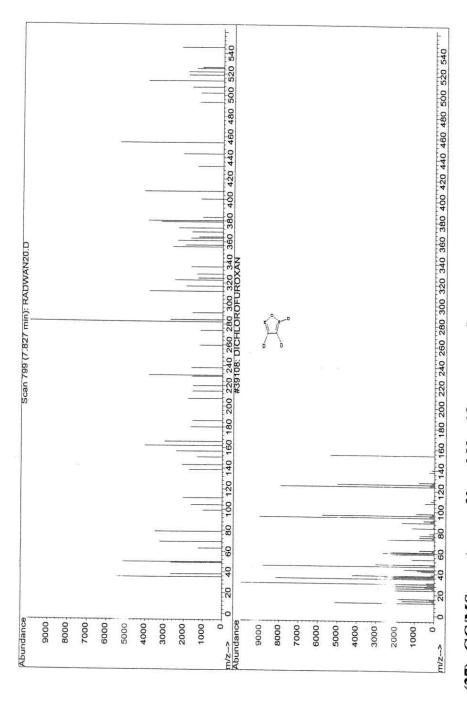


Fig. (27): GC/MS spectrum of band No. 13 compound. No.4: Dichlorofuroxan.

-1112-