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Antioxidant Capacity of Four Edible Plant Extracts and Theirlarvicidal Effect on the Third Instar *Cephalopinatitilator* Larvae

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THE larvicidal activities of *Ocimumbasilicum (O. basilicum), Citrus limon (C.limon), Syzygiumaromaticum (S. aromaticum),* and *Piper nigrum (P. nigrum)* extracts were evaluated against 3rd instar *Cephalopinatitillator* larvaeas an alternative to chemical drugs. In addition, the antioxidant capacity of these crude extracts was measured by four common methods. The *P. nigrum* seed extract possessed the highest antioxidant activity, highest total phenolic and flavonoid contents among all the investigated four plant extracts. The DPPH (1, 1-Diphenyl-2-Picrylhydrazyl) radical scavenging activity was 73.73±0.14% and the reducing power activity was 2.01±0.007). Ferric reducing power ability (FRAP) was 5327µM Trolox /100 g DW, while ABTS radical scavenging ability (2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) found to be 75.91±0.59% at the concentration (50 µg/ ml). According to the mortality percentages of *C. titilator* larvae and the $LC_{50, 90}$ of *S.aromaticum* extract were found to be more effective with an increase in dose followed by *C.limon, O.basilicum*, and *P. nigrum*. Also, by using light and scanning electron microscope (SEM) the morphological changes that occurred 24 hr at 1% of *C.limon* extract were filmed and the examined larva was exhibited extensive swelling of the integument. Also, posterior spiracles were showed severe damage and shrinkage of the internal structure. In conclusion, all the investigated plant extracts exhibited good antioxidant activity. The current study offers an opportunity for new compounds, which is a cheap alternative for the more costly larvicides.

Keywords: Antioxidant, *C.titilator,* Extracts, Larvicidal.

Introduction

The searching for natural food rich in antioxidants are increased that might help to prevent the oxidative damage. Recently, the treatment of diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease, and cancer are based on antioxidant-drug formulations [1-4]. Previous researches have shown that in plants, phenolics are the major antioxidants to prevent the oxidation of the susceptible substrate [5]. Plant phenolics includes diterpenes (carnosol and carnosic acid), flavonoids (quercetin and catechin), phenolic acids (gallic, protochatechuic, caffeic, and rosmarinic acid) and volatile oils (eugenol, carvacrol and thymol) [6]. Various herbs of families *Lamiaceae, Myrtaceae, Piperaceae* and *Rutaceae* have been used to screen antioxidant capacities in them such as *O. basilicum*, *S. aromaticum*, *P.nigrum,* and *C. limon* [7-9] . There is a little information on the insecticidal effects

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of afourmentioned plant extracts. Leaf extract of *O.scanum* (family: *Lamiaceae*) exhibitedlarvicidal propertiesto *Anopheles gambiae* larvae [10,11]. Also, *P.nigrum* L. has a plethora of traditional applications as an insecticide. The exposure of *Anopheles* and *A. aegypti* larvae to extracts of white pepper produced 100 % mortality [12,13,14,15]. Likewise, clove extracts (*S. aromaticum*) may be applied as an insecticide against the *Japonesseterminte Reticulitermessperatus* Kolbe [16]. Similarly, the clove extracts and itsessential oil possess 100% repelling properties against *Leptotrombidiumimphalu* larvae which could be an alternative to chemical repellents commonly correlating with harmful side effects [17]. Also, one of the most commonly consumed fruits is citrus. The nature of citrus has provided with elements that have insecticidal properties and itsplantextracts are effective against mosquitoes [18, 19].

C.titillator larvae (Clark 1797), family *Oestridea*, attacks camels and cause nasopharyngeal myiasis[20]. Camels infested with C. *titillator* larvae reduce theirphysiological functions, reduce its milk production and losses of their weight [21, 22]. Unfortunately, myiasis treatment was based on systemic parasiticides such as macrocyclic lactones (MCL). The use of macrocyclic lactone erased some safety and ecological crisis [23, 24]. Therefore, a search for new alternatives for traditional insecticides is very critical. Controlling of parasites could be effectively and safely by using botanicals [25, 26, 27].

The present investigation aims to evaluate the total antioxidant capacity of *O. basilicum, C. limon, S.aromaticum*and *P. nigrum* extracts and their larvicidal effects on the 3rd instar of *C. titillator* (L.)

Materials and Methods

Plant extractspreparation

O. basilicum and *C.limon* fresh leaves were collected from the green house of National Research Centre, Giza-Egypt. However the seeds of *S. aromaticum* and*P.nigrum* were obtained from acereal market in Egypt. The samples were grinded to a fine powder and prepared for methanolic extracts.

Estimation of total phenolic and total flavonoid contents

The methanolic extracts of *O.basilicum*, *C.limon*, *S.aromaticum* and *P. nigrum* were prepared to estimate the total polyphenol content according to Makkar et al.[28]. However, the total flavonoid was estimated according to Ordonez et al. [29].

Investigation of antioxidant capacity of plant extracts

DPPH free radical scavenging assay

Methanolic extracts of *O. basilicum, C.limon, S. aromaticum* and *P.nigrum* were prepared with ratio 85: 15 (methanol : water). One ml of freshly prepared ethanolic DPPH solution (20 µg/ ml^{-1}) was added to 0.5 ml of eachplantextract and stirred well. After 5 min of reaction at 517 nm, the decolorizing processes were recorded and compared with a blank control. BHT was used as a positive control. All plant extracts samples were analyzed in triplicate [30]. The following equation was used to calculate the activity of scavenge DPPH radical :

DPPH scavenging activity (%) =

[(control absorbance - sample absorbance) / controlabsorbance] × 100%

Reducing power assay

The reducing power of the methanolic extracts of *O. basilicum, C.limon, S. aromaticum* and *P. nigrum* were determined according to the method of Oyaizu [31]. Mixture were prepared which contain 0.5/ml from each extract, phosphate buffer saline (2.5 ml, 0.2 M, pH 6.6) and 1% potassium ferricyanide. These mixtures were incubated at 50°C for 20 min. After incubation, aliquots of trichloroacetic acid (10%) were added to the mixtures, then centrifuged at 1000 rpm for 10 min. The supernatant (2.5 ml) was mixed with 2.5 ml of distilled water and 0.1% of freshly prepared FeCl3 solution. The intensity of the blue-green color was measured at 700 nm. Increased absorbance of their action mixture indicated increased reducing power.

Antioxidant capacity FRAP assay

The antioxidant capacity FRAP assay was done with some modifications according to Benzie and Strain [32]. Stock solutions were prepared that contain 300 mM acetate buffer (pH 3.6), 10 mM of TPTZ (2, 4, 6-tripyridyl-s-triazine) in 40 mMHCl, and 20 mM $FeCl_3 \cdot 6H_2O$. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solutionand 2.5 ml $FeCl_3 \cdot 6H_2O$ solution and then warmed at 37°C before using. 500 µl from each extract was allowed to react with 2500 µl of the FRAP solution for 30 min in the dark condition. Readings of the colored product [ferrous tripyridyltriazine complex] were then taken at 593 nm. Results are expressed in µM Trolox/ 100 g dry matter.

Activity of ABTS radical scavenging

The ABTS radical cationdecolorization assay capacity of the extract and percentage inhibition calculated as ABTS radical scavenging activity [33].

$$(\%) = [(\text{Abs. control} - \text{Abs. sample})]/ (\text{Abs. control})] \times 100$$

Abs. control the absorbance of ABTS radical cation methanol; Abs. sample is the absorbance of ABTS radical cation sample extract.

Chemicals

DPPH, butylated hydroxyl toluene (BHT), 2, 4, 6-tripyridyl-s-triazine (TPTZ), (ABTS), potassium ferricyanide, trolox, ferrozine, $FeCl_2$ and $FeCl_3$ were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Folin-Ciocalteu reagent, sodium carbonate, glucose and aluminum chloride were purchased from Merck Company (Darmstadt, Germany).

Collection of Larvae

C. titillator larvae were collected from camel heads of both sexes, slaughtered at Cairo abattoir. The 3rd instar larvae were checked and identified in the laboratory then washed with distilled water for dipping assay according to Khater [34].

Effect of plant extracts on 3rd larval stages of C.titillator

Dipping test

The 3rd larval stages of *C. titillator* were immersed in three different freshly prepared concentrations of plant extracts (0.25, 0.5, and 1 % each). The test was carried out according to Khater et al. [26]. The dipping procedure was applied to ten groups of five larvae for each concentration, for a total of 50 larvae per concentration. The control group was immersed in distilled water. Each group of larvae was immersed for 60s in a 100ml solution of each extract. Then larvae were kept in Petri-dishes at 27 ± 2°C and 80 ± 5% relative humidity (RH). The mortality percentages were recorded at 3, 6, 12, 24 and 28hr. Mortality was determine after, the indicated period of time post- treatment by counting the number of a live larvae in each dish and the number of the dead ones was then deduced for each replicate. The LD_{50} and LD_{90} was computed based on the data obtained from the mortality percentage.

Scanning electron microscope (SEM)

The 3rd larval stages were dipped for 60s in 1% *C. limon* extract. After 24 hrs the treated and control samples were immersed in 2.5% glutaraldehyde for 24 hrs. Then washed in buffer and post fixed in 1% osmium tetra oxide in 0.1M cacodylate buffer before being dehydrated in an ethanol series [35]. Finally larvae samples were examined and photographed with SEM (JXA 840, Electron Probe Micanalyzer, Jeol, Japan).

Light microscopy

Ten of treated and control 3rd instar *C. tittilator* larvae were dissected to get cuticle. Cuticle sections were cut and stained with hematoxyline and eosin. Then observed under light microscopy and photographed with a digital camera (Olympus CX41 microscope).

Statistical analysis of data

Expression of data as mean ± standard error (SE) for at least five larvae in ten replicates for each concentration. IBM SPSS statistics 16 software were used for data analysis. For all concentrations, one-way analysis of variance followed by Duncan's test ($P \leq 0.05$) was used to assess the statistically significance of deference among treated groups.

Results

Total phenolic and flavonoid contents

The amount of total phenolic varied widely in samples extract and ranged from 5.12±0.144 to 12.74±0.117mg GAE/ g dry weight (DW) (Table 1). The highest level of phenolic was found in *P.nigrum* seed extract (12.74±0.117 mg GAE/g DW), while the lowest was in *C.limon* (L.) leaves extract (5.12±0.144 mg GAE/g (DW). Similarly total flavonoid contents recorded the same trend *P.nigrum* seed extract 6.34 ±0.255 mg querctine/g (DW, *O. basilicum* leaves extract 3.14±0.142 mg querctine /g (DW, *S. aromaticum* seed extract 3.05 ± 0.065 mg querctine /g (DWand the lowest amount was found in *C. limon* (L.) leaves extract (2.84±0.081 mg querctine /g (DW).

TABLE 1. Total phenolic and flavonoid content of *Ocimumbasilicum* (L.), *Citrus limon* (L.) leaves and *Syzygiumaromaticum, Piper nigrum* seeds.

Plant extract	Total phenolic (mg GAE/g D.W.)	Total flavonoid (mg QE/g D.W.)
O. basilicum	6.61±0.28	3.14±0.142
C. limon (L.)	5.12±0.144*	2.84±0.081*
S. aromaticum	6.2±0.28	3.05±0.065
P. nigrum	12.74±0.117**	6.34±0.255**

Each value represents the mean of 3 replicates (Mean ±SD).
*: The lowest level; **: The highest level.

Evaluation of total antioxidant capacity of plant extracts

DPPH radical scavenging activity

All the extracts possess good DPPH radical scavenging activity (Fig.1A). *P. nigrum* seed extract at the concentration of 50µg/ml exhibited the highest DPPH radical scavenging activity (73.73±0.14%) followed by *O. basilicum* leaves extract at the concentration (250 µg/ml) exhibited 57.12±1.3%, radical scavenging activity while *S. aromaticum* seed extract and *C. limon* (L.) leaves extractradical scavenging activity found to be 52.76±0.35 % and 50.43±0.91% respectively at the concentration (250 µg/ml).

Reducing power assay

Figure 1 (B) explained that seed extract of *P. nigrum* (50 µg /ml) had high reducing power activity (2.01±0.007) when compared to the extracts of *O. basilicum, S. aromaticum* and *C.limon* (L.) their reductive potential found to be 0.799±0.017, 0.76±0.026 and 0.566±0.028, respectively, at the concentration 250 µg/ml, which means that *P. nigrum* seed extract had the superiority as reducing power agents among the examined extracts.

Ferric reducing antioxidant power (FRAP) assay

Antioxidant activity increased proportionally to the polyphenol content. Antioxidant activity results of all extracts wereexpressed as µM Trolox/100 g in Fig.1 (C). The values obtained by FRAP assay were between 5327µM Trolox/100 gDW for *P.nigrum* seed extract (50 µg /ml) and 2055Mm Trolox/100 gDW for *C.limon* (L.) leaves extract (250µg / ml).

ABTS radical cationscavenging activity

ABTS scavenging activities of extracts were illustrated in Fig.1(D). The seed extract of *P.nigrum* (50 µg/ml) is thehighest among all the investigated plants it's found to be 75.91±0.59%. On the other hand *S. aromaticum* seed extract had lower ABTS radical cation scavenging ability (48.78±0.38%) at the concentration of 250 µg / ml. Phenolic and flavonoid constituents were rich in all the investigated extracts and exhibited good antioxidant activity measured by different methods.

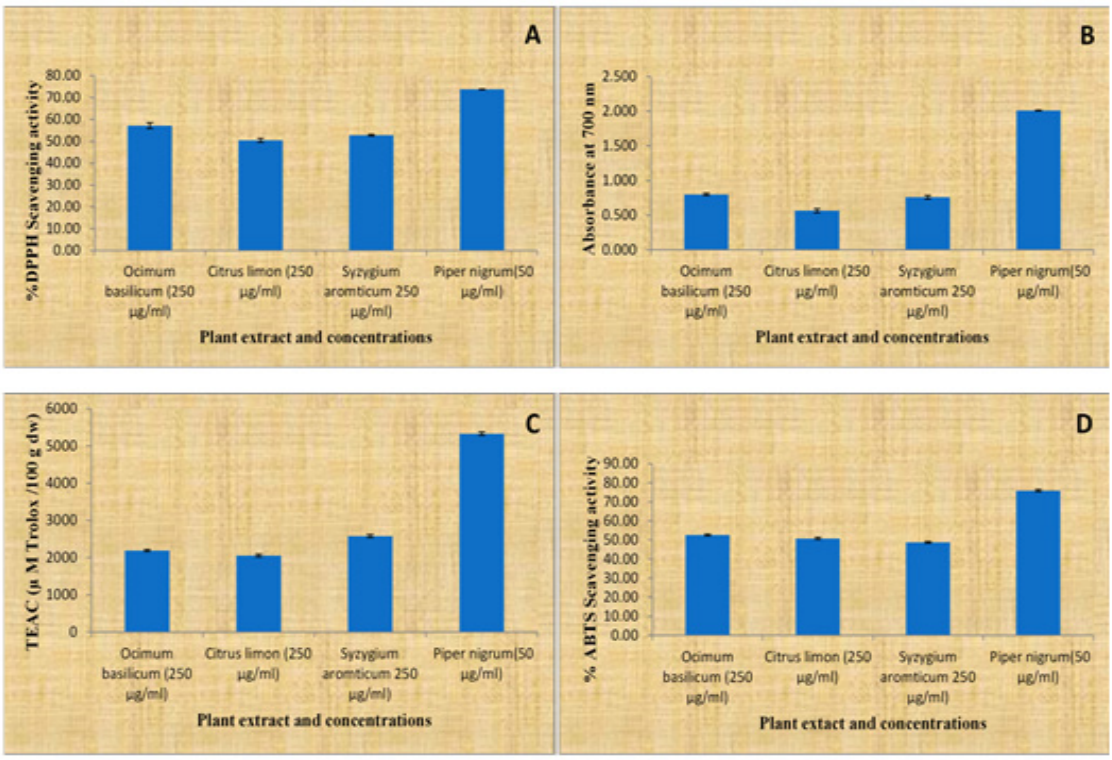

Fig.1. (A-D) . Antioxidant activity of *Ocimumbasilicum, Citrus limon* (L.) leaves and *Syzygiumaromaticum, Piper nigrumseeds* extracts using different antioxidant assays: Scavenging ability on DPPH radical (A) Reducing power (B) Antioxidant capacity FRAP assay, (C) Scavenging ability on ABTS radicals (D) Data are means ± standard deviation of triplicate experiments.

Effect of plant extracts on 3rd instar larvae of C. titillator

Four plant extracts were subjected to 3rd instar larval of *C. titillator,* under lab conditions. Table (2) and Fig. (2) are compared the mortality percentages caused by different concentrations of *C. limon, O. basilicum, S. aromaticum* and *P. nigrum* plant extracts. When high doses of plant extracts was used (1%) *S. aromaticum* caused high mortality at 12h, 24 and 48hr (24, 42, 42% mortalities, respectively) followed with *O.basilicum, C.limon* and *P. nigrum* extracts which caused mortalities (8.9, 24.4, 35.6%); (24.4, 31.16, 31.1%) and (8.9, 8.9, 11.1%), respectively at the same concentrations and time intervals. Significant toxicity (LC_{50} and LC_{90}) of *S. aromaticum* extract on larvae occurred at 24 hrs (Table 2, Fig. 2). According to LC_{50} and LC_{90} of *S. aromaticum* extract (0.4, 1, respectively) were found to be more effective with increase in dose followed by *O .basilicum* (0.6, 1.5), *C. limon* (0.6, 2.4) and *P.nigrum* (1, 2), respectively.

TABLE 2. Mortality percentages of 3rd instar Cephalopinatittilator (L.) at different concentrations of plant extracts.

	% Mortality of Larva(Means ±SE)								
	Time / hours								
	12h			24h*			48h		
Conc. (%)	0.25%	0.5%	1%	0.25%	0.5%	1%	0.25%	0.5%	1%
C. limon	2±2	10.9±3.1	24.4±5.5	6.0±4.3	21.8±3.3	31.1±3.5	6.0±4.3	21.8±3.3	31.1±3.5
LC_{50}		-			0.6			-	
LC_{90}		-			2.4			-	
O. basilicum	----	-	8.9±3.5	2.0±2.0	3.64±2.4	24.4±6.5	6.0±3.1	9.1±3.1	35.6±4.4
LC_{50}		-			0.4			-	
LC_{90}		-			1			-	
S.aromaticum	4.0±2.67	6.0±4.27[c]	24.0±6.53[b]	12.0±4.4[b]	10.0±5.4	42.0±4.8[a]	20.0±5.9	20.0±5.9	42.0±4.8
LC_{50}		-			0.1			-	
LC_{90}		-			0.5			-	
P.nigrum	4.0±4.0	-	8.9±3.5	----	3.64±2.4	8.9±3.5	-	3.64±2.4	11.1±4.8
LC_{50}		-			1			-	
LC_{90}		-			2			-	

No mortality was recorded in the control. Con.: Concentrations,*: significant, LC_{50}: Lethal concentrations required to kill 50% of the larvae exposed. LC_{90}: Lethal concentrations required to kill 90% of the larvae exposed. [a]$p<0.01$ significant increase than 0.5%, 0.25%; [b]$p<0.01$ significant increase than 0.5% ; [c]$p<0.01$ significant increase than 0.25%.

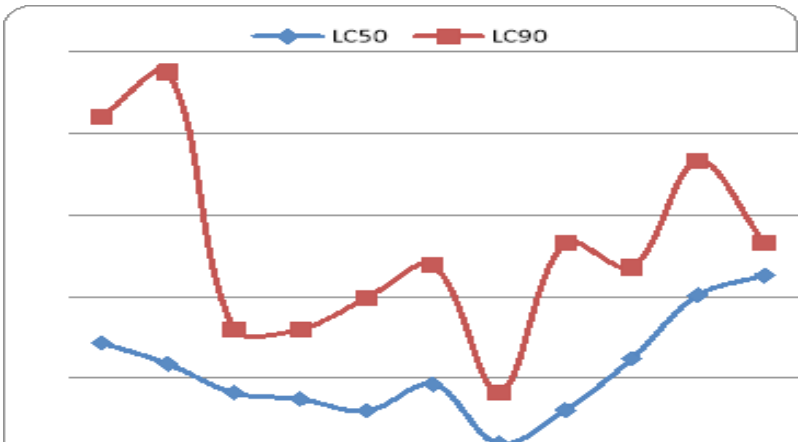

Fig. 2. Lethal concentrations (LC_{50}, LC_{90}) of the four plant extracts against 3rd instar *C. tittilator* (L.) at 24 hr. LC_{50}: Lethal concentrations required to kill 50% of the larvae exposed. LC_{90}: Lethal concentrations required to kill 90% of the larvae exposed.

SEM observations

SEM of normal control C.titillatorlarvae

Antennary lobes of 3rd instar *C. titillator* (L.) are small, the unarmed pseudocephalon bears an antenno-maxillary sensory complex formed by the antenna and the maxillary palp with a set of central small coeloconicsensilla and few other outlying sensilla (Fig.3a). The buccal funnel is already well structured with strong pair of mouth hooks or maxillae. Each maxillae is sharply pointed and ventrally curved; its surface ornamented by wrinkle areas with dorso-lateral grooves, and mandibles are absent. The pseudocephalon is followed by the first thoracic segment (Fig.3a), which is circled, anteriorly, by a band of several rows of small. Caudally directed spines. Ventrally, a band of small spines is found, in irregular rows, spines is found, in irregular rows, behind the fleshy spines on the 3rd thoracic segments where the number of rows and spines decreased in the distal segments. The abdominal segments have a very large number of curricular semi grooves (Fig.3b) with several pits and deep pores. The abdominal respiratory spiracles of 3rd instar *C. titillator* (L.) were located at the last posterior end of the larval body (Fig. 3c).It is formed of adorsal and ventrallip that joined together forming cuticle ring enclosing the posterior spiracles inside (Fig.3c). The spiracles plate appeared strongly sclerotized bearing numerous respiratory units which scattered in the spiracular plates. Each respiratory unit had a slit which surrounded by rima.

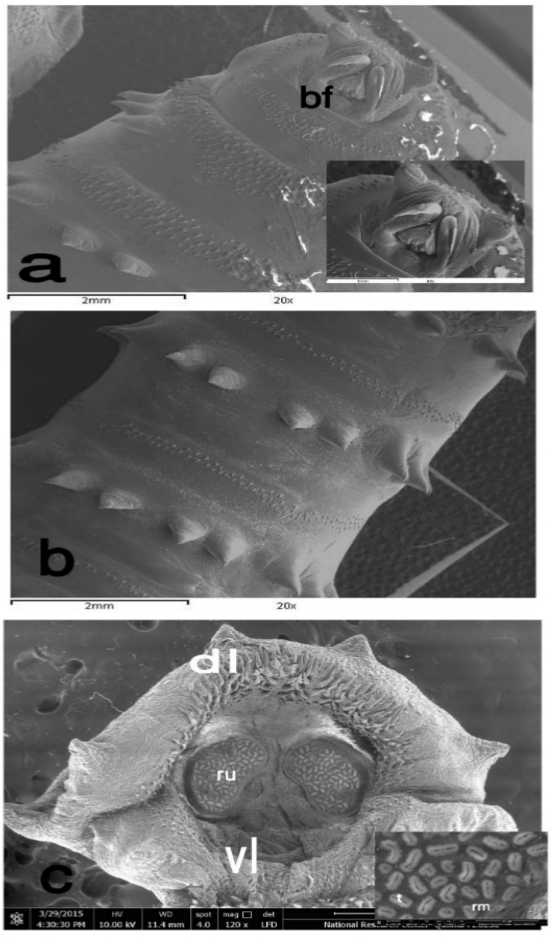

Fig. 3. (a-c). Scanning micrographs of normal control 3rd instar larvae of *C.tittilator*showing (a) anterior end and buccal funnel. (b) Normal appearance of integument and spines. (c) Normal kidney shaped posterior spiracles with spiracle units. Arrow: normal respiratory slite and rima. Abbreviation: bf: buccal Funnel, dl: dorsal lip, t: respiratory slit, vl: ventral lip, rm: rima, ru: respiratory unit.

SEM of C. titillator larvae after treatment with plant extracts

By using SEM was filmed morphological changes that occurred 24 hr at 1% of *limon* extract. Third instar larva exhibited remarkably aberrant appearances. Extensive swelling of the integument was evident in most specimens examined. There was sloughing and corrosion of the cuticle surface at the anterior and swelling of inter segmental spins. Also, posterior spiracles showed severe damage and shrinkage of the internal structure (Fig.4a-c).

Light microscopic observations

The integument consist of a single layer of cells and cuticle. The normalcuticle of 3rd instar *C.tittilator* (L.) is formed of epicuticle followed by procuticle. The procuticle consisting of exocuticle,endocuticle and inner epidermal cells (Fig.5a, b). The effects of 1% the of *C.limon*in cuticle were studied for morphological alterations. The epicuticle layer was wrinkled and corrugated (Fig.5c). As a result of cuticle swelling a moderate degeneration and atrophy were observed in fibrils of exocuticle and endocuticle. Also, the inner epidermal cell showed sever disruption (Fig. 5c).

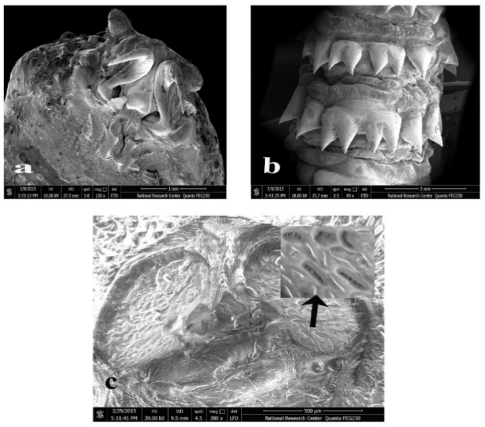

Fig. 4. (a-c). Scanning electron micrographs of the 3rd instar *C.tittilator* (L.) after 24 hr treatment with 1% *limon* extract.(a) anterior end showing sever corrosion, (b) mid region of treated larvae showing the integument and swelling of the intersegmental spines, (c) distortion of posterior spiracles,the respiratory units seemed to be sunken and the respiratory slits lost their linear shape.

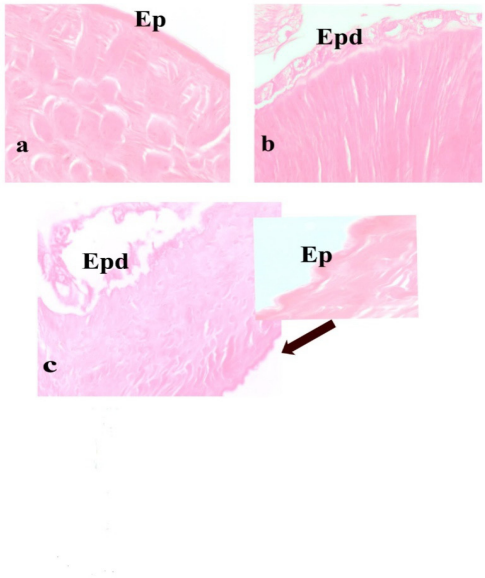

Fig.5. (a-c). a,b).cuticle of normal control 3rd inst ar larvae of *C.tittilator* (c) Cuticle of 3rd instar *C.tittilator* treated with 1% extract . Ep: Epicuticle, Epd: Epidermal layer (H & E).

Discussion

Free radicals can be formed in human metabolism to deactivate the viral and bacterial presence or environmental factors like pollution, smokes, and others. Radical chain reactions with DNA, proteins and cell membrane cause harmful effects to human body. Antioxidants, enzymes and vitamins are naturally available anti-free radical defense systems used to prevent oxidative damage and to protect the body from harmful pathogens [36].

The extracts showed positively correlated with total phenolic content and significantly higher inhibition percentage (stronger hydrogen-donating ability). The reduction in DPPH molecules numbers can be correlated with the hydroxyl groups. The different between antioxidant potential of the extracts may be due to the difference in chemical structures of their phenolic compounds, as suggested by previous work as regards the relationship between the chemical structure and antioxidant potential of phenolic compounds by means of the DPPH method [37, 38]. Also, Gülçin [39] suggested that the antioxidant activities of the individual compounds may depend on structural factors, such as, flavone hydroxyl, keto groups, free carboxylic groups, the number of phenolic hydroxyl or methoxyl groups and other structural features. The results of reducing power ability (absorbance at 700 nm) for *of O.basilicum*, *C.limon* (L.) leaves and *S.aromaticum*, *P.nigrum* seeds extracts revealed good capacity to reduce iron (III) and forming stable products. An increase in absorbance of the reaction mixture would indicate an increase in reducing capacity [39, 40] .Many studies focused on the relationship between reducing power values and the antioxidant activity of the phenolic compounds[41]. The results showed that antioxidant activity capability could lead to a significant correlations between antioxidant activities, phenolic and flavonoid contents in polar and semi-polar fractions.

FRAP assay is usually used to investigate the antioxidant capacity of plant. As shown from Fig.1A, B,C and D, the extracts of *O. basilicum*, *C. limon* (L.) leaves, *S. aromaticum*, and *P.nigrum* seeds have effective and powerful reducing power when using the FRAP method and compared to the standard (Trolox). Reducing powers of tested extracts were exhibited in the following order: *P.nigrum* seeds >*O.basilicum* leaves >*C. limon* (L.) leaves >*S. aromaticum* seeds. These results demonstrated the electron donor properties of tested extracts thereby neutralizing free radicals by forming stable products [42].

Another total antioxidant activity screening method is ABTS radical cationdecolorization assay. On average, the *O.basilicum*, *C. limon* leaves S. *aromaticum,* and *P.nigrum* seeds showed the inhibition of ABTS radical. In our study could readily scavenge ABTS action indicating the presence of phytochemical components such as flavonoids and phenols, which substantiate their antioxidant action. Phenols and flavonoids contribute to the quality and nutritional value in terms of modifying color, taste aroma and flavor .The phenolic compounds act as antioxidant agents ABTS doesn't discriminate between OH phenolic, providing a response related to total groups able to quench a radical reaction [43].

In the present searching for natural product as alternative of chemical drugs against nasophoryngeolmyiasis of camels. Our results indicated that, at 1% concentration the *S.aromaticum* and *C. limon* extracts caused significant mortality (42, 31.1%) after 24 hrs post exposure while *O. basilicum* and *P.nigrum* extracts caused 24, 8.9% respectively. Bagavan et al. [44] reported that there were a significant toxicity of *C.sinesis* peel, *O. sanctum*leaf , and *Rhinancanthusnasutus* leaf extractsagainst the larvae of *An. Subpictus*. Similarly, *C.aurantifolia* (L), *C.sinensis* (L), and *C. paradisii* (L) exhibited significant insecticidal activity against *Triboliumconfusum Jacquelin* and *Sitophilusgranarius* (L.). Extracts of the four plant species investigated in this work have shown significant LC_{50} and LC_{90} at 24hr on *C.titilator* larvae. These effects were found to be most pronounced in the extracts of *S. aromaticum* and *C.limon* compared to *O. basilicum*and *P.nigrum* extracts. Previous work with aqueous extracts of *S. aromaticum* and *Rhazyastricta*, caused highest rate of mortality, compared to neemwhen tested on *C.pipiens* L. Furthermore, *C. limon* and *Musa acuminate* alcoholic and aqueous extracts were found least effective against *P. citrella* larvae[45] .

Our investigations showed remarkable effects on morphological features of 3rd*C. titillator* (L.) that treated with extracts. Many changes have occurred in response to plant extracts. These changes consisted of severe swelling of the body wall and spines. The anterior end and spiracles plate of the larvae, showed wrinkled and irregular cuticular surface. El-Hawary and Sammour [46,

47] studied the bioactivity and mechanism of action of some wild plant extracts on *Aphiscraccivora*. Abdelgaleil and El-Aswad [48] demonstrated the modeof action of limonene on the cotton leafworm, *Spodopteralittoralis*. Limonene cause an increase in the spontaneous activity of sensory nerves and results in lack of coordination, twitching, and convulsions. As well, Lewis et al. [49] studied the mode of action of *Asimicin* against the fourth instar *Ostrinianubilalis*. *Asimicin* resulted in a significant reduction in respiration (concentration for 50 % inhibition = 0.55 nmol/mg protein). The cuticle of insect is mainly composed of a mixture of long-chain compounds which include waters, hydrocarbons, alcohols, free fatty acids, aldehydes, ketones and cyclic compounds [50]. In the present study the histological observations of the cuticle in case of 1% *C.limon* appeared to be more swollen than normal and the epicuticle layer was wrinkled and corrugated. Similar changes were observed in larvae of *Chrysomyiaalbiceps*which exposed tospinosad, *Zingiberofficinale* (root) and *Allium sativum* (fruit) [51]. As well as *Luciliasericata* treated with camphor and lavender oils [52].

Stadler and Butter et al. [53] proposed that mineral and vegetable oils caused a softening of the cuticle in adult cotton boll weevils *Anthonomusgrandis* BOh (Coleoptera: Curculionidae). Oils and extracts are able to penetrate the cell membranes, accumulate inside the cytoplasm and cause cell dehydration [54]. Acomplete removal of the cuticle wax layer, as well as hardening and stiffening of the cuticlewhen cottonseed oilcombined with diflubenzuron and applied at very low rates to boll weevils, *Anthonomusgr and isgrandis* Boheman[55].

Conclusion

All the examined plant extracts exhibited good and promising antioxidant activity by using several antioxidant assays (DPPH, Reducing power ability, FRAP and ABTS radical scavenging ability). This work displayed promising larvicidal properties of plant extracts and that the cuticle of larvae is subject to severe swelling. Whether this is due to direct uptake by the cuticle or an indirect effect via disruption of the abdominal spiracles remains to be determined. Finally the extracts of *S. aromaticum* ,*C. limon*, and *O.basilicum* have good natural larvicidal agents than *P.nigrum* which could be used in pharmaceutical and veterinary industries.

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قدره أربعة مستخلصات نباتية صالحة للأكل كمضدات اكسده وتأثيرها كمبيد ليرقات الطور الثالث سيفالوبينا تيتليتور

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تم تقييم كفاءه اربعه مستخلصات نباتيه من اوراق نبات الريحان و الليمون وبذور القرنفل والفلفل الاسود كمبيد للطور الثالث ليرقة السيفالوبينا تيتيلاتورز . بالإضافة إلى ذلك ، تم قياس قدرة مضادات الأكسدة لهذه المستخلصات الخام بأربعة طرق شائعة . اظهرت النتائج ان مستخلص بذور الفلفل الاسود يحتوي علي اعلي نشاط مضاد للأكسدة ، كما يحتوي علي أعلى محتويات للفينول والفلافونويد الكلية بين جميع المستخلصات النباتية الأربعة. كما اظهرمستخلص بذور القرنفل اعلي معدل وفيات ليرقات السيفالوبينا تيتيليتوريليه مستخلص اوراق الليمون ويليه الريحان ثم بذور الفلفل الذي اظهر نسب وفيات قليله باستخدام تركيزات مختلفه . ايضا تم تصوير التغييرات المورفولوجية لليرقات بعد ٢٤ ساعه من تعرضها لتركيز ١٪ لمستخلص اوراق الليمون باستخدام الميكروسكوب الضوئي والالكتروني الماسح. اظهرت اليرقات تورم ملحوظ واضرار بالغه .واخيرا أظهرت جميع المستخلصات النباتية التي تم فحصها نشاطًا جيدًا مضادًا للأكسدة. كما تقدم هذه الدراسة مركبات جديدة كبديل رخيص للمبيدات الحشرية الأكثر ضررا.