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INSECTICIDAL AND PHYTOCIDAL EFFECTS OF *SIMMONDSIA CHINENSIS* CONSTITUENTS

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ABSTRACT

Simmondsia chinensis seeds were extracted subsequently with different solvent systems. Seven resulted fractions were evaluated for their insecticidal activity against *Culex pipiens* 4th instar larvae and phytocidal activity on wheat. All fractions killed the treated larval population increasingly with increasing time of exposure and concentration. Fractions 1, 2, 6 and 7 caused LC_{50} equaled 463.6 and 296.4 µg/ml; 321 and 166.8 µg/ml; 403 and 292 µg/ml and 1100 and 927.7 µg/ml after 24 and 48 hours exposure, respectively. Fraction 5 was less effective. Fractions 3 and 4 were the most effective achieving LC_{50} values equaled 59.6 and 32.6 µg/ml comparing with 27 and 23.7µg/ml after 24 and 48 hours exposure, respectively. The phytocidal effect on wheat (*T. aestivum*) seeds was differed in systematic arrangement with increasing the concentration. Seed germination was inhibited by fractions 4, 5 and 7 slightly with EC_{50} values equaled > 2000 µg/ml. Fractions 1, 2, 3 and 6 moderately inhibited the seed germination with EC_{50} values equaled 347, 505, 541.6 and 723.2 µg/ml, respectively. The tested fractions harshly inhibited both root and shoot systems growth. Generally these fractions appeared to be more active on the shoot systems. Treatment of the pre-germinated seeds with these fractions reduced the growth inhibitions of root and shoot systems. The active fractions constituents were identified through GC-MS analysis. The major contained fatty acids were 11-ecosenic acid ($C_{20}H_{38}O_2$, 20.563%) and 5,13-docosadienoic acid ($C_{22}H_{40}O_2$, 13.620%) in fraction 3, however erucic acid ($C_{22}H_{42}O_2$, 40.279%) and arachidic acid ($C_{20}H_{40}O_2$, 24.517%) were the major contents of fraction 4. Some other fatty acids at 5.344–8.956% and 2.401–8.934% in fractions 3 and 4, respectively were differently identified proving the differences in their biological effects.

Key words: *Simmondsia chinensis*, Phytocidal, Insecticidal, Fatty acids, Identification

INTRODUCTION

Simmondsia chinensis (Link) Schneider**,** Buxaceae is a semiarid evergreen shrub in the desert south-western United States, north-western Mexico and some of the Middle East and Latin American countries (Borlaug *et al*., 1985; Bellirou *et al*., 2005). It tolerates saline, alkaline soils and minimal water requirements (Aly and Basarir, 2012). The mature plant produces 5-10 pounds of seeds for using its oil as a hair conditioner and restorer, in medicine, cooking and rituals by Indians and Mexicans. Comercially, It was used for lubrication, toiletry, cosmetic industries and pharmaceuticals (Cokelaere *et al*., 1992). Its defatted meal is a potential supplement for animal feeds and its oil is composed of esters that are composed primarily of C20 to C22 un-branched monoenic fatty acids and alcohols. It has been described as a liquid wax, which is slightly occlusive and has moisturizing properties. Jojoba seeds contain about 50–60% of unique wax ester oil composed mainly of straight chain monoesters in the range of C40–C44 (Ellinger *et al*., 1973). Significant differences among the oil, protein and carbohydrate contents in jojoba seeds were revealed. Anti-inflammatory activity has been exhibited by jojoba seeds extracts (Habashy *et al*., 2005). Kolodziejczyk *et al*. (2000) reported that its defatted seeds major constituents are proteins (31%) and carbohydrates (55%). Shrestha *et al*. (2002) added that its protein consists mainly of albumins (79%) and globulins (21%). This meal also contains approximately 15% of simmondsins (Van Boven *et al*., 2000) as simmondsin and seven of its derivatives have been identified form *S. chinensis* seeds (Bellirou *et al*., 2005) with insecticidal and anti-feeding activities against cotton leaf worm, *S. littoralis* Boisduval (Lepidoptera: Noctuidae) as well as their activity against plant pathogenic fungi of its seeds extracts (Abassy *et al*., 2007).

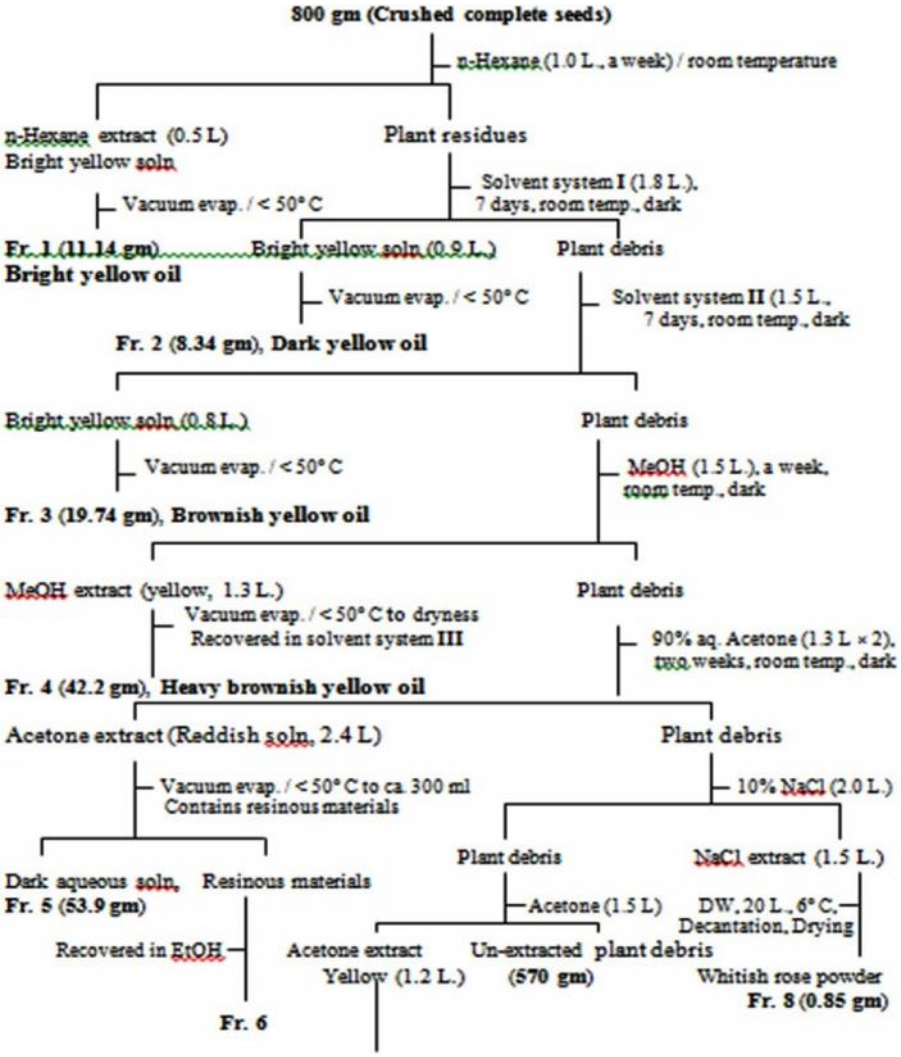
In this study, jojoba seeds were extracted subsequently with several solvent systems and the obtained seven fractions were screened for their insecticidal effect against the *Culex pipiens* 4th larval instar and phytocidal activity at different plant stages. The active fractions were analyzed for their constituent.

MATERIALS AND METHODS

Instruments and chemicals: GC Chromatographic measurement was carried out on HEWLETT 5890 PAKARD SERIES II Gas Chromatography, High Institute of Health, Alexandria University. El-Shatby, Alexandria, Egypt.

GC-MS Analysis was done on Thermo Scientific ISQ Trace GC Ultra coupled with HEWLETT 5989-B PAKARD mass spectrometer. This analysis was carried out in Institute of Research and Higher Studies, Alexandria University, Egypt.

Extraction of *Simmondsia chinensis* seeds: Based on Abdel-Aty and Abdel-Megeed (2015), complete seeds (800 gm) were crushed (pasted) and soaked in n-hexane (1.0 L) for a week at room temperature in the dark. The hexane bright yellow extract was concentrated under vacuum at < 50° C to bright yellow oil as fraction **1** (11.14 gm). The plant debris was successively soaked in solvent system **I** (petroleum ether (60–80): ethyl acetate at a volume ratio of 2:1) (1.8 L); solvent system **II** (petroleum ether (60 – 80): n-butanol at a volume ratio of 1:2) (1.5 L) and methanol (1.5 L) to give yellow oily solutions that were concentrated under vacuum at < 50° C giving a dark yellow oil (8.34 gm), a brownish yellow



Solvent systems I, Petr. Ether (60-80): EtOAc (2:1); **II,** n-BuOH**:** Petr. Ether (60-80) (2:1); **III,** n-BuOH**:** EtOH: EtOAc (1:1:1)

Fig. 1: Extraction and isolation of *Simmondsia chinensis* seeds constituents

oil (19.74 gm) and a heavy brownish yellow oil (42.2 gm) known respectively as fractions **2**, **3** and **4.** The remained plant tissues after methanol extraction were soaked twice in 90% aqueous acetone (1.3 L) for a week at room temperature to give a reddish solution, which was concentrated to ca. 300 ml of a heavy brown solution containing some resinous materials. After removal of the resinous materials, the aqueous solution (53.9 gm) was known as fraction **5**. The resulted plant debris was re-soaked at room temperature in the dark for a week successively in sodium chloride (10%, 1.5 L) and acetone (1.5 L). The sodium chloride extract was diluted with 20 L of distilled water and the diluted solution was decanted at 6°C. A whitish precipitate was obtained and dried over phosphorus pentoxide (P_2O_5) to fraction **7** as a whitish rose powder (0.852 gm). The acetone extract (1.2 L) was divided into an aqueous solution, which was concentrated to fraction **8** (2.14 gm) and some resinous materials combined with that was produced by 90% aqueous acetone extraction and recovered in ethyl acetate as fraction **6** (2.5 gm). Fractions **1**, **2**, **3**, **4**, **5**, **6**, **7** and **8** weighed 11.14, 8.34, 19.74, 42.2, 53.9, 2.5, 2.14 and 0.852 gm (oven dried extract) with 1.39, 1.04, 2.46, 5.27, 6.74, 0.31, 0.27 and 0.11 % of the un-extracted sample. The extraction process is illustrated in Figure (**1).**

Insecticidal activity: The WHO standard test method for mosquito larvae (WHO, 1963) was used to test the insecticidal activities of the obtained *S. chinensis* fractions against the early fourth larvae of mosquito (*Culex pipiens* L.) at concentrations of 0, 50, 100, 200, 500, 1000 and 2000 µg/ml. Control was concurrently conducted under the same conditions. The results were recorded as mortality percents after 24 and 48 hours exposure at each concentration of each tested fraction comparing with the un-treated population. LC_{50} (Lethal concentration caused 50% mortality) were calculated after 24 and 48 hours.

Phytocidal activity:

Effect on wheat (*Triticum aestivum*) seeds: The isolated jojoba oily fractions were checked for their phytotoxicity on wheat seeds using the cotton plug technique (Grodzinsky and Grodzinsky, 1973) at 100, 200, 500, 1000 and 2000 µg/ml. They were dissolved in dimethylsulfoxide (DMSO) at a concentration as high as 1% of the tested solution volume. Thirty seeds were used in each replicate. Three replicates were considered as a treatment. Control was concurrently conducted. After 12 days, number of germinated seeds was counted and the height of both root and shoot system's growth of the grown seedlings were measured. Results were compared with untreated seeds.

Effect on pre-germinated seeds: The separated fractions were re-examined for their phytocidal effects on root and shoot systems of wheat (*Triticum aestivum*) seedlings. The tested concentrations were 0, 100, 200, 500, 1000 and 2000 µg/ml. The procedure was achieved by using 1.5% plain agar (Zemanek, 1963) containing the required concentration of the tested fractions in test tubes, The tested fractions were dissolved in DMSO and control was done at the same conditions. The test tubes were watered with a constant volume until roots reached the bottom of the tube. The length of both root and shoot systems were measured and the growth inhibition percentages were calculated and the obtained effective concentration on 50% of the treated seedlings (EC_{50}) values were determined.

Chromatographic identification: Samples were prepared by sulfuric acid methanol reagent. The active oily fractions (Fractions **3** and **4**) were individually esterified for their fatty acid identification through methanolysis based on Ernesto *et al*. (2008). Fraction 3 (1.0 ml) and Fraction 4 (0.8 ml) were individually dissolved in 5 ml of benzene in a screw sealed glass tube. A methanolic solution (1%) of sulfuric acid (7 ml) was added to each tube and the mixture was heated at 90°C and the esterified fatty acid derivatives sample was evaporated to dryness at 45°C. Then, 1.0 ml of n-hexane was added and the vial was heated at 80°C for 3 min.

GC Chromatographic measurement was carried out on HEWLETT 5890 PAKARD SERIES II Gas Chromatography using column HP-5. Inlet temperature was 260 °C and FID detector was used. The used temperature program started at 80 °C for a minute and elevated to 235°C with a range of 10°C/ minute. Sample concentration was 50 mg/ml and 1.0 µl was injected. The run was continued for 25 minutes.

GC-MS Analysis of the active fractions: GC-MS Analysis was done on Thermo Scientific ISQ Trace GC Ultra coupled with HEWLETT 5989-B PAKARD mass spectrometer. The used column was TG-5 MS (30 m × 0.32 mm × 0.25 µm) and the inlet temperature was 260 °C. The sample (0.5 µg) was charged in 1 µl of dichloromethane (DCM) with splitless. The carrier gas was Helium (He) and the analysis temperature program started at 45 °C for 2 minutes and increased with 15 °C/ min rate to 165 °C, which was fixed for 45 minutes. Quadrable (220 °C) as an ion source and MS detector (its temperature is 300 °C) were used. Sample was bombarded with (70 e.v) electron volt and the mass range was 40 - 500.

Statistical analysis: Inhibition percentages in germination and seedling heights were recorded. LC_{50} and EC_{50} values were calculated using Costat software (1986) according to probit analysis (Finny, 1965).

RESULTS AND DISCUSSION

Insecticidal activity: The insecticidal activity of the separated *S. chinensis* fractions against the *C. pipiens* 4th instar larvae differed according to the tested fraction, concentration and exposure time. Fraction **1**, isolated with normal hexane caused mortality percents ranged from 6.7 to 100% and from 2.6 to 100% at the concentration range of 100-2000 µg/ml and 50-1000 µg/ml after 24 and 48 hours exposure, respectively with lethal concentration of 50% of the treated population equaled 463.6 and 296.4 µg/ml, respectively. While fraction **6** (isolated from the acetone originated fraction) was near to fraction 1 in its mortal effect with LC_{50} values of 403 and 292 µg/ml after 24 and 48 hours exposure, respectively without significant differences between each other, fraction **2** (isolated from the solvent system I) significantly exceeded the previously mentioned two fractions (**1** & **6**) exhibiting mortality percents increasingly with increasing the tested concentration to complete lethality of the treated population at 2000 µg/ml achieving 321 and 166.8 µg/ml LC_{50} values after 24 and 48 hours, respectively. Although, there was no significance between fraction **3** (isolated from the solvent system **II**) and fraction **4** (isolated from methanol) in their effects against the treated insect, they were the most effective fractions exhibiting 59.6 and 32.6 µg/ml LC_{50} values after 24 hours exposure comparing with 27 and 23.7 µg/ml after 48 hours exposure, respectively. Fraction **7** (isolated from acetone) was lesser in its effect causing LC_{50} values of 1100 and 927.7 µg/ml, respectively after 24 and 48 hours exposure, respectively. Fraction **5** (originated from the aqueous acetone extract) was the weakest fraction in its effect as it needs to increase its concentration to kill 50% of the treated population.

From the illustrated data, all fractions killed the treated population increasingly with increasing time of exposure and concentration (Table **1**). The active fractions should be followed for discovering their insecticidally active constituents comparing with El-Hag *et al*. (1999) who considered *Rhazya stricta* methanol and other extracts as larvicidal against *Culex pipiens* larvae with 251 and 467µg/ml LC_{50} values. On the other hand, Ahmed and El-Hamshary (2005) proved the ethanol extract of *Iris psuedacorus* leaves as larvicide when caused LC_{50} values of 10.36 and 7.36 µg/ml after 24 and 48 hours exposure against *C. pipens* 2nd larval instar. These data indicated that our separated fractions could be considered as active insecticidal constituents on the treated insect.

Phytocidal activity: The isolated fractions inhibited the wheat (*T. aestivum*) seeds in a function of the tested fraction and the applied concentration. All the tested fractions caused their inhibition in systematic arrangement with increasing the tested concentration. Treatments of wheat seeds with the isolated fractions gave different results from pre-germinated seeds. Seed germination was slightly inhibited by fractions **4**, **5** and **7** with EC_{50} values equaled > 2000 µg/ml. Fractions **1**, **2**, **3** and **6** moderately inhibited the seed germination with EC_{50} values equaled 347, 505, 541.6 and 723.2 µg/ml, respectively. All fractions were more effective inhibiting the growth of both root and shoot systems than seed germination. Fractions **1**, **2**, **3**, **4**, **5**, **6** and **7** harshly inhibited the root system growth with EC_{50} values equaled 28.5, 28.8, 21.6, 21.0, 250, 7.5 and 200.3 µg/ml, while they achieved EC_{50} values equaled 10.8, 29.7, 6.8, 20.3, 175.5, 12.5 and 87.1 µg/ml in the same array (Table **2**). Generally these fractions appeared more active on shoot systems except fractions **2** and **6**, there was no significant difference between their effects on root and shoot systems.

On the other hand, treatment of the pre-germinated seeds with these fractions reduced the growth inhibitions of root and shoot systems. This effect may be due to short exposure time than in case of seed treatment. The tested fractions showed their inhibition in systematic arrangement with increasing the tested concentration reaching complete prevention of the treated seedling to growth at 2000 µg/ml in case of fractions **1**, **3** and **4**. However fraction **7** needs higher concentrations to reach 50% inhibition (> 2000 µg/ml EC_{50} value), fractions **1**, **2**, **5** and **6** were moderately effective with EC_{50} values equaled 450, 684, 511 and 1176 µg/ml against the root system growth and 247, 342, 355 and 792 µg/ml, respectively on the shoot system growth. Both fractions 3 and 4 were highly toxic without significant difference between them. They exhibited EC_{50} values equaled 162 and 184 µg/ml on root system and 172 and 216 µg/ml against shoot system growth, respectively (Table **3**).

3- GC- Analysis: Gas chromatographic analysis of the two active fractions illustrated their fatty acid contents. Fraction **3** differed from fraction **4** in their fatty acid contents, which may be the main reason of their different biological effects. As shown in Table (**4**), tetracosanic acid ($C_{24}H_{48}O_2$), behenic acid ($C_{23}H_{46}O_2$), docosahexenoic acid ($C_{22}H_{40}O_2$), ecosapentadecenoic acid ($C_{20}H_{30}O_2$), hexadecanoic acid ($C_{16}H_{32}O_2$) and dodecanoic acid ($C_{12}H_{24}O_2$) were found at 0.524, 2.491, 6.633, 3.259, 1.496 and 0.038%, respectively in fraction **3**, while they were not recognized in fraction **4**. On the other hand, decanoic acid ($C_{10}H_{20}O_2$) was only recognized in fraction **4** at 3.291%. Some other fatty acids were found in both fractions at < 5% as octanoic acid, octadecadienoic acid, linoleic acid, oleic acid and arachidonic acid at 0.012, 3.209, 1.404 and 3.857% in fraction **3** comparing with 1.369, 0.647, 1.563 and 0.981% in fraction **4**. These fatty acids perhaps appeared

Table (1). Insecticidal activity of *S. chinensis* fractions against *C. pipiens* 4th larval instar; shown as mortality% and LC_{50} values

Fraction	Time (hr)	Mortality % at different concentrations (µg/ml)							LC_{50} 95% CL (µg/ml)	Slope ± SE	χ^2	DF
		0	50	100	200	500	1000	2000				
1	24	0	0	6.7 ± 2.1	15.3 ± 2.9	48.1 ± 3.9	78.7 ± 4.6	100	463.6 412– 522	2.74 ± 0.03	10	4
	48	0	2.6 ± 1.3	13.8 ± 4.2	40.9 ± 4.2	49.7 ± 8.4	100	100	296.4 262– 335	2.66 ± 0.04	18	3
2	24	0	0	3.76 ± 0.1	37.6 ± 8.0	55.3 ± 4.8	90.2 ± 10	100	321.0 284– 363	2.49 ± 0.03	16	4
	48	0	8.3 ± 1.7	37.4 ± 4.8	64.9 ± 4.3	79.6 ± 3.9	90.9 ± 8.7	100	166.8 144– 193	2.04 ± 0.03	12	4
3	24	0	47.5 ± 2.1	61.9 ± 1.6	84.6 ± 11.4	94.3 ± 5.2	100	100	59.6 47.4–75	1.9 ± 0.04	2.9	3
	48	0	70.8 ± 8.4	81.4 ± 8.7	92.3 ± 6.8	100	100	100	27.0 17 – 42.7	1.76 ± 0.09	2.6	2
4	24	0	58.7 ± 3.7	76.0 ± 9.0	82.2 ± 6.9	90.5 ± 1.4	96.7 ± 5.8	100	32.6 20 – 50.4	1.24 ± 0.02	3	4
	48	0	75.2 ± 7.1	83.3 ± 1.4	95.3 ± 5.0	100	100	100	23.7 14.2– 40	1.82 ± 0.10	2.6	2
5	24	0	0	0	0	6.4 ± 2.4	13.3 ± 1.5	24.2 ± 4.8	> 2000			
	48	0	0	0	0	20.0 ± 5.0	12.42 ± 4.2	40.9 ± 3.4	> 2000			
6	24	0	9.33 ± 1.1	15.3 ± 0.2	43.3 ± 2.9	58.3 ± 7.6	68.3 ± 2.9	80.4 ± 4.0	403.0 332–490	1.32 ± 0.01	2.1	4
	48	0	9.3 ± 1.1	15.3 ± 0.2	58.3 ± 2.9	73.3 ± 5.7	77.8 ± 6.9	91.3 ± 1.2	292.0 249–343	1.68 ± 0.01	7.2	4
7	24	0	0	0	13.3 ± 2.9	33.2 ± 3.3	39.1 ± 6.9	69.3 ± 3.0	1100.0 910–1333	1.77 ±0.027	9.5	3
	48	0	0	0	23.3 ± 2.9	39.1 ± 6.9	41.1 ± 1.0	73.1 ± 6.7	927.7 762–1130	1.6 ± 0.02	19	3

Table (2). Phytocidal effects of *S. chinensis* isolated fractions on *T. aestivum* seeds.

Fraction	Effect on	Effect at different concentrations (µg/ml)						EC_{50} 95% CL (µg/ml)	Slope ± SE	χ^2	DF
		0	100	200	500	1000	2000				
1	% Ger.	100	80.3 ± 5.8	70.0 ± 5.0	47.8 ± 7.6	21.7 ± 2.9	0	347.0 298 – 402	2.0 ± 0.024	15	3
	Root (cm)	11.40 ± 3.1	2.53 ± 0.51	1.33 ± 0.15	1.03 ± 0.12	0	0	28.5 13 – 61.4	1.3 ± 0.05	14	3
	Shoot (cm)	13.20 ±3.36	1.50 ± 0.81	1.10 ± 0.21	0.80 ± 0.1	0	0	10.8 2.5 – 44.4	1.1 ± 0.07	6	3
2	% Ger.	100	86.4 ± 2.9	70.3 ± 5.8	54.6 ± 2.9	40.7 ± 5.8	10 ± 2.9	505.0 425 - 601	1.6 ± 0.02	8	3
	Root (cm)	11.4 ± 3.1	2.70 ± 0.65	1.50 ± 0.85	0.77 ± 0.15	0.27 ± 0.12	0	28.8 13.5 – 61	1.34 ± 0.05	1.1	3
	Shoot (cm)	13.2 ±3.36	2.40 ± 0.31	1.90 ± 0.10	0.60 ± 0.15	0.13 ± 0.11	0	29.7 14.3 – 61	1.4 ± 0.06	2	3
3	% Ger.	100	93.3 ± 5.8	76.7 ± 10.4	43.3 ± 17.3	31 ± 5.0	20.7 ± 2.9	541.6 450 – 652	1.5 ± 0.02	9.4	3

	Root (cm)	11.4 ± 3.1	2.87 ± 0.15	2.57 ± 0.25	1.47 ± 0.29	1.30 ± 0.23	0.20 ±0.07	21.6 7.4 – 61.4	0.87 ±0.03	4.6	3
	Shoot (cm)	13.2 ±3.36	2.00 ± 0.5	1.70 ± 0.40	1.50 ± 0.15	0.73 ± 0.31	0	6.8 1- 43	0.74± 0.03	4.9	3
	% Ger.	100	90.0 ± 10	73.3 ±10.4	70.0 ± 5.0	66.7 ± 2.9	56.7 ± 5.8	> 2000			
4	Root (cm)	11.4 ± 3.1	3.45 ± 0.76	2.56 ± 0.40	2.34 ± 0.23	1.30 ± 0.17	0.67 ±0.12	21.0 6.3 – 67.9	0.74± 0.02	2.3	3
	Shoot (cm)	13.2 ±3.36	3.23 ± 0.12	2.70 ± 0.44	2.00 ± 0.51	0.90 ± 0.23	0.62 ±0.18	20.3 6.4 – 63.1	0.8 ±	1.7	3
	% Ger.	100	90.0 ± 10	86.7 ± 5.8	86.7 ± 15.3	73.3 ± 5.8	56.7 ± 5.8	> 2000			
5	Root (cm)	11.4 ± 3.1	8.60 ± 1.14	7.10 ± 1.5	3.90 ± 0.1	2.30 ± 0.2	0.17 ±0.12	250.0 210 - 299	1.75 ± 0.023	6.3	
	Shoot (cm)	13.2 ±3.36	12.00 ± 0.56	6.13 ± 1.9	3.70 ± 0.25	1.31 ± 0.12	0.27 ± .06	175.5 141 - 218	1.6 ± 0.02	6.2	3
	% Ger.	100	90.0 ± 10	76.7 ± 5.8	60.7 ± 2.9	50.0 ± 8.7	20.0 ± 10	723.2 594 - 880	1.48 ± 0.02	5	3
6	Root (cm)	11.4 ± 3.1	1.70 ± 0.25	1.00 ± 0.05	0.80 ± 0.4	0.40 ± 0.1	0	7.5 1.3 - 41	0.91 ± 0.05	2.5	3
	Shoot (cm)	13.2 ±3.36	2.40 ± 0.06	1.30 ± 0.15	1.00 ± 0.1	0.70 ± 0.06	0	12.5 3.3 - 46	0.96 ± 0.04	3.9	3
	% Ger.	100	100	96.7 ± 5.8	93.3 ± 5.8	86.7 ± 5.8	80.0	> 2000			
7	Root (cm)	11.4 ± 3.1	6.30 ± 0.82	5.80 ± 0.89	4.80 ± 1.23	4.30 ± 0.95	3.93 ± 1.5	200.3 93.2 – 424	0.42 ± 0.015	0.2	3
	Shoot (cm)	13.2 ±3.36	6.50 ± 0.83	5.90 ± 1.04	5.00 ± 1.59	4.60 ± 1.48	4.00 ± 1.8	87.1 24.9 - 293	0.4 ± 0.02	4	3

Table (3): Inhibitory effects of *S. chinensis* fractions on *T. aestivum* seedling root and shoot systems growth

Fraction	Effect on	Inhibition % at different concentrations (µg/ml)							EC_{50} 95% CL (µg/ml)	Slope ± SE	χ^2	DF
		0	50	100	200	500	1000	2000				
1	Root	0	6.3 ± 1.6	8.3 ± 1.4	24.6 ± 1.9	42.1 ± 3.6	70.8 ± 7.2	100	450 391– 518	2.04 ± 0.02	22	4
	Shoot	0	22.2 ± 4.8	27.8 ± 4.9	30.6 ± 3.4	63.5 ± 4.8	80.5 ± 2.7	100	247 208– 292	1.6 ± 0.01	23	4
2	Root	0	0	9.2 ± 2.9	15.4 ± 0.8	42 ± 0.3	61 ± 2.9	80.2 ± 2.8	684 583– 802	1.8 ± 0.02	3.6	4
	Shoot	0	11.1 ± 4.8	23.6 ± 2.4	41.7 ± 1.7	54.2 ± 4.2	70.2 ± 2.4	90 ± 2.5	342 285 – 411	1.4 ± 0.01	4.7	4
3	Root	0	10 ± 1.5	30 ± 5.5	71.1 ± 8.4	83.3 ± 2.9	91.7 ± 4.7	100	162 142 – 188	2.13 ± 0.02	15	4
	Shoot	0	13.9 ± 4.8	31.9 ± 2.4	63.9 ± 4.8	77.8 ± 6.9	86.1 ± 4.8	100	172 147 – 202	1.8 ± 0.02	11	4
4	Root	0	28 ± 3.9	40.8 ± 3.8	48.4 ± 4.0	62.5 ± 2.5	73.3 ± 2.9	100	184 149 – 230	1.21 ± 0.01	20	4
	Shoot	0	27.8 ± 4.8	41.7 ± 8.3	48.6 ± 2.4	55.5 ± 4.8	68.1 ± 1.3	100	216 167 – 282	0.95 ± 0.01	8.7	4
5	Root	0	0	16.7 ± 2.9	37.5 ± 2.5	45.8 ± 7.2	62.5 ± 2.5	83.3 ± 7.6	511 428 – 610	1.53 ± 0.01	16	4
	Shoot	0	6.9 ± 2.4	25 ± 1.4	45.8 ± 4.3	53.3 ± 5.8	63.9 ± 4.8	91.7 ± 0.9	355 296 – 426	1.42 ± 0.01	16	4
6	Root	0	0	4.2	16.7	37.5	43.8	58.4	1176	1.45	8.3	4

				± 1.4	± 3.8	± 2.5	± 6.3	± 9.5	930 –1490	± 0.02		
	Shoot	0	2.8	8.3	16.7	26.4	63.9	75	792	1.66	7.4	4
			± 2.5	± 0.4	± 4.2	± 6.4	± 7.9	± 5.0	660 – 949	± 0.02		
	Root	0	0	0	6.2	11.7	25.7	33.3	> 2000			
7					± 1.2	± 1.4	± 1.3	± 7.2				
	Shoot	0	2.8	5.5	11.7	16.4	22.3	41.7	> 2000			
			± 0.7	± 1.3	± 3.5	± 2.9	± 5.5	±3.4				

as metastases. The major contained fatty acids were 11-ecosenic acid ($C_{20}H_{38}O_2$, 20.563%) and 5,13-docosadienoic acid ($C_{22}H_{40}O_2$, 13.620%) in fraction **3**, however erucic acid ($C_{22}H_{42}O_2$, 40.279%) and arachidic acid (($C_{20}H_{40}O_2$, 24.517%) were the major content of fraction **4.** Other fatty acids were varied from 5.344 – 8.956% and from 2.401 – 8.934 in fractions **3** and **4**, respectively.

The obtained data go with (Abbassy *et al*., 2007; Miwa, 1984; Yaron *et al*., 1982) as they revealed that jojoba waxes are rich in a mixture of esters of long chain fatty alcohols and long chain fatty acids (C_{20} and C_{22}) with two double bonds as cis-11-eicosenoic acid (66.35%) followed by cis-9-octadecenoic (16.99%) and cis-13-docosenoic (14.24%) acids. The other detected fatty acids, cis-9-hexadecenoic, octadecanoic (18:0), eicosanoic (20:0) and docosanoic (22:0) acids were found in trace amounts (0.1%). These minor fatty acids may be metastases. The alcohols have been identified as mixtures of cis-11-eicosenol, cis-13-docosenol and cis-15-tetracosenol (C-24). The obtained major fatty acids percent (in the isolated fractions only) were less than the reviewed values (calculated in the whole johoba wax) because of extraction of some of them in fractions **1**and **2**.

On the other hand, the insecticidal effect of the active jojoba oily fractions may be due to decreasing daily O_2 consumption and CO_2 release (Bream *et al*., 2001). This suggests jojoba oil may have been used as a general insecticide. Regarding the phytocidal effects of jojoba wax that may be due to their fatty acids contents as the majority of the green oils, soaps (e.g., pelargonic acid), or acetic acid control weeds by destroying the leaf cuticle or causing cell leakage that rapidly leads to death. Also, fatty acids enhance the herbicides absorption as Brand *et al*. (2005) found that Trifluralin's absorption was enhanced in the presence of oleic acid whereas the greatest absorption of atrazine and alachlor occurred with palmitic acid and the control media, respectively.

Regarding the environmental impacts, it is known that the LD_{50} of crude jojoba wax is greater than 160 g/kg in mice. Jojoba oil is widely used in cosmetics, such as moisturisers, sunscreens, shampoos and conditioners as well as number of skin and scalp disorders. Other early-mentioned medical uses, such as curing the suppression of the urine, helping in weight loss, improvement of liver functions, elevating body immunity, remedy for cancer and promotion of growth of hair are also reported (Yaron, 1987). Few flavonoids include quercetin 3,3'-dimethyl ether, iso-kaempferide and quercetin-3-methylether were isolated and evaluated for their hepatoprotective and antioxidant activity from Jojoba pericarp. Jojoba seeds have also been used in cleaning of aquatic system mainly for the removal of access ferric ions (Al-Anber *et al*., 2014). International demand for jojoba oil is increasing continuously.

Table (4): GC-MS analysis of fractions 3 and 4

No	t_R (min.)	Identified fatty acid	Molecular Weight	% Area	
				Fr. 3	Fr. 4
1	2.76	Caprylic acid (Octanoic acid) (C8:0) $C_8H_{16}O_2$	144.211	0.012	1.369
2	3.18	Capric (Caprinic) acid (Decanoic acid) (C10:0) $C_{10}H_{20}O_2$	172.265	---	3.291
3	5.18	Lauric acid (Dodecanoic acid) (C12:0) $C_{12}H_{24}O_2$	200.318	0.038	---
4	9.67	Palmitic acid (Hexadecanoic acid) (C16:0) $C_{16}H_{32}O_2$	256.424	1.496	---
5	11.55	Linoleic acid (9,12-Octadecadienoic acid) (C18:2 ω 6) $C_{18}H_{32}O_2$	280.445	3.209	0.647
6	11.71	Oleic acid (9-Octadecenoic acid) (C18:1 ω 9) $C_{18}H_{34}O_2$	282.461	1.404	1.563
7	11.81	Stearic acid (Octadecanoic acid) (C18: 0) $C_{18}H_{36}O_2$	284.477	5.344	2.610
8	12.63	Unknown		---	1.723

9	13.85	Cis 5,8,11,14,17-Ecosapentadecenoic Acid (C20: 5 ω 3) $C_{20}H_{30}O_2$	302.451	3.259	---
10	13.96	Arachidonic acid (Ecosatetradecenoic acid) (C20: 4 ω 6) $C_{20}H_{32}O_2$	304.467	3.857	0.981
11	14.25	Cis,cis,cis (11,14,17)-Ecosatrienoic acid) (C20: 3 ω 3) $C_{20}H_{34}O_2$	306.483	8.300	4.554
12	14.32	8,11,14-(Ecosatrienoic acid) (C20: 3 ω 6) $C_{20}H_{34}O_2$	306.483	8.956	3.706
13	14.39	(Eicosadienoic acid) (C20: 2 ω 6) $C_{20}H_{36}O_2$	308.499	7.584	2.401
14	14.43	5-Eicosenic acid (11-Ecosenic acid) (C20: 1ω 9) $C_{20}H_{38}O_2$	310.514	20.563	8.934
15	14.94	Arachidic acid (Ecosanoic acid) (C20: 0) $C_{20}H_{40}O_2$	312.530	6.685	24.517
16	16.60	Docosahexaenoic acid (C22: 6 ω 3) $C_{22}H_{32}O_2$	328.488	6.633	---
17	17.01	5,13-Docosadienoic acid (C22: 2) $C_{22}H_{40}O_2$	336.552	13.620	3.425
18	17.72	Erucic acid (Cis-13-Docosenoic acid) (C22: 1) $C_{22}H_{42}O_2$	338.568	6.028	40.279
19	20.34	Docosanic acid (Behenic acid) (C23: 0) $C_{23}H_{46}O_2$	354.610	2.491	---
20	21.44	Legoceric acid (Tetracosanic acid) (C24: 0) $C_{24}H_{48}O_2$	368.637	0.524	---
Total				100.00	100.00

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