J. Pest Cont. & Environ. Sci. 12 (1/2): 39-54 (2004).

Insecticidal activity of an isolated fraction mixture from willow bark (*Salix safsaf*).

Ahmed S. Abdel-Aty
Department of Pesticide Chemistry, Faculty of Agriculture, Alexandria University.

ABSTRACT

The air-dried bark of safsaf tree, willow (*Salix safsaf*) was extracted with aqueous acetone. After the complete evaporation of acetone, some fractions were separated from the resultant extract by using partitioning between different solvent systems. One of these fractions (fraction **II**) was found to be biologically active. In this study it was tested for its insecticidal activity against the 4th instar of cotton leaf worm, *Spodoptra littoralis* (Boisd), and on the 4th instar of *Culex pipiens*. Fraction **II** exceeded its original extract on *C. pipiens* in its lethal effect with LC_{50} equal 1450, 560, 240, 220 and 125 µg / ml in comparing with 2100, 1300, 1100, 500 and 130 µg / ml after 1, 2, 3, 4 and 5 days exposure and with LT_{50} equal 4.6, 2.1, 1.5 and < 1.0 days in comparison to 4.8, 4.2, 3.1 and 2.54 days in case of the original at 200, 500, 1000 and 1500 µg / ml. Fraction **II** was more effective in reducing the pupation with EC_{50} equal 270 µg / ml comparing to 960 µg / ml due its original extract. Although fraction **II** showed weak lethal effect, it exhibited a great anti-feeding activity against *S. littoralis* in concentration and time dependant effect with EC_{50} equal to 160 and 1500 µg / ml after 24 and 48 hours exposure, respectively. Based on GC-Ms analysis, this fraction appeared to be contain fatty acids; namely hexadecanoic acid (Palmitic acid, $C_{16}H_{32}O_2$), octadecanoic acid (Stearic acid, $C_{18}H_{36}O_2$) as saturated fatty acids and cis-oleic acid (9-Octadecenoic acid, $C_{18}H_{34}O_2$) as an unsaturated fatty acid as well as a flavonoid derivative of apigenin.

INTRODUCTION

Various medicinal uses from the willow trees have been developed to help cure stomach pains, fever and headaches. Aspirin, for example, is a derivative of the willow species (Julkunen and Sorsa, 2001). They showed also that

applying the bark powder to burns; brewing tea from the leaves to treat rheumatism, which also acts as a mild laxative. Some water extracts from *Salix caprea*, *Calluna vulgaris* and *Potentilla erecta* were the most potent cyclooxygenase inhibitors and were active to inhibit the prostaglandin biosynthesis and platelet activity factor (PAF) induced exocytosis *in vitro* (Tunon *et al.*, 1995). Saturated fatty acids (C-15 and C-31) and flavonoids (apigenin, kaempherol and luteolin) were identified from *S. lindleyana*, which has antipuretic properties (Thapliyal and Bahuguna, 1993). 2'-Cinnamoylsalicortin as a new phenolic glycoside, was found by Nichols - Orians *et al.*, (1992) in *S. sericea* which likely to posses biological activity. An allelopathic effect of *S. babylonica* leaf leachates was shown on rice seed inhibiting its germination (Koul *et al*, 1991). Leaves and flowers of *S. boldtiana* showed their antitumor and antimalarial effect through DNA interaction mechanism (Pezzuto *et al.*, 1991). Its leaves and branches were shown to have anti-inflammatory effect when used as bathing agents depending on the extraction temperature with improving the blood circulation (Shimizu *et al.*, 1993). Some phenylpropanoids like triandrin and n -coumaric alcohol derived from *S. viminalis* showed stimulating action in mice as spontaneous motor and antihyprotic activities (Sokolov *et al*, 1990). Kolodziej (1990) isolated four dimeric and five trimeric procyanidins from medicinal willow (*S.* spp) bark, claimed to have analgesic and antipyretic effects. Malterud *et. al.*, (1985) found that the isolated flavonoids from the wood of *S. caprea* were potent inhibitors of wood-destroying fungi and inhibited some of rot-producing fungi.

The aqueous extract of willow (*S. safsaf*) bark was fractionated to some biologically active fractions by partitioning between different solvent systems. Fraction **II**, which was isolated from n-hexane layer, was found to be more active to inhibit of root growth of wheat and squash seedlings with IC_{50}'s equal 170 and 230 ppm, respectively. It was effective against the rice weevil, *Sitophilus oryzae* with LC_{50} equal 140 ppm during 2.4 days. It gave high lethal effects against the terrestrial snail, *Theba pisana* with LC_{50} equal 56 ppm (Abdel-Aty, 2003).

The aim of the present study was planned to investigate the insecticidal activities of an isolated fraction mixture from *Salix safsaf* against *C. pipiens* and *S. littoralis*.

MATERIALS AND METHODS

Isolation of the tested fractions:

Based on Abdel-Aty (2003), The original fraction (15.43 % of the un extracted materials) was obtained from *S. safsaf* (Willow) bark by soaking in 70 % aqueous acetone for a month at room temperature and complete evaporation of acetone as a solid. The original extracted solid was re-dissolved in water and partitioned with petroleum ether (30-40 °C) 7 times. The petroleum ether free-aqueous layer was cooled to give a brown precipitate that was filtered off. The filtrate was extracted with ethyl acetate 8 times using a separatory funnel followed by evaporation of the resulted aqueous layer before extraction with n-hexane. Adding toluene to the n-hexane layer gave a resinous material that was dried using the hair dryer and over sodium hydroxide to give a brown powder identified as fraction **II** (5.9 % of the un-extracted materials).

Treated insects:

The 4th instar of laboratory strains of cotton leafworm, *S. littoralis* (Boisd.) and *C. pipiens* were cultured in the breeding sector of Pesticide Chemistry Department, Faculty of Agriculture, Alexandria University.

Tested fractions:

Fraction **II** and its original extract of *S. safsaf* (Willow) bark comparing with Lannate (methomyl), S-methyl-N-[(methyl carbamoyl)oxy] thioacetimidate as a comparative commercial insecticide.

Effect of the isolated fraction on *Culex pipiens*:

The larvae of *C. pipiens* were treated, in aqueous media, with the tested fraction at concentrations of 5, 10, 25, 50, 100, 200, 500 and 1000 µg / ml for 24 hours. Sixty larvae were divided into three replicates of each treatment. Number of alive larvae was distinguished and the mortality percent was calculated after 24 hours according to Abbott (1925). After 96 hours, the effect of the tested fractions on the pupation activity of the treated larvae was noticed

and calculated comparing with control. Lannate was used as a comparative commercial insecticide. Long-term effect was also studied for five days as mortality percent was calculated in comparison to the original extract. Both the lethal concentration of 50 % of the treated population (LC_{50}) and the lethal time of 50 % of the treated population (LT_{50}) were calculated on probit paper.

Effect of the isolated fraction on *S. littoralis*

A laboratory strain of cotton leafworm, *S. littoralis* (Boisd.), was rarred on castor bean leaves according to Eldefrawi *et al.*, (1964). Sixty larvae (in the 4th instar) were treated in three replicates for each treatment. The tested concentrations were 5, 25, 50, 100, 200, 500, 1000, 2000 and 3000 µg / ml using the leaf dipping technique as reported by Kubo and Nakanishi (1977) with slight modifications. The castor bean leaves were cut into equivalent circles 2.5 cm in diameter and immersed in the tested solutions for 30 seconds and dried before introducing to insects in plastic pots. The experiment was carried out at 25 ± 2 °C and 70 % relative humidity. Control was carried out under the same experimental conditions. After 24 and 48 hours, the alive number was counted and the mortality percents were calculated. The antifeeding activity was observed by comparing the average consumed food of each larva in control and treatment, determining the percentage of feeding inhibition according to the formula presented by Abivandl and Benz (1984):

$$\% \text{ FI} = 100 \times (C-T) / C$$

Where:

FI, feeding inhibition; C, consumed food/control larva; T, consumed food/treated larva.

GC-MS Analysis of the active fraction:

It was done on HEWLETT 58590 PAKARD SERIES II Gas Chromatography coupled with HEWLETT 5989 B Mass spectrometer. This analysis was carried out in Central Lab. Unit, the High Institute of Public Health, Alexandria University.

The used column was HP-5 MS (5 % Dimethyl, 95 % Diphenyl-polysiloxane) and the inlet temperature was 260 °C. The sample (1.75 µg) was charged in 1 µl of dimethylformamide (DMF) at a split ratio of 50:1. The carrier gas was Helium (He) and the analysis temperature programme started at 80 °C for a minute and increased with 10 °C / min rate to 235 °C, which was fixed for 25 minutes. Quadrable (226 °C) as an ion source and MS detector (its temperature is 270 °C) were used. Sample was bombarded with (70 e.v) electron volt and the mass range was 40- 450.

RESULTS AND DISCUSSION

Insecticidal activity against *C. pipiens*:

The isolated fraction **II** was weakly effective with mortality percents equal to 0, 7, 13, 20, 30 and 48 % on the mosquitoes larvae after 24 hours exposure at 50, 100, 200, 500 and 1000 µg / ml, respectively in comparison to methomyl as a comparative commercial insecticide, which completely killed the treated population at 100 µg / ml (Table 1). This preliminary result encouraged the study of its long-term effect, and it was found that the effects of the isolated fraction **II** exceeded its original extract of *S. safsaf* on *C. pipiens* larvae for 5 days in its lethal effect with lethal concentration of 50 % of the treated population (LC_{50}) equal 1450, 560, 240, 220 and 125 µg / ml comparing with 2100, 1300, 1100, 500 and 130 µg / ml after 1, 2, 3, 4 and 5 days exposure Table (2). Their mortal effects were also time of exposure dependant as the lethal time of 50% of the treated population (LT_{50}) were 4.6, 2.1, 1.5 and < 1.0 days in case of fraction (**II**) in comparison to 4.8, 4.2, 3.1 and 2.54 days in case of the original extract of *S. safsaf* (willow) bark at concentrations of 200, 500, 1000 and 1500 µg / ml, respectively indicating that the toxic effects on mosquitoes is referred to its content of this fraction components. It is found that the studied samples need more than 5 days exposure time to kill more than 50 % of population at 10, 50 and 200 µg / ml. At 3000 µg / ml they proved to be extremely toxic with LT_{50} < 1.0 day. At the same time, the two studied fractions showed sub-lethal effects on the treated larvae pupation. Fraction **II** was the most effective fraction in reducing the pupae population with effective

concentration on 50 % (EC_{50}) equal 270 µg / ml comparing to 960 µg / ml in case of the original extract (Table 3).

Table (1): Effect of the separated fraction from *S. safsaf* (Willow) on*C. pipiens* larvae; shown as mortality percents .

Compound	Mortality % at different concentrations (µg / ml)									LC_{50}
	0	5	10	25	50	100	200	500	1000	
Lannate	0	67	70	83	93	100				< 5
Fraction II	0	0	0	0	7	13	20	30	48	> 1000

Effect on *S. littoralis*:

Although the tested fraction (Fraction II) showed no lethal effect at less than 500 µg / ml followed by a very low mortal effect against the treated larvae within the exposure period as it gave 10 % and 15 % mortalities after 24 and 48 hours respectively at 5000 µg / ml (Table 4), It exhibited a great antifeeding activity against the untreated larval population in concentration and time dependant effect. It caused feeding inhibition percents ranged from 9.8 to 78.4 after 24 hours exposure in comparison to control with effective concentration on 50 % of population feeding activity (EC_{50}) equal to 160 µg / ml. This effect was diminished to range from 2.8 to 61.1 % inhibition after 48 hours exposure comparing with the untreated population with effective concentration on 50 % of population feeding activity (EC_{50}) equal to 1500 µg / ml. This reduced effect may due to the adaptation of the treated worm to the used toxicant or due to being hungry. This fraction was found to be less toxic than the used insecticide (Lannate) as it caused 58 and 39.8 inhibition percents at 200 µg / ml in comparison to 99.1 and 100 in case of Lannate (Table 5).

Table (2): Effect of the separated fraction from *S. safsaf* (Willow) on *C. pipiens* larvae; shown as mortality percents, LC_{50} and LT_{50} values.

Fraction	Conc. (µg/ml)	Mortality % at different times (Days)					LT_{50} (Days)
		1	2	3	4	5	
Control		0	0	0	0	0	
Original extract	10	0	0	12.5	16.7	22.9	> 5
	50	0	4.2	14.6	22.9	31.3	> 5
	100	0	10.4	16.7	27.1	43.8	> 5
	200	6.3	18.8	31.3	43.8	58.3	4.8
	500	8.3	25.0	31.3	50	77.1	4.2
	1000	18.8	27.1	41.7	62.5	91.7	3.1
	1500	29.2	41.7	50	75.0	100	2.54
	3000	62.5	72.9	100	100	100	< 1.0
	LC_{50} (µg/ml)	2100	1300	1100	500	130	
Fraction II	10	0	2.8	10.4	27.1	43.8	> 5.0
	50	8.3	16.7	25.0	31.3	43.7	> 5.0
	100	14.6	25.0	31.8	41.7	48.0	> 5.0
	200	18.8	31.3	41.7	45.9	54.2	4.5
	500	35.4	47.9	62.5	74.0	93.8	4.2
	1000	45.8	54.2	70.8	91.7	97.7	3.1
	1500	50.0	70.8	91.7	100	100	< 1.0
	2000	62.5	72.9	100	100	100	< 1.0
	3000	85.4	100	100	100	100	< 1.0
	LC_{50} (µg/ml)	1450	560	240	220	125	

Table (3): Effect of the separated fraction on the mosquito larvae pupation after 96 hours.

Compound	Pupation % at different concentrations (µg / ml)									EC_{50}
	0	5	10	25	50	100	200	500	1000	
Original extract	100	100	100	100	95	85	78	60	35	960
Fraction II	100	90	85	80	67	60	53	27	0	270

Table (4): Effect of fraction **II** from *S. safsaf* (Willow) on the cotton leaf worm (*S. littoralis*); shown as mortality percents.

Compound	Time of exposure	Mortality % at different concentrations (µ g / ml)										
		0	5	25	50	100	200	500	1000	2000	3000	5000
Lannate	24 hr	0	0	55	65	80	100					
	48 hr	0	0	60	75	90	100					
Fraction II	24 hr	0	0	0	0	0	0	0	5	5	5	10
	48 hr	0	0	0	0	0	0	5	5	5	15	15

GC-MS- analysis of fraction II

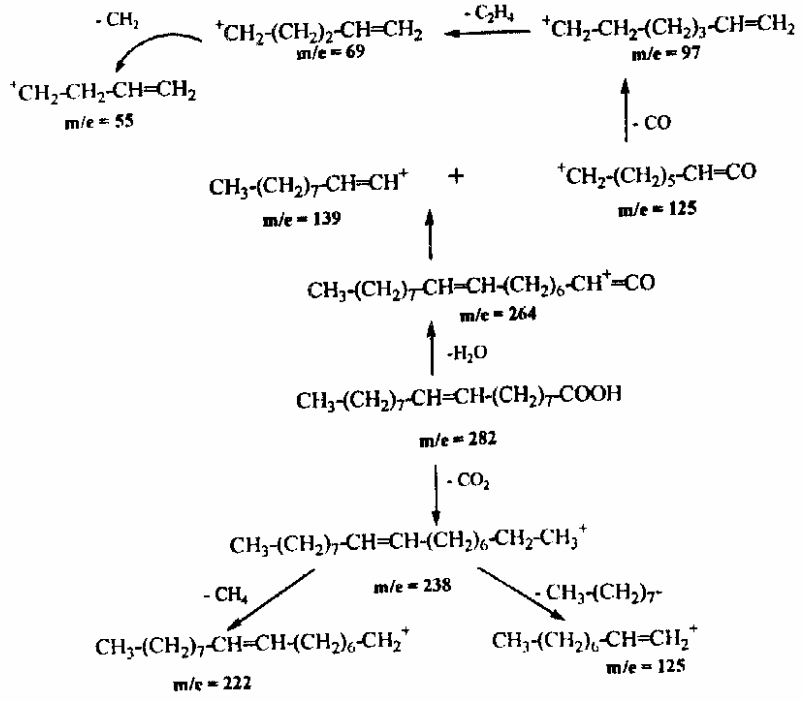
GC-MS analysis of fraction **II**, separated from the *S. safsaf*, willow bark is shown in Table (6), from which it was found that the major constituent appeared at a retention time (t_R) of 15.32 to 17.72 minutes. This region contains fatty acids, which is 65.06 % of the total peaks area.

Table (5): Effect of fraction II from *S. safsaf* (Willow) on *S. littoralis* palatability; shown as average food consumption and feeding inhibition percents.

Compound	Time of exposure		Consumed food of each larva (mg) and %FI at different concentrations (µg/ml)										
			0	5	25	50	100	200	500	1000	2000	3000	5000
Lannate	24 hr	A	21	5.59	3.68	0.736	0.294	0.2	-	-	-	-	-
		B	0	73.4	83.5	96.5	98.6	99.1	-	-	-	-	-
	48 hr	A	8.8	4.42	2.21	0.442	0.175	0	-	-	-	-	-
		B	0	49.8	74.9	95	99.2	100	-	-	-	-	-
Fraction II	24 hr	A	21	18.95	14.93	12.98	11.78	8.84	7.36	5.89	5.30	4.91	4.55
		B	0	9.8	29.0	38.2	44.0	58.0	65.0	72.0	74.8	76.7	78.4
	48 hr	A	8.8	8.56	8.14	7.68	7.36	5.30	4.82	4.62	4.28	3.80	3.42
		B	0	2.8	7.5	12.8	16.4	39.8	45.2	47.5	51.4	56.9	61.1

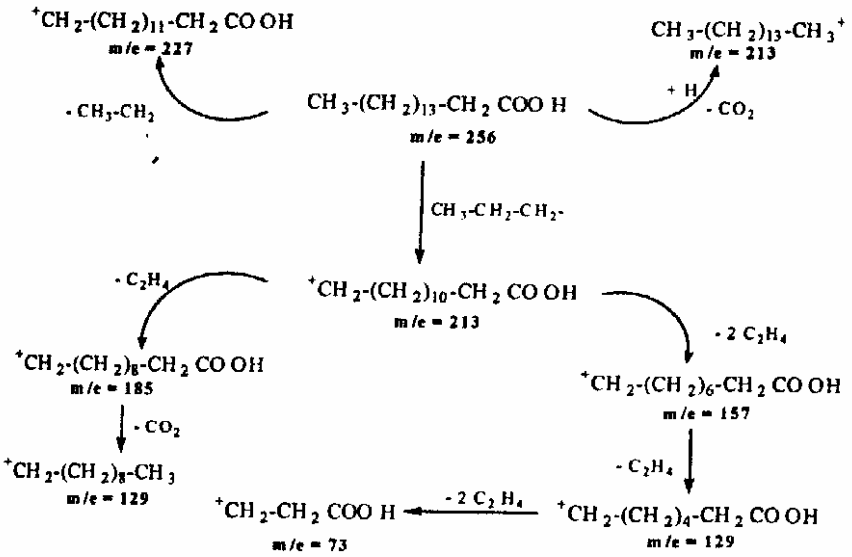
A: Average consumed food of each larva (mg); **B:** feeding inhibition percents (%FI)
- = No alive larvae was recorded

At 15.32 minutes retention time (10.22 % area) the compound having a molecular weight equal to 282.26 appeared to be cis-oleic acid with 98 % matching quality (The highest quality of the predicted compounds and the other appeared molecules should be its molecular fragments). Its mass spectrum showed its fragments at m/e = 264, 238, 222, 125, 97, 69, 55 and 44 which are the same of the standard compound emphasizing that this compound is cis-oleic acid (9-octadecenoic acid). This fragmentation process could be explained as follows:



Fragmentation pathways of cis-oleic acid (9-Octadecenoic acid)

The compound with molecular weight of 254.23 (its structure formula is $C_{16}H_{30}O_2$) identifi-ed as palmitoleic acid with 55 % matching quality. At a retention time of 15.49 minutes (17.0 % area) with 98 % matching quality a compound with molecular weight of 256.24. The fragments of this isolated compound appeared at m / e = 256, 213, 129, 73, 57 and 43, the same of the standard palmitic fatty acid as follow:



Fragmentation pathways of hexadecanoic acid (Palmitic acid)

From the fragmentation pathways surely that this compound is hexadecanoic acid (palmitic acid). In the same manner, Tetradecanoic acid (Myristic acid) with molecular weight equal 228.21, ($C_{14}H_{28}O_2$), Pentadecanoic acid ($C_{15}H_{30}O_2$) with molecular weight of 242.23 and heptadecanoic acid (Margaric acid) (M.w = 270.26, $C_{17}H_{34}O_2$) with 92, 78 and 60 % matching

quality. These acids could be considered as the major compound fragments by loss one or more CH_3 group. The compound ($C_{18}H_{36}O_2$, 284.27) was eluted at 15.71 minutes retention time with 10.81 % of the total peaks area and with 96 % matching quality with stearic acid (octadecanoic acid). Its fragmentation pathways emphasized its structure as the isolated compound gave 284, 256 (M-28), 213 (M-73), 185 (M-99), 129, 97, 57 and 44 m / e fragments that are the same of the standard stearic acid fragments. Heptadecene-8-Carbonic acid-(1) and cis-oleic acid with 96 % and 98 % matching quality (12.42 % of the total area) were predicted at 17.22 minutes. Because of cis -oleic appearance before at 15.32 minutes with the same quality, it could be thought that the eluted molecule is heptadecene-8-carbolnic acid (1). Stearic acid (octadecanoic acid, 95 % quality and 7.93 % area) was eluted at and 17.42 minutes retention time accompanying with tetradecanoic acid ($C_{14}H_{28}O_2$, 42 % matching quality) and pentadecanoic acid ($C_{19}H_{30}O_2$, 38 % matching quality), which could be considered as its fragments.

Another compound having the structural formula of $C_{16}H_{12}O_5$ was appeared and it was identified as follows:

It can be suggested that from its formula, it is a methylated flavonoid structure because the skeleton of the flavonoid is C_{15} only. It has only five oxygen atoms that means no oxygen atom on the carbon atom number 3 (3C-H not 3C-OH). Twelve hydrogen atoms indicate the presence of (2C=3C) double bond. So among flavonoids have been isolated from the plant *Salix* sp, this compound could be identified as a methylated derivative of apigenin that was isolated by Thapliyal and Bahuguna (1993).

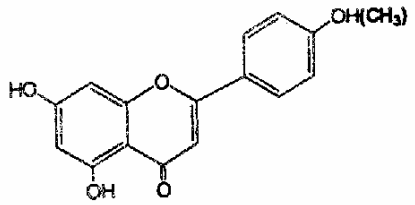


Table (6): GC-Ms analysis of fraction II, isolated from the willow bark, *Salix safsaf*

No	Retention time (min.)	% Area	Molecular weight	Match. quality	Structure formula	Identified compound
1	15.32	10.2	282.26	98	$C_{18}H_{34}O_2$	Cis-Oleic acid
			254.23	55	$C_{16}H_{30}O_2$	Palmitoleic acid
2	15.49	17.0	256.24	98	$C_{16}H_{32}O_2$	Palmitic acid
			228.21	92	$C_{14}H_{28}O_2$	Myrsitic acid
			242.23	78	$C_{15}H_{30}O_2$	Pentadecanoic acid
			270.26	60	$C_{17}H_{34}O_2$	Heptadecanoic acid (Margaric)
3	15.71	10.8	284.27	96	$C_{18}H_{36}O_2$	Stearic acid
			298.29	43	$C_{19}H_{38}O_2$	Nonadecanoic acid
4	17.22	12.4	282.26	96	$C_{18}H_{34}O_2$	Heptadecene-8-Carbonic acid-(1)
			282.26	98	$C_{18}H_{34}O_2$	Cis-Oleic acid
5	17.42	7.93	284.27	95	$C_{18}H_{36}O_2$	Stearic acid
			228.21	42	$C_{14}H_{28}O_2$	Tetradecanoic acid
			242.23	38	$C_{19}H_{30}O_2$	Pentadecanoic acid
6	17.72	6.73	284.07		$C_{16}H_{12}O_5$	Apigenin- methyl

It could be concluded that this fraction contains fatty acids mainly cis-oleic, palmitic, stearic and myristic acids as well as the methylated derivative of apigenin flavonoid. These results agree with Thapliyal and Bahuguna (1993) as

they isolated fatty acids (C15 to C 31) and apigenin among other flavonoids from *Salix lindleyana*.

REFERENCES

Abbott, W. W. (1925). A method of computing the effectiveness of an insecticide. J. Econ Entomol., 18: 265-267.

Abdel-Aty, A. S. (2003). Preleminary pesticidal studies of Willow bark (*Salix safsaf*). J. Pest. Cont. & Environ. Sci., 11 (1): 131-143.

Abivandl and Benz (1984). Tests with the extracts of 21 medicinal plants for antifeedant activity against larvae of *Pieris brassicae* L. (Lep, Ppieridae). Bull. Soc. Entomol. Swisse., 57: 383-392.

Eldefrawi, M. E.; A. Toppozada; N. Mansour and M. Zeid (1964).Toxicological studies on the Egyptian cotton leafworm *Prodenia litura* (F.) I- Susceptibility of different larval instars of Prodenia to insecticides. J. Econ. Entomol., 57: 591-593.

Julkunen, T. R. and S. Sorsa (2001). Testing the effects of drying methods on willow flavonoides, tannins and salicylates. J. Chem. Ecol., 27 (4): 779-789.

Kolodziej, H. (1990). Oligomeric flavan-3-ols from medicinal willow bark. Phytochemistry, 29 (3): 955-960.

Koul, V. K.; A. Raina; Y. P. Khanna; M. L. Tickoo and H. Singh (1991). Evaluation of allelopathic influence of certain farm grown tree species on rice (*Oryza sativa* L.c.v. PC. 19). Indian Journal of Forestry, 14 (1): 5457.

Kubo, I. and K. Nakanishi (1977). In "Host plant resistance to pests." Hedin. P. A. Ed., American Chemical Society: Washington, ACS Symp. Ser. No. 62, P.165.

Lowry, R. R. and I. J. Tinsley (1976). Rapid colorimetric determination of free fatty acids. Journal of American Oil Chemistry Society, 53: 470 -472.

Malterud, K. E.; T. E. Bremnes; A. Faegri; T. Moe and E. K. S. Dugstad (1985). Flavonoids from the wood of *Salix caprea* as inhibitors of wood-destroying fungi. J. Natural Products, 48 (4): 559563.

Nichols-Orians, C. M.; T. P. Clausen; R. S. Fritz; P. B. Riechardt and J. Wu (1992). 2'-Aphenolic glycoside from *Salix sericea*. Phytochemistry, 31 (6): 2180-2181.

Pezzuto, J. M.; C. T. Che; D. D. McPherson; J. P. Zhu; G. Topcu; C. A. J. Erdelmeier; G. Cordell (1991). DNA as an affinity probe useful in detection and isolation of biologically active natural products. J. Natural Products, 54 (6): 1522-1530.

Shimizu, M.; T. Matsuzawa; K. Hase; Y. Tsurumi; T. Seki; M. Morohashi; K. Torizuka; K. Terasawa; T. Honda and N. Morita (1993). Studies on bathing agent: I. Anti-inflamatory effect of bathing agent used for skin disease. Shoyakugaku Zasshi, 47 (1): 1-4

Sokolov S.Ya.; V. P. Boiko; V. A. Kurkin; G. G. Zapesochnaya; N. V. Rvantsova and N. Grinenko (1990). Comparative studies of the stimulating properties of some phenylpropanpids. Khimiko Farmasevticheskii Zhurnal, 24 (10): 66-68.

Thapliyal R. P. and R. P. Bahuguna (1993). Fatty acids and flavonoids of *Salix lindleyana*. International Journal of Pharmacognosy, 31 (2):165-166.

Tunon, H.; C. Olavsdotter and L. Bohlin (1995). Evaluation of anti-inflamatory activity of some swedish medicinal plants. Inhibition of prostaglandin biosynthesis and PAF-induced exocytosis. J. Ethnopharmacol, 48 (2): 61-76.

Received 21 / 6 / 2004
Accepted 4 / 8 / 2004

النشاط الإبادى الحشرى لمكونات الجزء الفعال فى لحاء الصفصاف

أحمد صبرى عبد العاطى
قسم كيمياء المبيدات – كلية الزراعة – جامعة الإسكندرية – مصر

تم استخلاص لحاء اشجار الصفصاف بعد تجفيفه هوائيا بواسطة الأسيتون المائي. تم تبخير الأسيتون كاملاو فصل المستخلص إلى أجزاء Fractions بتوزيع المستخلص الأساس بين مذيبات مختلفة إحدى هذه القطفات (Fraction II)أظهرت سمية نباتية على بادرات القمح و الكوسة و كذلك على حشرة سوسة الأرز و القوقع الأرضي *Theba pisana*

تم تقييمها (Fraction II)على العمر الرابع لكل من يرقات العوص و يرقات دودة ورق القطن ووجدانها تعدت فى تأثيرها المستخلص الاساس على يرقات البعوض حيث كان التركيز المميت لـ ٥٠% من العشيرة المعاملة هو ١٢٥، ٢٢٠، ٢٤٠، ٥٦٠، ١٤٥٠ جزء فى المليون مقارنة بـ ١٣٠، ٥٠٠، ١١٠٠، ١٣٠٠، ٢١٠٠ جزء فى المليون فى حالة المستخلص الاساس Un fractionted extract أيضا كانت Fraction II أكثر نشاطا مثبطة لتعذير اليرقات بتركيز مؤثر على ٥٠% من العشيرة المعاملة قدره ٢٧٠ جزء فى المليون مقارنة بـ ٩٦٠ جزء فى المليون فى حالة المستخلص الاساسى. بالرغم من ان Fraction II اظهرت تاثيرا سميتا ضعيفا على دودة ورق القطن الا انها أحدثت تاثيرا قويا كمانع للتغذية معتمدا على التركيز ووقت التعرض حيث حققت التركيز المؤثر على ٥٠%من القدرة على التغذية هو ١٦٠، ١٥٠٠ جزء فى المليون بعد ٢٤، ٤٨ ساعة على الترتيب .

تم التعرف على مكونات هذه الـ Fraction الفعالة بفصل و تقدير مكوناتها مستخدما جهاز التحليل الكروماتوجرافى – مطياف الكتلة GC-Ms analysis .

وجد ان هذه الـ Fraction تحتوى على احماض دهنية مشبعة مثل حمض البالميتيك Palmitic (C_{16} H_{32} O_2) وحمض الاستياريك Stearic (C_{18} H_{36} O_2) وكذلك حمض السيس اوليك اسيد cis-Oleic acid كحمض دهنى غير مشبع (C_{18} H_{34} O_2) اضافة الى مشتق للفلافونويد Flavonoid denivative وهو ميثايل ابيجينين methlyted apigenin derivative.