PHYSICO- AND PHYTOCHEMICAL ANALYSIES OF SOME *CITRUS* PEEL EXTRACTS, AND THEIR ACTIVITY AGAINST THE COTTON LEAFWORM, *SPODOPTERA LITTORALIS* (BOISD.)

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Abstract

Peels of fresh navel orange (*citrus sinensis*) and lemon (*citrus limon*) fruits (Rutaceae) were extracted by absolute methanol (MeOH) and Hexane (Hex) as solvents differing in their polarities. The purpose was to detect some chemical and physicochemical characters of their crude extracts, and evaluate their bioactivity against 4^{th} larval instar of the cotton leafworm, *Spodoptera littoralis* using different methods of treatments. MeOH proved to be significantly ($P<0.01$) more efficient than Hex to extract phytochemicals (proteins, carbohydrates, phenols, flavonoids and alkaloids) from lemon and orange. In addition, total antioxidant capacity was 26.4 and 3.85µM/gm for orange peel extracted by MeOH and Hex, respectively. It seems that extraction ability could affects physicochemical characteristics, since % yield and specific gravity of MeOH extracts, either from lemon or orange, were significantly higher than Hex extracts. pH was <2.85 and >4 for Hex and MeOH extracts, respectively. Toxicity of citrus peel crude extracts against *S. littoralis* larvae showed that treatment by contact method was more efficient than ingestion, and Hex extracts were more toxic than MeOH extracts in both treatments. Citrus peel extracts showed relatively strong contact action, moderate repellency, weak ingestion and antifeedant activity against the 4^{th} instar larvae. It is suggested that Hex could extracts toxic substances more than MeOH, out of the studied phytochemicals. Citrus peel waste is promising as a natural biopesticide, and its activity is determined by the solvent used for extraction, citrus species and method of treatment.

Key words: *Citrus*, peel waste, phytochemicals, physicochemicals properties, biopesticides, Lepidoptera, *Spodoptera littoralis*.

INTRODUCTION

Citrus are known as one of the major fruit crops in the world, and the genus *Citrus* of family Rutaceae includes different species such as *Citrus sinensis* (orange), *Citrus limon* (lemon), *Citrus paradise* (grapefruit), *Citrus aurantium* (sour orange) and *Citrus aurantifolia* (lime). Citrus peel waste though is seasonal, yet, is a big problem to the processing industries and pollution monitoring agencies (Kumar *et al.*, 2012). However, citrus peels are a potential source of valuable essential oils and plant secondary metabolites. So there is an increased attention in extracting valuable products from citrus waste materials. Essential oils of citrus peel were used as food preservatives, phytomedicine and antioxidant agents (Javed *et al.*, 2014).

The properties of the extract (either crude or oil) determine its bioactivity. Barkatullah *et al.* (2012) emphasized that study of various physicochemical characteristics explores the practical importance of herbal oils in daily life. Lario *et al.* (2004) determined some physicochemical properties of the raw residues from lemon juice, all of which depend on the chemical composition of the residue. However research has been limited mainly to oils characteristics (Javed *et al.*, 2014), effect of physical or physicochemical conditions during processing on peel properties (Pandharipande and Makade, 2012) or physicochemical characterization of the orange juice waste water of a citrus by-product (Viuda-Martos,2011).

The adverse effects of chemical control have been motivating the demand for alternatives in pest control, such as the use of natural products (Dutra *et al.*, 2016). Crude extracts or, in most cases, essential oils of citrus fruit peels were tested against different species of insect pests such as mosquitoes (Escartin and Mariani, 2014), Lepidoptera such as *spodoptera litura* (Loh *et al.*,2011), hymenoptera(Vogt *et al.*,2002), Coleoptera (Dutra *et al.*,2016).

Few reports about effect of citrus peel crude extract on lepidoptera including the cotton leafworm *S. littoralis* were recorded. It is known that crude extracts are more stable than oils and could be kept for longer time. Secondly oils are monoterpenes to irritate human skins, and finally crude extracts are less expensive to produce than oils (Oshagi *et al.*, 2003).

Generally all parts of *citrus* plants were tested for their bioactivity such as leaves (Warikoo *et al.*, 2012). It was found that the activity of extract of three fruit tissues (flavedo[peel], albedo and flesh) of *C. aurantium* against adults of the olive fruit fly was limited to the peel, and this activity was significantly higher than that of the whole fruit extract (Siskos *et al.*, 2007).

In the current study peel of fesh navel orange (*C. sinensis*) and lemon (*C. limon*) fruits were extracted using hexane and methanol as solvents differing in their polarity to address three objectives (1) Measurements of some physicochemical properties such as % yield, pH and specific gravity. proteins, phenols, carbohydrates, antioxidant capacity, alkaloids and flavonoids contents were measured as phytochemicals characteristics of the extracts. (2) Detecting the most efficient solvent in extracting the active biochemical components (3) Toxicity of lemon and orange crude peel extracts against the cotton leafworm *S. littoralis* larvae. This study reports the activity against one of lepidopteran pests, and the fundamental scientific basis for the use of citrus peels waste as natural bioactive product to combat harmful insects.

MATERIALS AND METHODS

Plant material :

Navel orange and lemon fruits were fresh and of eating quality, and purchased from local market during citrus season (October - January). They were brought to the laboratory, and the fruits were cleaned thoroughly by washing with tap water in order to clean dust or any particles. Fruits were inspected carefully to find any kind of diseases or pest infestation, and the infested ones were discarded. Fruits were kept in a refrigerator (2-8°C) for few days till extraction.

Extraction :

About 10 kg of navel orange or 5 kg of lemon fruits were dissected to get peels. The peels were weighed and cut into small pieces. Each 1 kg of peels was homogenized with 1 liter of absolute methanol (MeOH) or Hexane (Hex) from Analar chemicals co., for 5 min in electric blinder. The resultant homogenate was macerated for 72 hr, at room temperature, in a double amount of the used solvent to ensure efficient extraction. The homogenate was filtered using filter paper (Whatman No. 1). The filtrates were concentrated to dryness under vacuum pressure using rotary evaporator (Labconco, Germany) at 35°C. The resultant plant residue was considered as crude extract. It weighed, collected in a glass stoppered tubes and stored at -10°C in a deep freezer till use.

- **Physicochemical analysis** :

The physicochemical properties of lemon and orange crude extracts that determined were %yield, pH and specific gravity.

1- %yield :

The percentage of crude extract yield was calculated using the following equation (AOAC, 2000).

Percentage yield (W/W) = $\frac{\text{weight of crude extract}}{total\ weight\ of\ material\ used\ for\ extraction} \times 100$

2- **Specific gravity determination**:

The specific gravity was performed at room temperature by comparing the weight of a given extract volume to the weight of water of the same volume using the following formula (AOAC, 2000):

Specific gravity of test sample = $\frac{W2-W0}{W1-W0}$

Where :

W_0 = weight of empty bottle

W_1 = weight of water + bottle

W_2 = weight of sample + bottle

3- **pH determination** :

pH of the different crude extracts was measured with pH meter DM-20(Digmid). The electrode was put into 5 ml of the extract without dilution for 2 min before recording readings. pH meter was calibrated using the appropriate buffer before analysis.

- **Phytochemical analysis :**

Various quantitative studies were carried out on hexane and methanol of lemon and orange crude peel extracts (50%, W/V) to determine proteins, carbohydrates, phenols, flavonoids and alkaloids. The produced colors of reactions were read using U/V ultraviolet/visible spectrophotometer (Spectronic 1201, USA).

1- Protein assay :

Total proteins were determined by the method of Bradford (1976) using Coomassie Brilliant blue G-250 (Sigma, Germany) as the dye. Five milliliters of protein reagent were added to 100 and 20 µl of Hex or MeOH extracts, respectively and mixed thoroughly. The absorbance at 595 nm was measured after 2 min and before 1 h against blank prepared from 50 µl of phosphate buffer (0.1 M, pH 6.6) and protein reagent. Bovine serum albumin (Stanbio laboratory, Texas, USA) was used as the standard.

2- Determination of total carbohydrates :

Total carbohydrates were quantified in peel crude extracts by the phenol-sulphuric acid reaction of Dubois *et al.* (1956). To 100 and 1 µl of Hex and MeOH extracts, respectively, 0.5 ml of phenol (20%,W/V) (RhÔne. Poulenc, France) was added. Then 5 ml of conc. H_2SO_4 were added rapidly with shaking. The tubes were allowed to stand 20 min, then the absorbance of characteristic yellow orange color was measured at 490 nm against blank, using glucose (Aldrich chemical Co.) as the standard.

- **Quantification of total phenols :**

The amount of total phenols in peel crude extracts was determined by Folin-Ciocalteu (FC) method as modified by singleton and Rossi (1965). Gallic acid (GA, 5%) from Sigma was used to construct a calibration curve. Ten or 1 µl of Hex or MeOH extracts were placed in test tubes. Then 0.5 ml of FC reagent were added and waited for 4 min. one milliliter of Na_2CO_3 (7.5%, W/V) was added, and kept for 2h in darkness and finally the absorbance of each sample was measured at 760 nm.

- **Total flavonoid content :**

Total flavonoid content was measured by the aluminium chloride colorimetric ($Alcl_3$) method (Zhishen *et al.*, 1999). Ten microliters of each extract was added to 100 µl of sodium nitrite (5%, W/V) and allowed to stand for 5 min. then 100 µl $Alcl_3$

(10%, W/V) were added and incubated for 5 min , followed by the addition of 1 ml sodium hydroxide NaOH (1M) and volume was made up to 5 ml with distilled water . After 15 min, the solution was mixed completely and the absorbance was measured against blank at 510 nm. Total flavonoid content was expressed as µg Catechin (BOH chemicals ltd., poole, Eng land) equivalent (CE) per ml of crude extract.

- **Total alkaloids determination :**

Bromocresol green (BCG, Aldrich chemicals) dye was used to estimate total alkaloids (Li *et al*., 2014). BCG solution was prepared by heating 69.8 mg BCG with 3 ml NaOH (2N) and 5 ml of distilled water until completely dissolving. The solution was then completed to 1 liter with distilled water.

Ten microliters of the crude extracts were thoroughly mixed with 3 ml of BCG solution. Thirty minutes later, 5 ml of chloroform were added, and shaken for 2 min. The lower layer was separated after 30 min. The extraction was continued for three times. A set of reference standard solutions of 0.1% atropine (Merck, Dermstadt) was prepared, and followed the steps described above. The absorbance of color was read at 418 nm. The total alkaloid content was expressed as Atropine equivalent (AE)/gm crude extract.

- **Total antioxidant capacity :**

The determination of the antioxidant capacity was measured using biodiagnostic kit No. TA 2513. Antioxidants in the sample react with a known quantity of exogenous hydrogen peroxide. The antioxidants in the sample eliminate a certain amount of the provided hydrogen peroxide. The residual hydrogen peroxide is determined colorimetrically by an enzymatic reaction which envolves the conversion of 3,5-dichloro-2-benzensulphonate to a colored product read at 505 nm.

- **Insect colony :**

Bioassays were conducted using 4th larval instar of the cotton leafworm, *S. littoralis* obtained as egg masses from an established laboratory colony in pest physiology laboratory , plant protection research institute, Sharkia branch (Egypt). Larvae were reared on castor bean leaves. The colony was maintained at 25±2°C, 60-70%RH, and photoperiod 12:12 (L:D)h.

- **Bioassay:**

Crude peel Hex and MeOH extracts of lemon and orange were tested against the newly moulted 4th larval instar of the cotton leafworm to determine their activity using different methods of treatment , the more efficient extraction solvent , and the more active citrus peel. Serial concentrations of the crude extracts (1, 2, 5, 10, 15, 20%) were prepared for all treatments except otherwise mentioned, using the same solvent of extraction.

1- Contact method :

The Petri dish residual exposure bioassay (Siskos *et al.*,2007) was used to evaluate the insecticidal activity of different citrus peel crude extracts against the larvae; In glass Petri dishes (bottom, internal diameter 9 cm; height, 1.5 cm). Serial concentrations of each extract were prepared, and 1 ml of each was spread on the bottom, and the Petri dishes were rotated manually until solvent evaporation to achieve an even distribution of the sample. Three replicates of 10 randomly selected larvae were used for each extract. The larvae were introduced into Petri dishes, and exposed to the extract for 30 min. The insects were exposed to the extract through contact with the cuticle and probably also with prologs. Then, the larvae were transferred to clean dishes supplied with their food (castor bean leaves). Mortality was recorded after 72 hr post-treatment. Control dishes were treated by the same manner with solvent only; the same number of larvae was introduced into these dishes, and controls were run simultaneously with the treatments.

2- Feeding method :

Feeding method of treatment was carried to test effect of extracts through feeding or ingestion using leaf dipping technique i.e larval feeding on leaves pre-treated with each extract. Clean castor bean leaves were dipped in serial concentrations of each peel crude extract (1, 5, 10, 20 and 40%) for 30 seconds, and left to dry at room temperature. Control leaves received solvent only. Leaves were introduced into Petri dishes containing 10 larvae replicate 3 times. Larvae fed on treated leaves for 24 h, and then replaced by new untreated ones. Mortality counts were performed after 72 h post-treatment.

3- Antifeedant test :

Tests for feeding deterrence were carried out using the leaf dipping method. Citrus peel crude extracts were diluted by the specified solvent to sub lethal concentrations (1%). Leaf pieces (5gm each) were soaked in the prepared extract, air dried and singly placed into small covered cups, each of which contained single larvae for 24 h to estimate the amount of leaves eaten. Results of dead larvae were discarded. Percent antifeedant activity calculated as follows:

$$\%\text{antifeedant activity} = \frac{\textit{leaf weight consumed in control} - \textit{leaf weight consumed in treatment}}{\textit{leaf weight consumed in control} + \textit{leaf weight consumed in treatment}} \times 100$$

4- Repellency :

Choice test was used to estimate the repellent action of citrus peel crude extracts against *S. littoralis* larvae. Repellency assay was carried out in glass Petri dishes (bottom, internal diameter 9 cm; height, 1.5 cm). Whatman filter paper was cut into two equal halves, and 200 µl of each extract (1%) was applied to filter paper one half as uniformly as possible. The other half received 200 µl of solvent (Hex or

MeOH) only. The two halves were then air dried to evaporate solvents completely. Three replicates of 10 larvae were used for each extract. The larvae were placed at the center of filter paper disc, and number of insects on both treated and untreated halves was recorded after 6 h of start of experiment at room temperature.

Percent of repellency was calculated as follows:

$$\% \text{ repellency} = 1 - \frac{No.of\ larvae\ in\ treated\ half}{No.of\ larvae\ in\ untreated\ half} \times 100$$

- **Statistics :**

The concentration-mortality data for LC_{50} determination were analyzed by the Probit procedure (Finney, 1971) using Ldp-line program (http://www.ehabsoft.com/ldpline/). Mortality counts were corrected by Abbott's formula (1925). All determinations were made in triplicates. The data are presented as the mean ± S.E., and analyzed with one-way analysis of variance (ANOVA). Means were separated using the Duncan's multiple range test. A probability value of $p<0.01$ was considered to denote the statistical significance difference. All statistical analysis were done using the software package Costat program. The Abbott's formula is as follows : corrected mortality(%)= $\frac{p-p_{\circ}}{100-p_{\circ}} \times 100$

Where: p is the percent mortality of treated insects

$p_{\circ}$ is the percent mortality of control insects.

RESULTS AND DISCUSSION

- **Physicochemical characteristics: -**

Percentage yield, PH and specific gravity of lemon and orange peel crude extracts were determined using Hex and MeOH as the extraction solvents (Table,1) .

Table 1. Physicochemical characteristics of citrus peel crude extracts.

Parameters	*Citrus sinensis*		*Citrus limon*	
	Hex	MeOH	Hex	MeOH
Yield ($g100g^{-1}$)	1.57 ± 0.03^{c}	7.84 ± 0.13^{b}	1.25 ± 0.01^{c}	11.8 ± 0.12^{a}
Specific gravity ($25^{0}c$)	0.80 ± 0.009^{d}	1.11 ± 0.01^{a}	0.93 ± 0.008^{c}	1.06 ± 0.02^{b}
pH	2.85 ± 0.07^{c}	4.80 ± 0.09^{a}	2.32 ± 0.041^{d}	4.11 ± 0.076^{b}

- Results are the mean ± S.E.
- Different letters in subscripts, within row, indicate a significant ($P<0.01$) difference.

The results showed that when MeOH was used for citrus peels extraction, either for lemon or orange, the yield was much higher than Hex. % yield was 7.84 and 11.8 for MeOH extraction of orange and lemon peels, respectively. It was 1.57 and 1.25 for orange and lemon, respectively, extracted by Hex. On the contrary, Hex extracts were more acidic than MeOH ones. PH was 2.85 and 2.32 for orange and lemon peels extracted by Hex, while it was 4.80 and 4.11, respectively, in the case of

MeOH extraction. Citrus peels extracted by MeOH characterized by that their specific gravity were > 1, while those extracted by Hex were < 1.

- **Chemical composition :-**

Phytochemical content of citrus peel crude extracts is illustrated in table (2). The results emphasized the relatively great ability of MeOH in extracting phytochemicals from lemon and orange peels. It was more efficient than Hex in withdrawing main metabolites (proteins and carbohydrates) or secondary metabolites such as phenols, Flavonoids and alkaloids. Also, it was more able to extract Flavonoids, alkaloids and phenols from orange peel than lemon.

Carbohydrates were the major component extracted by MeOH. They were 511 and 515 mg/gm crude extract for orange and lemon peels, respectively. Total proteins amount extracted by MeOH were less than 5% as compared to carbohydrates content. They equaled to 19.1 and 25.1 mg/gm, respectively.

Table 2. phytochemical content of citrus peel crude extracts.

Components	*Citrus sinensis*		*Citrus limon*	
	Hex	MeOH	Hex	MeOH
Proteins (mg/gm)	0.2±0.008 ^{c}	19.1±0.45 ^{b}	0.17±0.026^{c}	25.1±1.11^{a}
Carbohydrates (mg/gm)	3.3±0.08^{b}	511±8.67^{a}	5±0.31^{b}	515±15.6^{a}
Phenols (mg GAE/gm)	0.7±0.05^{c}	14.3±0.31^{a}	0.27±0.04^{c}	8.79±0.34^{b}
Flavoniods (mg CE/gm)	7.7±0.6^{c}	77.3±3.69^{a}	6.5±0.34^{c}	33.6±2^{b}
Alkaloids (mg AE/gm)	2.5±0.11^{c}	14.5±0.86^{a}	2.2±0.11^{c}	6.3±0.23^{b}

- Results are the mean ± S.E.
- Different letters in subscripts, within row, indicate a significant difference (P <0.01).

There were significant differences (P<0.01) between lemon and orange peels extracted by MeOH (Table , 2) orange peel total phenols, flavonoids and alkaloids were 14.3 mg/gm, 77.3 mg CE/gm and 14.5 mg AE/gm, respectively, while they were 8.79, 33.6 and 8.3, respectively , for lemon peel.

On the other hand, the results showed that Hex extraction of all of the studied phytochemicals quantities were considered as minor as compared to MeOH. (Table,2) In addition, there were non-significant differences (P<0.01) between lemon and orange peel extracted materials amounts by Hex.

- **Antioxidant activity:-**

The results (fig.1) shows over all antioxidant capacity of citrus peel crude extracts. MeOH had the higher ability than Hex to extract antioxidant components specially from orange peel (P<0.01). Total antioxidant capacity was 26.4 and 21.2

µM/gm extract for orange and lemon extracted by MeOH, and was 3.85 and 1.6 µM/gm extract for orange and lemon extracted by Hex, respectively.

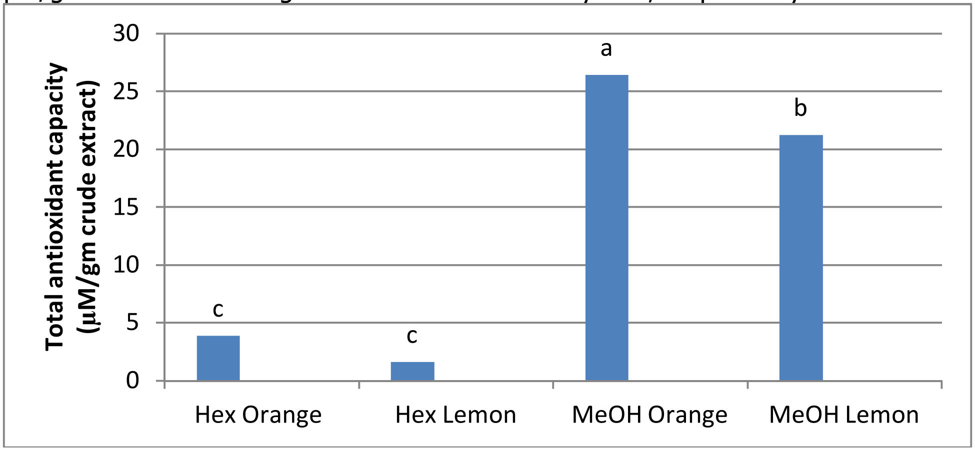

Fig. 1. Total antioxidant capacity of citrus peel crude extracts. Means (columns) ± S.E. having the same letters are not significantly different ($P<0.01$, Duncan`s multiple range test).

- **Toxicity of citrus peel extracts :-**

Toxic action of citrus peel extracts was tested against 4th larval instar of the cotton leafworm, *S.littoralis* using different methods of treatment (contact and ingestion). In general, treatment by contact method was more efficient than ingestion (feeding), and Hex extracts were more toxic than MeOH extracts in both treatment methods.

Exposure of larvae for 30 min to contact with crude extracts caused satisfactory results (Table,3). LC_{50} of MeOH extracts were 4.15 gm% (orange) and 3.32 gm % (lemon), while those of Hex extracts were 2.31 gm % (orange) and only 0.6 gm % (lemon).

Feeding of larvae for 24h on leaves treated by the extracts caused much lower toxicity than contact method (fig.2). LC_{50} values are illustrated in table (4). LC_{50} of MeOH extracts were 58.62 gm% (orange) and 91.11 gm% (lemon), while those of Hex extracts were 7.67 gm% (orange) and 22.24 gm% (lemon).

Table 3. Toxicity values of citrus peel crude extracts on 4th larval instar of *S.littoralis* treated by contact method.

Extraction solvent	Citrus species	Lc_{50}(gm%, W/v)	95% fiducial limits	slope	X^2
MeOH	*C. sinensis*	4.15	3.18-5.32	2.82	1.036
	C. limon	3.32	2.44-4.34	2.49	3.06
Hex	*C. sinensis*	2.31	1.65-3.28	1.99	0.29
	C. limon	0.60	0.37-0.86	1.79	1.43

- α=0.05
- the results 72h post-treatment

- **Antifeedant activity:**

Feeding of 4th larval instar larvae n leaves treated by 1% of lemon and orange peel crude extracts for 24 h caused slight feeding deterrence to larvae, specially for lemon extracts. The data (table, 5) shows that the treated larvae consumed 20.2, 26.2, 21.1, 23.6 and 25.29 mg/larva for MeOH extract of orange and lemon, Hex extracts of orange and lemon, and control, respectively. Orange MeOH and Hex extracts showed significant difference (P<0.01) as compared to control. The calculated antifeedant activity was 20.2, 16.6, 6.7 and 0.00% for orange extracted by MeOH and Hex, and lemon extracted by Hex and MeOH, respectively.

- **Repellent action :**

All treatments of the cotton leafworm larvae by citrus peel extracts showed moderate repellent action (table, 5). The larvae in treated filter paper disc half showed 43.3, 60, 36 and 55 % repellency for orange and lemon peel extracted by MeOH , and for orange and lemon peel extracted by Hex, respectively. One percent (gm%) of the tested extracts did not cause significant difference between the different extracts of the same citrus species.

Table 4. Toxicity values of citrus peel crude extracts on 4th larval instar of *S.littoralis* treated by ingestion method.

Extraction solvent	Citrus species	Lc_{50}(gml%, W/V)	95% fiducial limits	slope	X^2
MeOH	*C. sinensis*	58.62	42.07-115.4	2.40	0.28
	C. limon	91.11	65.5-170.4	2.13	0.59
Hex	*C. sinensis*	7.67	4.73-10	2.52	1.15
	C. limon	22.24	15.6-52.1	1.97	0.027

- α=0.05
- the results 72h post-treatment

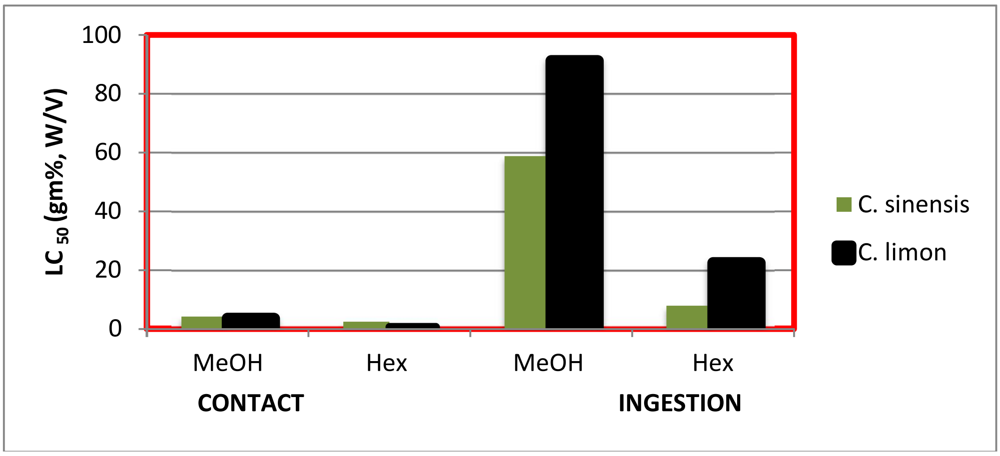

Fig. 2. comparison between different methods of treatment by citrus peel crude extracts against 4th larval instar of *S. littoralis*

Table 5. Activity of citrus peel crude extracts against 4th larval instar of *S. littoralis*

Extraction solvent	Citrus species	Antifeedant test		% repellency
		No. of mg of consumed leaves	%antifeedant activity	
MeOH	*C. sinensis*	20.2±0.63[c]	20.2	43.4±0.86[b]
	C. limon	26.2±0.52[a]	0.00	60±0.86[a]
Hex	*C. sinensis*	21.1±0.29[bc]	16.6	36±2.89[b]
	C. limon	23.6±0.46[ab]	6.7	55±1.73[a]
Control	-	25.29±0.63[a]	-	-

- larvae were treated by the extracts for 24 h (antifeedant test), and for 6 h (repellency test). Results of dead larvae were discarded. Results are the mean± S.E. . LSD= 2.95 and 10.30 for antifeedant and repellency tests, respectively. Means bearing different subscripts, within column, are significantly different p (<0.01).

Physicochemical analysis that were performed showed that % yield and specific gravity of citrus peel extracted by MeOH were significantly higher than those extracted by Hex. MeOH can extract compounds representing a wide polarity range. High molecular weight polar compounds were mainly extracted with medium –and low-polarity compounds by methanol extraction (Silva *et al.*, 1998). Hex is known as a non polar solvent. Chemical components and their proportions in the residue affected by the solvent type which in turn determine physicochemical properties (Lario *et al.*,2004). It seems that phytochemicals extraction ability of MeOH, as proved by the present study, could be reflected upon physicochemical characteristics of citrus extract. Yield is might an important factor to be considered, because it expresses the amount of extract produced from a given plant mass, which is important from an economic point of view (Dutra *et al.*, 2016).

Hex peel extracts had low specific gravity (<1). Specific gravity is the ratio of the density of a respective substance to the density of water at 4°C (Bamgboye and Adejumo, 2010). Hex was used to extract peel oils. Specific gravity of oils are less than 1 for most of oils except few containing oxygenated aromatic compounds (Osagie *et al.*, 1986). On the other hand, Hex extracts were more acidic than MeOH ones, specialy for lemon peel extracts. Lario *et al.* (2004) found that pH of the raw residue from lemon juice industry was 3.96. while the pH of orange juice waste water was 4.56 (Viuda Martos, 2009). They added that all of which (physicochemical properties) depend on the chemical composition of the residue. Therefore, it is important to know exactly which compounds present.

Concerning chemical properties, the data revealed that MeOH was more able than Hex to extract antioxidant chemicals specialy from orange peel. Orange peel extracted by MeOH had the highest phenol and flavonoids content, which might be responsible for that capacity. Phenolic compounds and flavonoids act as radical scavengers due to their hydrogen –donating capacity (Vidua-Mortos *et al.*,2009). Abd El-aal and Halaweish (2009) illustrated that orange peel extracts exhibit strong antioxidant activity. Therefore the use of these extracts in food is recommended to suppress lipid oxidation.

The present paper quantified some important phytochemicals such as proteins, carbohydrates, phenols, flavonoids and alkaloids. MeOH was the more efficient solvent in extracting these components. Previous studies showed that the secondary metabolites such as phenols, flavonoids, alkaloids and terpenoids are present in *citrus* species (Javed *et al.*, 2014). The qualitative phytochemical study of the *C. limetta* peel extracts, showed the absence of carbohydrates using hexane and petroleum ether as solvents (found in minor quantities in the present data), and the presence of terpenoids and flavonoids as the common phytochemical constituents in the extracts suggesting their possible role in the toxicity against mosquitoes (Kumar *et al.*, 2012).

The study proved that peel waste could be toxic against the cotton leaf worm, *S. littoralis*. It seems that the toxic chemicals present in the intact fruit are mainly produced and/or accumulated in the peel tissue of citrus fruit (Siskos *et al.*, 2007). Citrus peels showed relatively strong contact, moderate repellency and weak feeding and antifeedant activity against 4th instar larvae of *S. littoralis*.

Review study reported that essential oils from four *Citrus* spp. had strong toxicity using contact bioassay against *Callsobruchus maculates* (Dutra *et al.*, 2016). The antifeedant tests (leaf dip method) conducted on the second instar larvae of *S. litura* showed that the antifeedant effect of *C. hystrix* DC leaves essential oils was 15.63% when oils used by 0.60 ml/L. Gc-Ms analysis revealed that major component was B-citronella(66-85%) that has insect repellent properties. Other components such as B-citronel (6.59%), linalool (3.9%) and citronellol (1.76%) might have repellent activity against the larvae (Loh *et al.*, 2011).

Our investigation showed that lemon peel either contact or repellent actions were more potent than orange peel. Mensah *et al*. (2014) found that volatile oils of lime, sweet orange and lemon had strong repellent activity on carpenter ants. Orange recorded the lowest activity of 82.5% and lemon recorded the highest activity of 95%. Lemon was more toxic than orange. The Lc_{50} of lemon and orange was 4.54 and 17.02%, respectively.

The results indicate that toxicity of the extract differ according to the solvent type. Hex (weak polar) was more potent than MeOH (strong polar) extract specially in the contact action. Other activities such as repellency and antifeedant showed insignificant differences between the solvents. Salvatore *et al*. (2004) reported that lemon peel extracts obtained with different solvents contain compounds of different polarities. Methanol and ethyl acetate extracts contain, among others, non volatile compounds, while diethyl ether (weak polar) contains mainly volatile monoterpenic hydrocarbons, alcohols and aldhydes limonene is the major monoterpene constituent

(64-70%). The diethyl ether extract from fresh lemon peel was the most toxic to Mediterranean fruit fly larvae. It seems that Hex as weak polar solvent extract essential oils of peels that might be added to the toxicity of the extract against the cotton leaf worm larvae. It is suggested that Hex could extracts toxic substances more than MeOH, out of the studied phytochemicals.

Lemon and orange peel extracts mode of action is mainly reported to their effect on the nervous system. This might explains that why contact was more efficient than ingestion treatment. Orange oil (Cold press extract) contains the monotepenoid d-limonene. The mode of action of d-limonene is similar to that of pyrethrum, affecting sodium flux in the peripheral neurons (Vogt *et al*., 2002). Contact treatment by limonene caused decreased locomotion of the red palm weevil adults, trembling of appendages which demonstrating the effect of limonene on the center of locomotion in the nervous system neurotoxins insecticides (Abdullah, 2009). Monoterpenes cineole and limonene are toxic by penetrating the insect body via (a) the respiratory system (fumigant effect) (b) the cuticle (contact) or (c) the digestive system (ingestion) (Prates *et al*., 1998).

It could be concluded that the extraction solvent type is a principal factor in determining chemical composition and physicochemical properties of citrus extracts. Citrus peel waste is promising as a natural biopesticide, and its activity is determined by the solvent used for extraction, citrus species and method of treatment. Lemon extracted by Hex, used for repellency or contact action is the most efficient against the cotton leafworm, *S. littoralis* larvae.

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"الخواص الفيزيوكيميائية والكيميائية لمستخلصات قشور بعض الموالح و فاعلية هذه المستخلصات على دودة ورق القطن"

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تم في هذه الدراسة استخدام مذيبين عضويين مختلفي القطبية وهما الميثانول و الهكسان (١٠٠%) وذلك للحصول على المستخلصات الخام من قشور صنفين من الموالح و هما البرتقال أبو سرة و الليمون. وكان الغرض من ذلك هو دراسة بعض الصفات الفيزيوكيميائية و الكيميائية و فاعلية هذه المستخلصات على دودة ورق القطن.

و قد أوضحت النتائج ان الميثانول (عالي القطبية) له قدرة معنوية عالية عن الهكسان (ضعيف القطبية) في استخلاص المكونات النباتية الهامة مثل البروتينات و الكربوهيدرات و الفينولات و الالكالويدات و الفلافونيدات و مضادات الاكسدة وذلك من قشور البرتقال والليمون على حد سواء. و كان هذا له تأثير على اختلاف الصفات الفيزيوكيميائية للمستخلصات فقد وجد ان كمية المستخلص المئوية و الكثافة النوعية في مستخلصات الميثانول اعلى من مستخلصات الهكسان ولكن كان الاس الهيدروجيني لمستخلصات الميثانول اكبر من ٤ واقل من ٢,٨° في مستخلصات الليمون.

اثبتت دراسات السمية لقشور الموالح على العمر اليرقي الرابع لدودة ورق القطن ان المعاملة بطريقة الملامسة للمستخلص اعلى كفاءة من تغذية اليرقات على المستخلص ، و كانت مستخلصات الهكسان اكثر سمية من مستخلصات الميثانول مما يرجح ان الهكسان قد يستخلص بعض المواد و التي قد تختلف كميا او نوعيا من تلك التي يستخلصها الميثانول. كما أن مستخلص الليمون كان أكثر فاعلية من مستخلص البرتقال عند استخدامه عن طريق الملامسة و كان له نشاط طارد اعلى من البرتقال. و بصفة عامة فان قشور الموالح المستخدمة كان لها كفاءة عالية عن طريق الملامسة و تأثير طارد متوسط لليرقات ولكنها ضعيفة نسبيا عند استخدامها عن طريق الأكل او كمثبطات تغذية.

وبناء على هذه الدراسة يمكن القول ان قشور الموالح و التي تعتبر من النفايات يمكن استخدامها في مجال المكافحة كمواد آمنة و صديقة للبيئة كما أن فاعليتها تتوقف على نوع المذيب ، صنف الموالح المستخدم ، و طريقة معاملة اليرقات .