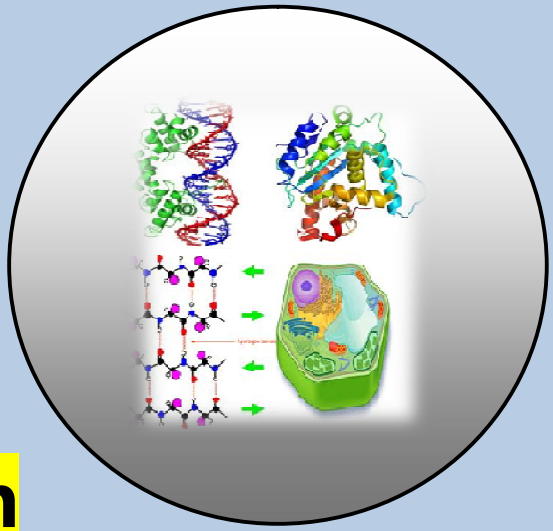
Effectiveness of *Nigella sativa*, Profenophos and their Mixture on some Biochemical and Histological Parameters of *Spodoptera littoralis* (Boisd) and Albino Rat

By

Hanan H. Osman and Hosam H. Osman

ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)

Index Copernicus International Value
IC Value of Journal 4.21 (Poland, Europe) (2012)
Global Impact factor of Journal: 0.587 (2012)



J. Biol. Chem. Research
Volume 31 (1) 2014 Pages No. 446-463

Journal of Biological and Chemical Research
(An International Journal of Life Sciences and Chemistry)

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 31, No. 1: 446-463 (2014)
(An International Journal of Life Sciences and Chemistry)

ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)

Dr. Hanan H. Osman
http:// www.jbcr.in
jbiolchemres@gmail.com
info@jbcr.in

RESEARCH PAPER

Received: 04/02/2014 Revised: 08/03/2014 Accepted: 15/03/2014

Effectiveness of *Nigella sativa*, *Profenophos* and their Mixture on some Biochemical and Histological Parameters of *Spodoptera littoralis* (Boisd) and Albino Rat

***Hanan H. Osman and **Hosam H. Osman**

***Plant Protection Research Institute, Agric. Res. Cent., Dokki, Giza, Egypt**
****Anatomy Department, College of Medicine and Medical Science, Taif University, KSA,**
**** Anatomy Department, College of Medicine , Al Azhar Universty, Cairo, Egypt.**

ABSTRACT

The aim of this work was devoted to minimize the usage of conventional insecticides, reduce the environmental pollution, and protect human-beings and domestic animals due to insecticides applications, so the present work was conducted by comparing the biochemical and histological effects of the compounds LC_{50} of Profenophos , LC_{50} of Nigella sativa (black cumin) seed oil (N.S) , and mixture of LC_{25} of Profenophos and (N.S) against fourth instar larvae Spodoptera littoralis under semi field condition. The results showed effectiveness of Profenophos and N.S which led to death of 50% after one and seven days after treatment of larva respectively, while the mixture showed top toxic death 63% of the larvae. The estimated Co-toxicity factor was 27.2, so there were potentiation between N.S mixed with Profenophos against 4th instar S. littoralis larvae. we also noted that the mixture caused damage to the insect by inhibition of choline esterase,total protein and lipid while caused significant increase in chitinase enzyme activity and this parallel with histological disorders in the mid gut of insect and destruction of the walls of the cells of the body when compared with Profenophos ,(N.S) and the control group. The present study also done by treat the rat with 1/10 of LD_{50} of Profenophos 1/10 of LD_{50} of (N.S) and 1/10 of $LD_{50}$of mixture of Profenophos and (N.S) for three months and comparing the histological effects of them on the liver and kidneys of rat. The results showed that the mixture caused improvement in histological changes of the liver and kidneys of rat as compared with rat of control group and rat exposed to a Profenophos, (N.S).We concluded that the addition of (N.S) on the Profenophos increased efficiency against insect and reduced its adverse effect on human so we recommend that addition of some plant extracts on insecticides to reduce the environmental pollution and its side effects on the vital organs of human and domestic animals.

Key Words: Spodoptera littoralis, Albino Rat, Nigella Sativa Profenophos, Biochemical and Histological Effects.

INTRODUCTION

The cotton leaf worm, *S. littoralis* (Boised) (Lepidoptera: Noctuidae) is swarming polyphagous, foliage feeding insect that is distributed throughout the world. This insect is one of the major cotton pests which cause considerable damage to many crops (Khawas and Abd El-Gawad, 2002). The mixtures are usually applied in the field to enhance the spectrum of the control so using them as a counter measure for resistance management in insect pests has been advocated by several researchers (Mushtaq 2004) . Also plant extracts have more attention in controlling many pests possess distinct toxicity and lead to antifeedant activity and inhibition growth of some pests. *Nigella sativa (N.S.)* is one of these species (Mozaffarian 1998). Many studies have reported that plants are one of the richest sources which can be used to pest control (Schmidt and Assembe, 2002). Natural plant extracts play an increasing prominent role as alternatives to synthetic pesticides due to the increasing concern on health hazards and environmental pollution (Sharma et al., 2006). The essential oils of the T. ammi, A. graveolens and N.sativa killed the larvae and adults of the T. castaneum by vapour action (Mukesh2007).Most of the evaluated plant oils were found to have an insecticidal effect on the 4th instar larvae of *S. littoralis* (Mesbah et.al.2006). It was found also four plant extracts were used to evaluate their insecticidal effect on 4th instars of *S. littoralis* larva and they showed significant biochemical and histopathological effects (Sayed et.al 2011). *N.S.* is a plant grows spontaneously and widely in several Southern Mediterranean and Middle Eastern countries (Tariq, 2008). *N.S.* seed *(N.S.S.)* are often used as a spice but are also used extensively in the traditional medicine of many countries (Meddah et al., 2009). *N.S.S.* has over 100 different chemical constituents, including abundant sources of all the essential fatty acids. Although it is the oil that most often used medicinally, the seeds are a bit spicy and are often used whole in cooking curries, pastries and Mediterranean cheeses (Tariq, 2008) *N.S.* has been used traditionally for the treatment of many diseases owing to the reported antiviral (Salem and Hossain, 2000), anti-inflammatory (Hajhashemi et al., 2004) and immunomodulatory activities (Tekeoglu et al., 2007). *N.S.* decrease blood glucose and improve antioxidant activities of glutathione peroxidase (GPx) (Zohra et.al.2012). The effect of *N.S.* and Arugula oils on *Spodoptera* against the fourth instar larvae was studied .One day after feeding , data generally showed that all treatments reflected a significantly lower effects than those recorded after 7 days, and the highest concentration (10%) caused the highest percentage of mortality for all treatments.(Abd ELatif et al 2009). The acute and chronic toxicity of *Nigella sativa* fixed oil was investigated in mice and rat by (Zaoui et al 2002) who reported that *N.S.* increase the hepatic enzymes levels, including aspartate-aminotransferase,, alanine-aminotranferase, in rat and mice. Organophosphorus insecticides (O.Ps) are widely used for a variety of agricultural and public health applications (Maroni et al., 2000).O.Ps produce a wide range of toxicity in mammals by inhibiting acetylcholinesterase (AChE), and the consequent accumulation of the neurotransmitter acetylcholine (ACh) in synaptic junctions (Attia, 2000).

O.Ps decrease the activity of some enzymes e.g glutathione peroxidase and has histopathological effects on heart in rats (Hatice and Yusuf 2011). *Profenofose* (curacron) O-(4-bromo-z-chlorophenyl) o-ethyl Spropyl phosphorothioate is an organophosphorous insecticide widely used to control various white fly and mites on vegetable (Habiba et al., 1992).

So the present work was conducted to evaluate the efficiency, biochemical and histological effects of *Profenophos, N.S.* and their combination on 4th larvae of cotton leaf-worm *S. littoralis,* and also evaluate the possible the histological effects of *Profenophos* and their combination on the liver and kidneys in rats treated with them.

MATERIAL AND METHODS

Rearing technique of insect: *Spodoptera littoralis* (Boisd.) eggs obtained from the laboratory culture of plant protection Research Institute, Agricultural Research Center (Cairo, Egypt). This strain was reared in the laboratory under constant laboratory conditions of 27±2 o C and 65±5 % RH (EL- Defrawy et al., 1964).

I- Tested compounds: they used in the present experiments were:

a- The tested plant extracts: *Nigella sativa L. (N.S.)* which belongs to family *Ranunculaceae* it is the fresh crude extract which used in this study was obtained from seeds production of *Nigella sativa plants.* Seeds (samples of 100 g) were extracted by addition of 20 ml of emulsifier polyethylene glycol 600 diluted in xylene to 80 ml crude plant extracts. The *Nigella* volatile oil containing melanthin, nigelline, damascene and tannin.

b- Insecticide:

Common name: *Profenofos*

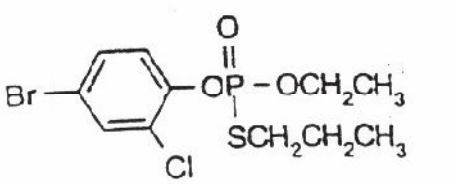
Trade name: curacron 72% EC from family of organophosphhorus it acts on the nerve System of insects by inhibiting many enzymes.

Source: was obtained from Syngenta Co. Giza, Egypt.

Chemical name:

O (4-bromo-2-chlorophenyl) o-ethyl-4-propylphosphorothiate

Chemical structure:



c- Mixture *N. sativa plus Profenophos* on *S. littoralis:* This mixture containing the combination of LC_{25} *Profenofos* and LC_{25} of *N.S.*

II- Laboratory Bioassay:

Different 5 concentrations of *Profenophos and N.S* were prepared in distilled water. For each concentration, leaves of cotton leaves were washed, dried and immersed in tested solution for 20 seconds, then allowed to dry under laboratory conditions. Each treatment was replicated three times (10 larvae/replicate), including controls 4th instar larvae.

For the control test, larvae were fed on leaves immersed in distilled water after drying. Mortality was assessed after 24 h exposure to *Profenophos.* 72h exposure to N. sativa then calculated median lethal concentration (LC_{50}) for each compound and prepare mixture with LC_{25} of both compounds.

Semi Field Experiment: - Seedlings of cotton plant (*Gossypium barbodense L.),* obtained from the pots of Plant Protection Research Institute were exposed to (LC_{50}) from tested compounds for 1 hour then, collected from pots and transfer directly to the laboratory for feeding the larval of *S. littoralis .* Forty newly molted 4th larvae of *S. littorsalis,* ten larvae were put in glass jar as a replicate fed on cotton leaves treated with (LC_{50}) of each *N.S, Profenofos* and their mixture. In control test, leaves were treated with distilled water only.

III- Statistical analysis: The mortality percentages of treated larvae were corrected against those of the control by Abbott's formula (Abbott, 1925). The data were then subjected to probit analysis (Finney, 1971) to give values of LC_{50}.

IV- Joint action studies: Binary mixture of the *Nigella oil and Profenophos* were prepared according to their toxicity equivalent LC_{25} values. The combined action of the mixture was expressed in as the "co-toxicity factor" according to (Mansour et al. 1966), and subsequently the type of interaction (joint action) was estimated.

$$\text{C0-toxicity factor} = \frac{\text{Observed mortality \% - Expected mortality \%}}{\text{Expected mortality \%}} \times 100$$

The Co-toxicity factor Differentiate the results as following:

A positive factor of 20 or more is considered as potentiation, a negative of 20 or more is considered as antagonism, while intermediate values (-20 & +20) indicated additive effect.

V-Preparation of samples for biochemical studies on *S.littoralis*:

After the detection of the median lethal concentration (LC_{50}) values using the 4th instar larvae were fed to reach the 6th instar. Samples were collected after homogenizing the 6th instar larvae representing 1 gm larval body weight, in 5 ml distilled water by using chilled glass Teflon grinder. The homogenate was centrifuged for 10 min. at 5000 rpm, the supernatant fraction being used for the enzyme assay (El- Sheikh 2012).

a- Determination of acetyl-cholinesterase (AchE) activity:

Acetyl-cholin esterase was measured according to method described by (Simpson et al. 1964) using acetylcholine bromide (AchBr) as substrate. The activity of AchE in the haemolymph was expressed as µg Acetyl¬-choline bromide/min/ml.

b- Determination of chitinase activity: Colloidal chitin was prepared according to (Bade and Stinson., 1981).

c- Determination of Total lipids content: it was determined in the homogenat of late 6th instars of *S. littoralis* was determined by the phosphovanillin method of (Baronos and Blackstock 1973).

d- Determination of Total proteins content: it was determined in the homogenate of late 6th instars of *S. littoralis* with the folin phenol reagent according to the method of (Lowry et al. 1951).

VI-Histological studies of *Spodoptera littoralis:* The effect of LC_{50} of *Profenofos, N.S* and mixture on the histological structure of the mid-gut of 4th instar *S .littoralis* was determined by fixing treatment and control larvae overnight in Bouin's solution. Specimens were then dehydrated in a series of ethanol, cleared in xylene and embedded in parablast. Embedded tissues were sectioned on a rotary microtome at 5 µm for the histopathological study, these sections were stained with hematoxyline and eosin Stained sections were finally mounted on glass slides with DePeX mounted medium. Microscopic examination and photography were done using aLeitz microscope. (Hassan et al 2011)

Laboratory animals

Forty adult albino rats weighting (200 - 250 g) were obtained from the farm of General Organization of Serum and Vaccine (Helwan Farm). The animals were housed in plastic cages in an air conditioned room where regular alternate cycles of 12 hr light and darkness were maintained and supplied with pelleted diet and tap water ad libitum. Animals were observed and signs of intoxication were recorded. The rats were equally divided into four groups each group containing ten rats and orally treated as follows:

I- Group (1): 10 rats were administrated with normal diet and water daily for 12 weeks

II- Group (2): 10 rats were given *profenofos* at a dose of 22.5 mg/kg body weight in 0.4 ml tap water through oral intubation. Dosages represent 1/10 LD_{50}. LD_{50}value of *profenofos* (225.17 mg/kg b.W.) was determined orally according to (Weil, 1952). The treatment was carried out for 12 weeks.

III- Group (3): 10 rats were given *N.S* at a dose of 2 ml/kg body wt. through oral intubation. Dosages represent 1/10 LD_{50}. The treatment was carried out for 12 weeks

IV- Group (4): 10 rats were given mixture of 1/10 LD_{50} of *profenofos* 22.5 mg/kg body weight in 0.4 ml tap water with 1/10 of LD_{50} of *N.S*. 2 ml/kg body weight/day for 12 weeks.

Histological Preparations on Rat

The animals were sacrificed and autopsy performed immediately; liver and kidney tissues were removed from the control and experimental groups were rapidly excised after the previously mentioned duration, cut into small pieces and dropped in Bouins fluid in which they were kept for appropriate time. After fixation, they were subjected to the normal procedure for paraffin embedding. Sections were cut at the thickness of 5 microns and stained with Haematoxylin-Eosin, (Bancroft and Gamble, 2002)

1-Toxicological effect of *Profenofos* and *Nigella sativa* on *S. littoralis*

The data in table (1) showed the efficiency of *Profenophos and N.S* against the 4th instars of *S. littoralis* under semi-field conditions. In general, data revealed that, *Profenophos* has higher activity against the larvae, with LC_{50} = 9.342 ppm than *N.S* $LC_{50} = 15.4 \times 10^3$ ppm, in comparison with control group.

RESULTS

Table 1. Efficacy of *N. sativa* and *Profenophos* against *S. littoralis*.

Treatment	LC_{25}	Lower	Upper	LC_{50}	Lower	Upper	Slope
N. sativa	$7.7x10^3$	$1.9x10^3$	$3.7\ x10^3$	$15.4\ x10^3$	$6.5\ x10^3$	$9.2\ x10^3$	1.6002 ±0.2267
Profenophos	4.6707	2.2838	6.5674	9.342	6.6773	11.6828	2.2404 ±0.4560

Table 2. AchE activity of *Spodoptera littoralis* treated with LC_{50} of *Profenophos*, *N. sativa* and combination of them.

Tratment	*(ug AchBr/min/g.b.wt* ± SE	%activity
N. sativa	13.14 ±0.472	-13.03
Profenophos	11.83 ±0.579	-21.70
Mixture	9.64 ±0.465	-36.20
Control	15.11 ±1.609	-

Table 3. Effect of LC_{50} of *Profenophos* , *N. sativa* and combination of them on the total lipid,protein,of *S. littoralis* homogenate

Tratment	Total protein ± SE	decrease %	Total lipid ± SE	decrease %
N. sativa	18.961 ±1.295	15.934	28.55 ±0.809	5.212
Profenophos	16.845 ± 1.039	25.315	22.335 ± 1.732	25.846
Mixture	14.945 ± 0.714	33.739	19.413 ± 0.892	35.547
Control	22.555 ± 1.732	-	*30.12* ±1.125	-

Table 4. Chitinase activity of *S. littoralis* treated homogenate with LC_{50} of *N. sativa*, *Profenophos* and combination of them.

Tratment	(Ug NAGA / min/g.b.wt.).)± SD	% activity
N. sativa	43.733 ±2.002	11.820
Profenophos	60.411 ± 2.768	54.464
Mixture	63.912 ±2.7645	63.416
Control	39 .110 ±2.800	-

2-Joint action analysis for mixture against *S. littoralis*

Observed % mortality =63.6 Expected % mortality= 50

Calculated Co-toxicity factor = 27.2

So there was potentiation between *N.S* mixed with *Profenophos* against 4th instar *S. littoralis* larvae. (Co-toxicity factor = + 20 or more).

3 - Biochemical studies on *S. littoralis:* The mixture was proved to be the most effective of the tested groups compared with *Profenophos* alone, *N.S* alone and control group

a. Determination of cholinesterase enzyme activity.

The data in table (2) revealed that the cholinesterase was significantly decreased in all treatments. The most decrease was happened with the mixture treatments (-36.20%) followed by *Profenophos* treatment (-21.70%) and then *N.S* treatment (-13.03%) lower than in the control. Results in Table 2 also showed that there is a synergism between *Profenophos* and *N.S* which might be caused by inhibition of cholinesterase enzyme.

b. Determination of total lipid and protein.

The data in table (3) revealed that the total protein was significantly decreased in all treatments. The most significant decrease was happened with the mixture treatments (33.739) followed by *Profenophos* treatment (25.315) and then *N.S* treatment (15.934) lower than in the control, and the total lipid was significantly decreased in all treatments. The greatest decrease was happened with the mixture treatments (35.s547) followed by *Profenophos* treatment (25.846) and then *N.S* treatment is not significant (5.212) lower than in the control.

c. Determination of chitinase enzyme activity.

The data in table (4) revealed that the mixture was the most effective compounds, which caused a significant increase in the chitinase activity (63.416) as compared with, *Profenophos* (54.464), *N.S.* (11.820) as compared with the control

4- Histopathological observation of *Spodoptera littoralis*:

Group 1 (control group): By observing the control group, it was found that the mid gut lining consists of a pseudo-stratified epithelium, simple column type composed of four layers, with one layer covering the connective tissue membrane, two layers of muscular fiber and a single layer of epithelial cells resting on the base membrane **(Fig. 1).**

Group 2: By observing the group 2, it was found that the midgut of *S. Littoralis* treated with LC_{50} of *Profenophos* induced severe histological damage in the integument of the 4th instar larvae, there were also severe destructed epithelial cells, detached and folded of epithelium cells as compared to third and control groups **(Fig. 2). Group 3:** By observing the group 3, it was found that the midgut *S. Littoralis* treated with LC_{50} of *N.S*. induced histological damage in the integument of the 4thinstar larvae as compared to control group there were also mild destructed epithelial cells and mild increase goblet cells.**(Fig .3),**

Group 4: By observing the group 4, it was found that the mid gut *S. Littoralis* treated with LC_{25} of mixture induced severe histological damage in the integument of the 4th instar larvae. As compared to control, second and third groups, there were also severe destructed epithelial cells, detached and folded of epithelium cells **(Fig. 4).**

Histopathological Observations of rat:

A- Histopathological Observations of liver of rat: Rats of the control group (group 1): Light microscopic examination of the liver control rats showed normal hepatic architecture without any abnormalities **(Fig.5).**

Rats of group 2: Liver sections of rats administrated orally with the *Profenofos* shown lost its characteristic architecture compared with the control group, the cytoplasm of the hepatocytes was characterized by having coarse, pink, increased number of vacuoles and Inflammatory cellular infiltration was abundant around the central vein with small fragmented pycnotic cellular nuclei (**Fig.6).**

Rats of group 3: Liver sections of rats administrated orally with the *N.S* showing slight dilatation, of the central vein (CV), hepatocytes (H) showed small number of vacuoles (V) in the cytoplasm, some pycknotic nuclei (n).**(Fig. 7).**

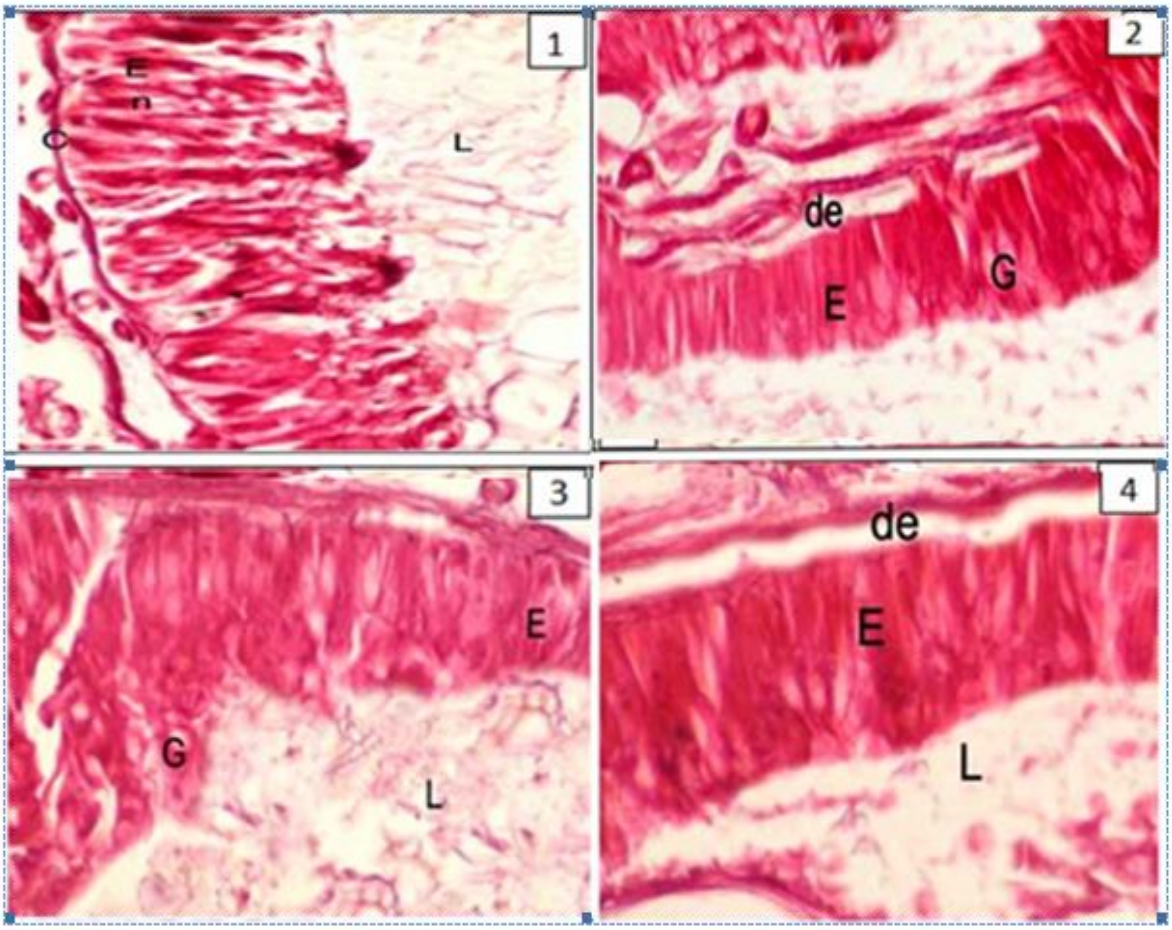
Rats of group 4: Liver sections of rats administrated orally with mixture of *Profenofos* and *N.S* showed some protective effects as compared to the group1, 2, and 3 as marked diminution of hydropic degeneration, some hepatocytes still showed hypertrophy, and the kupffer cells showed mild hypertrophy **(Fig.8).**

B- Histolopatological Observations of kidney of rat: Rats of the control group (group 1): The Kidney of control rats, had normal renal structure of cortex and medulla, the cortex showed a normal structure of renal glomeruli. The proximal convoluted tubules are lined with typical thick cubic epithelium. The distal convoluted tubules showed lower cubic epithelium, the glomerular capsule are lined with a flat epithelium, **(Fig.9)**

Rats of group 2: Light microscopic examination in the kidney of rats (group 2) showed that there were many areas of tubular damages ranged from mild to severe in the kidney were observed in all treatment animals, vascular glomerular enlargemenst, tightly filling the glomerular capsular space and flat epithelium lining the Bowman's capsule. **(Fig.10).**

Rats of group 3: Light microscopic examination in the kidney of rats of (group 3) showing small number of swollen glomeruli, tightly filling the glomerular capsular space **.(Fig.11).**

Rats of group 4: Light microscopic examination in the kidney of rats of (group 4) showed decreased in the vasculature of the renal glomeruli, appearance of the glomerular capsular space**. (Fig.12).**



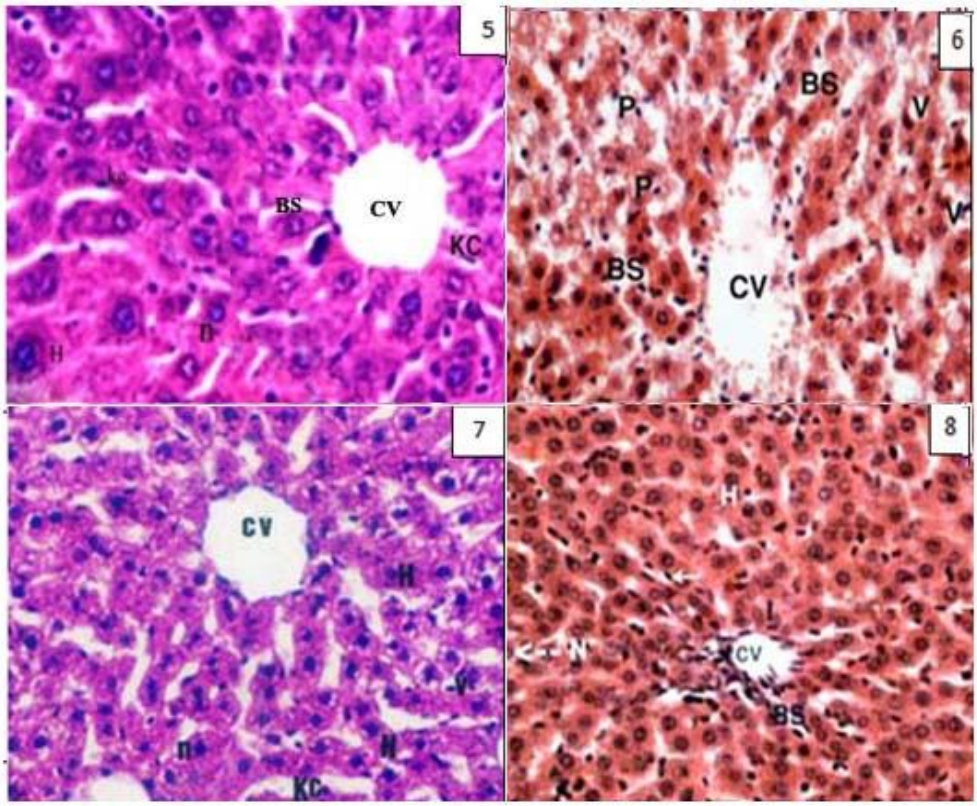
List of figures of *Spodoptera littoralis*:

Fig. 1: Longitudinal ssection of the midgut of *S. littoralis* (control group) showing: the epithelial cells (E) covered by cuticle (C), the cells have nucleus (n) and the lumen (L). (H.E.400)

Fig. 2: Longitudinal section of the midgut of *S. littoralis* (group 2) showing: sever degenerated epithelial cells (E) with detachment areas (de), mild increase of goblet cells (G). (H.E.400)

Fig. 3: Longitudinal section of the midgut of *S. littoralis* (group 3) showing: the mild degenerated epithelial cells (E), mild increase of goblet cells (G) and the lumen (L). (H.E.400)

Fig. 4: Longitudinal section of the midgut of *S. littoralis* (group 4) showing: sever congested and destructed epithelial cells (E) with detachment areas (de). With lumen (L) (H.E.400)



List of figures of rat

Fig. 5: Light photomicrograph of liver, transverse section from control rat, showing cell cords radiating from the central vein (CV). The hepsatocytes (H) cords with defined cell lining and rounded nucleus (n) sepsarated by blood sinusoids (BS) which containing Kupffer cells (kc). (H.E.400)

Fig. 6: Light photomicrograph of transverse section of liver rat (group 2), showing more dilatation of central vein (CV) and blood sinusoids (BS) with degenerated vacuolated cytoplasm(V) with multiple picnotic nuclei(P) (H.E.400)

Fig 7: Light photomicrograph of liver, transverse section of liver rat (group 3), showing dilatation, of the central vein (CV), hepatocytes (H) showed multiple vacuolated cytoplasm (V), pycnotic cellular nucleus (n) (H.E.400)

Fig 8: Light photomicrograph of transverse section of liver rat (group 4), showing less nearly normal central vein (CV) and hepatocytes (H) with normal nuclei (dotted arrow N) and adjacent less inflammatory cell infiltration (I) note that there were increase in Kupffer cell (arrow) (H.E.400)

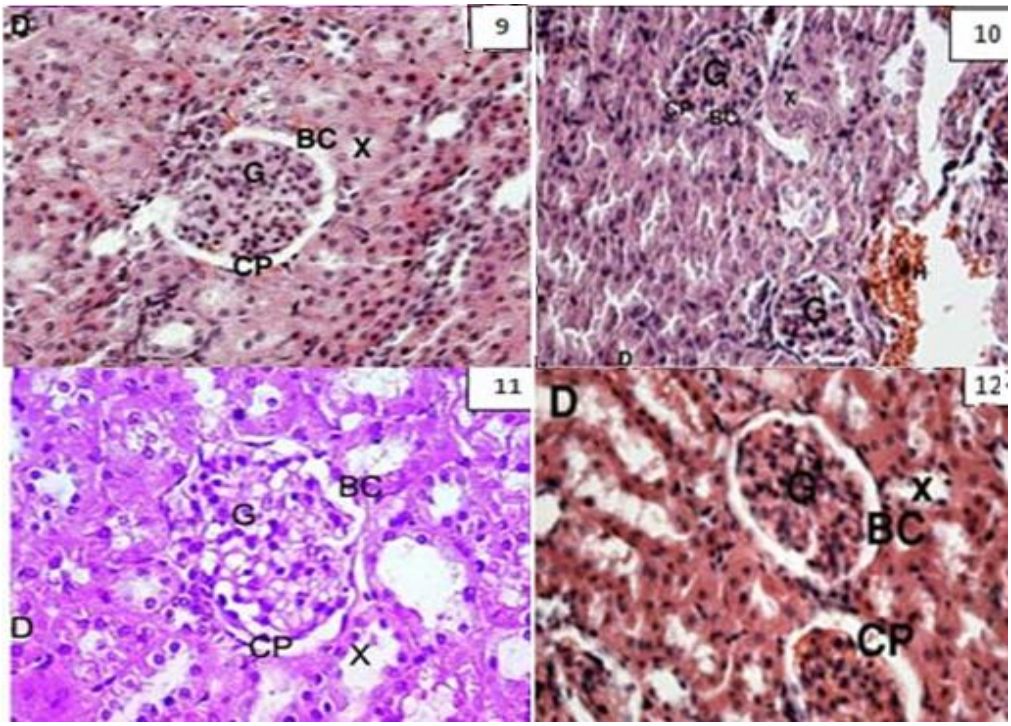


Fig 9: Light photomicrography of Kidney (cortical part) of a control rat. The renal glomeruli (G) has flat epithelium lining the glomerular capsule (BC) with distinct capsular space (CP) , the proximal (X) are lined with typical thick cubic epithelium and distal (D) convoluted tubules are lined with the relatively low simple cubic epithelium**.** (H.E.400)

Fig 10: Light photomicrograph of transverse section of Kidney rat (group 2), showing a vascular glomeruli (G) are enlarged, tightly filling the glomerular capsular space (CP),with flat epithelium lining the Bowman's capsule (BC) Some cells of the proximal (X) and distal (D) convoluted tubular epithelium show features of oedema. Capillaries are dilated and filled with blood cells (H). (H.E.400)

Fig 11: Light photomicrograph of transverse section of kidney rat (group 3), showing small number of vascular glomeruli (G) was slightly enlarged, tightly filling the glomerular capsular space (CP), with flat epithelium lining the Bowman's capsule (BC). Small number of of the proximal (X) and distal (D) convoluted tubular epithelium were edematous. (H.E.400)

Fig 12: Light photomicrograph of transvserse section of kidney rat (group 4), showing a decreased in the vasculature of the renal glomeruli (G), appearance of the glomerular capsular space (CP). Decrease the oedema of both the proximal (X), and distal (D) convoluted tubular epithelium. Lack of the fibroses in the Bowman's capsule (BC). (H.E.400).

DISCUSSION

The data in table (1) showed that the *Profenophos* highly active against the larvae, than *N.S*. in comparison with control treatment. These results are parallel with the results of (Ashraf et al 2012) who reported that *Profenofos* was the most toxic than *Dipel-2x and Neemix*. Our results were also parallel with the results of Carlini and Grossi-de-Sá (2002) who reported that *N.S.* environmentally safe compounds as pest control agents. The LC_{50} of *N. sativa oil* was found 9.46 and 10.84 ml for larvae and adult respectively (Mukesh2007).

The present study revealed that there was potentiation between *N.S* mixed with *Profenophos* against 4th instar *S. littoralis* larvae,these results were agreement with (Sameeh et al 2004) who reported that there was potentiation between Plants oil mixed with malathion against Mosquitoes larva. Also, it worth mentioning that the combined action of the mixed pesticide with plant oils showed synergistic action against the 4th larval instar of *S. littoralis* (Mesbah et.al 2006). Many trials for using several different plant extracts against larvae of *S. littoralis* were mentioned in the present study. In general, all these trials recorded inhibitory effects of the plant extract against the larvae, reducing of consumed food amount, by sorghum seedlings extract (Hafez et al., 2003).

The data in table 2 also showed that there is a synergism between *Profenophos and N.S* which might be caused by inhibition of Ach. E this result is parallel with (Kulkrani & Hodgson 1980 and Gunning et al. 1999). In such cases, *Profenophos and N.S* mixtures provide a level of synergism by competitive substrate inhibition. The mechanism of this synergism is as follows: Ahmed et.al (2011) reported that the *N.S* is detoxified in larvea esterases and oxidases, Rattan (2010) reviewed that the mechanism of action of plant secondary metabolites on insect body and documented several physiological disruptions, such as inhibition of acetylecholinestrase (by essential oils), also *Profenophos* is anticholinstrase this explain the synergism between *Profenophos* and *N.S.* The proteins help to synthesize microsomal detoxifying enzymes which assist in the detoxification of toxicants that enter into the insect body. It is the most important components of the biochemical milieu of insect that bind with the foreign compounds. In general, the problem of protein synthesis is intimately related to the metabolism of nucleic acids. (Wilkinson 1976) and (Ahmed et al. 1985).

The data in table (3) revealed that there was significant decrease in total proteins, and lipid in all groups ,the most significant decrease was happened with the mixture treatments followed by *Profenophos* and then *N.S* treatment lower than the control. These results were in parallel with the results of (Pavela et al. 2008) who decided that decreasing in larval growth by extract of *Reynoutria sp*. plants also results were in agreement with the results of (Rawi et al 2011) who decited that crude extracts on *Spodoptera littoralis* larvae caused marked decrease in total lipids, total protein and glucose contents and with (Marie et al 2009) who reported that Jojoba and Sesame oil extracts caused decrease in total lipid on *Spodoptera littoralis* likewise (Shonoda et al., 2012), tested the efficacy of the botanical extract *(myrrh),* chemical insecticide and their combination on the cotton leafworm *Spodoptera littoralis*, results showed the strong efficacy of the botanical extract which could be used alone or in combination with LC_{50} of the insecticide.

The data in table (4) revealed that the mixture caused a significant increase in the chitinase activity as compared with, *Profenophos , N.S.* and control, these mean that the mixture has a powerful action on inhibition of chitin production as compared with, *Profenophos, N.S* and control groups therefore the larvae which treated with the mixture are unable to successfully molt into the next stage than the other larvae which were treated with *Profenophos , N.S* and larvae of control groups these results were in coincidence with the results of (Ahmed et al 2011) who reported that the inhibition of in chitinase enzyme will inhibits the production of chitin, therefore the larvae are unable to successfully molt into the next stage. The reasons for employing mixtures of insecticides with plant extract in agriculture may give the best control of a complex of pests with varying susceptibilities to the different components of the mixture insects that are resistant to one or more insecticides may be susceptible to a combination of toxicants or synergism may be exhibited by the combination (All et al. 1977 and Ahmad et al. 2011).

Histopathological Observation of *Spodoptera littoralis*

Group 1(control group): the present work revealed that mid gut lining of *Spotopetra littoralis* in Fig. 1 were in coincidence with results of (Antonia et al 2010) who described the normal structures of the mid gut of *Spotopetra littoralis*

Group 2: By observing the group 2, it was found that the mid gut of *S. Littoralis* treated with LC_{50} of *Profenophos* induced severe histological damage in the integument of the 4^{th} instar larvae, as seen in Fig. 2, these results matched with those reported by Ezz El-Din et al. (2009), who mentioned that the most effective tested insecticide was organophosphorus (O.P) against 4^{th} instar larvae of *S. littoralis* using topical application. Also they found that the O.P was more potent and toxic than Spinosad. Similar results were obtained by (Ebeid, et al. 2012), where they found that O.P was highly toxic to adults of *S. littoralis*.

Group 3: By observing the group 3, it was found that the midgut *S. Littoralis* treated with LC_{50} of *N.S.* induced histological damage in the 4^{th} instar larvae as compared to control group as in Fig .3, these results were in coincidence with results of (Abd ELatif et al 2009) who reported that the effect of *Nigella* and *Arugula* oils on *Spodoptera* against the 4^{th} instar larvae showed that all treatments reflected a significantly lower effects than those recorded after 7 days and the highest concentration (10%) caused the highest percentage of mortality for all treatments, also (Rawi et al 2011) added that the crude extracts on *Spodoptera littoralis* larvae caused highly histopathological disturbances in the mid gut and body wall cells of *Spodoptera littoralis*.

Group 4: By observing the group 4, the present work revealed that the mid gut *Spodoptera littoralis* treated with LC_{25} of mixture induced severe histological damage in the integument of the 4^{th} instar larvae. As compared to control, second and third groups, as in Fig. 4, similar results were obtained from (Sayed et al 2011) who reported that the treatment with LC_{10} and LC_{25} of the most potent extracts *Azadirachta. indica, Citrullus colocynthis Ammi majus and Mentha microphylla* extract post formulation on *S. littoralis* larvae, showed cytoplasm granulation cells and separation of epidermis of mid gut of *S. Littoralis* . Khan et al 2011 also reported that the effect of crude leaf extract of *Datura Alba*,

Which is a medicinal plant on the *American Cockroach, Periplaneta Americana* is disruption of the cellular structures of the *Cockroach* midgut, fed on different doses of leaf extract of *Datura. Alba.* Don-Perdo, 1989 and Mukesh 2007 added that the mode of action of essential oils on larvae, oviposition and death of the adults, may be due to the suffocation and inhibition of different biosynthetic processes of the insect metabolism

Histopathological Observations of Rat

The present study revealed that *Profenophos* caused coarse, pink, increased number of vacuoles and Inflammatory cellular infiltration was abundant around the central vein with small fragmented pycknotic cellular nuclei in the liver of rat these results were parallel with the results of (Zama et.al 2007) who reported that O.P caused degeneration in liver tissue and in liver enzymes and with (Manal and Ola 2008) who added that *Profenophos* caused congested blood vessels of the livers with granular degeneration and necrosis with inflammatory cells in hepatic parenchyma of rat treated with *Profenophos*,while microscopical examination of the kidneys of rat treated with *profenefos* showing vascular glomerular enlargement, tightly filling the glomerular capsular space and flat epithelium lining the Bowman's capsule of kidneys of rat these results were parallel with the results of (Manal and Ola 2008) who reported that *Profenophos* caused haemorrhage in interstitial spaces, proliferative glomeruonephritis and toxic tubular nephritis of kidneys of rat.

The present study revealed that *N.S* caused mild dilatation, of the central vein, small number of vacuoles in the cytoplasm of the liver of rat, and small number of swollen glomeruli in the kidneys also mixture of *Profenophos* and *N.S* showed some protective effects as compared to the group1, 2, and 3 as marked diminution of degeneration in the liver and kidneys of rat these results were in coincidence with the results of (Tarek 2012) who reported that supplementation with green tea resulted in mild in vacuolization, swelling and degeneration in the endothelium of glomerular tuft and the epithelium of lining tubules. Mixture has an antioxidant which mops up free radicals produced in the body and shows the ability to scavenge superoxide, hydrogen peroxide, and hydroxyl radicals (Young and Woodside, 2001). Hence the rats in group 3 and 4 in liver and kidneys were nearly similar in histological features, so, the present study suggested that the mixture showed hepatoprotective and renal protective effects than O.P these result were parallel with (Atef and Wafa'a 2010) who reported that N.S can be considered as a promising therapeutic agent agains hematotoxicity, immunotoxicity, hepatoxicity, nephrotxicity and cardiotoxicity induced by diazinon.

CONCLUSIONS AND RECOMMENDATION

Taken together, our results indicated that the addition of *Nigella sativa* oil increase the effectiveness of *Profenophos* on larva of and reduce the harmful effect on liver and kidneys of rat. So we recommended that addition of plant extract on insecticides to reduce the harmful effect of insecticides on human and animal.

ACKNOWLEDGEMENTS

The authors thank Professor Saied Ahmed Omara, Professor of entomology department of cotton leaf worm, for spending quite a precious time and professor Gamal El Meizen Professor of entomology, head of department of cotton leaf worm ,Res. Inst., Agric. Res. Cent., Dokki, Giza, Egypt for spending quite a precious time for the proper achievement of this work.

REFERENCES

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

Abd ELatif, M. E., Abd El-Nabi, L. M. A. ,Hussein, E. H. and Abd El-Hafez, Z. A. 2009. Effect of two methods of *Nigella sativa* and *arugula* oils extraction on oil chemical composition and its effiucacy on *Spodoptera littoralis* larvae, *J. Agric. Res. Kafrelsheikh Univ., 35 (4) 1069-1081*

Ahmed E. M. A.E.M. and Shehata E. M. S. 2011. Toxicity and Biochemical Impacts of Some New Insecticide Mixtures on Cotton Leafworm *Spodoptera littoralis* (Boisd.) *Plant Protect. Sci. Vol. 47, No. 4: 166–175*

Ahmed, M. S., ALI, A. F. and Shakoori, A. R. 1985. Effect of dieldrin on The whole body protein content of Periplaneta Americana.*Pak. J.Zool.17 (1):105-109.*

All. J.N., Ali M., Ho'rnyak, E.P. and Weaver, J.B. 1977. Joint action of two *pyrethroids* with methyl parathion, methomyl, and chlorpyrifos on Heliothis zea and H. virescens in the laboratory and in cotton and sweetcorn. *Journal of Economic Entomology, 70: 813–817.*

Antonia, R. R., Doroty, M. D., Rosemary, M., Karla, R. A. P.,Aline V. B. and Reginaldo, B. d. C. 2010. The effect of sub-lethal doses of Azadirachta indica (Meliaceae) oil on the midgut of *Spodoptera frugiperda* (Lepidoptera, Noctuidae) *Revista Brasileira de Entomologia 54(3): 505–510.*

Ashraf A. H. M., Hanan. H. O. and Samya, Z. S. 2012. Estimate the efficiency of different compounds against laboratory and field strains of *Spodoptera littoralis* under laboratory conditions. *J. Egypt. Ger. Soc. Zool. 64 (E):Entom. 113-130.*

Atef, M. A.A. and Wafa'a, A.A.T. 2010. Preventive Effects of Black Seed *(Nigella Sativa)* Extract on Sprague Dawley Rats Exposed to *Diazinon. Australian Journal of Basic and Applied Sciences, 4(5): 957-968.*

Attia, A. M. 2000. Possible involvement of beta-adrenergic receptors in the enhancement of nocturnal pineal N-acetyltransferase activity due to parathion administration. *Toxicol*., *Vol. 142 (2):79-86*

Bade,M.L. and Stinson, A. 1981. Biochemistry of insect differentiation. A system for studying the mechanism of chitinase activity in vitro. Archs Biochem. Biophyscs. 206 : 213-221

Bancroft, J.D. and Gamble, M. 2002. Theory and Practice Histological Techniques, 5th ed., Churchill Livingstone. New York, Edinburgh and London, pp. 126 and 173-175.

Baronos, H. and Blackstock, J. 1973.Estimation of lipids in marine animals and tissue: Detailed investigations of the sulphophosphovanillin method for total lipids. *J. Exp. Mar. Biol*. *Ecol*., 12: 103-118.

Carlini C.R, Grossi-de Sá, M.F. 2002. Plant toxic proteins with insecticidal properties. A review on their potentialities as bioinsecticides. *Toxicon*. 40: 1515-1539

Don-Perdo, K.N. 1989. Mechanism of the action of the some vegetable Oils against Sitophilus zeamais (Motsch) (Coleoptera: Curculionidae) on wheat. *J. Stored Prod. Res*. 25: 217-223.

Ebeid, A.R. and Gesraha, M.A. 2012. Impact Of Three Commercial Insecticides On Some Biological Aspects Of The Cotton Leafworm, Spodoptera littoralis (Lepidoptera: Noctuidae). *Journal of Applied Sciences Research*, 8(5): 2620-2625

El-Defrawi, M.E., Toppozada, A., Mansour, N. and Zeid, M . 1964. Toxicological studies on the Egyptian cotton leafworm Prodenia litura 1-Suceptibility of different larval instars to insecticides. *J. Econ .Entomol*. 57: 591-593.

El- Sheikh, T. A. A. 2012. Comparative toxicity and biochemistry of organophosphates and pyrethroid compounds on both laboratory and field strain of the Cotton Leafworm Spodoptera littoralis (Boisd.) Egypt. *Acad. J. biolog. Sci*., *Vol.4 (1), 141-151*.

Ezz El-Din, H.A., El-Gahreeb, A.M., El-Sayed, A.M.K. and bdu-Allah, G.A.M., 2009. Toxicity of spinosad and abamectin compared with some conventional insecticides against parent field strain of cotton leaf worm, *Spodoptera littoralis* (Boisd.). *J. Agric. Sci. Mansoura Univ.,* 34: 5221-5229.

Finney, D. J. 1971. Probit Analysis.3rd. Cambridge Univ. Press, London.

Habiba, A. R.; Ali, M. H. and Ismail, M. M. S. 1992."Biochemical effects of profenofos residues in potato."*J. Agric. Food. Chem.* 40(8): 552-556.

Hajhashemi,V., Ghannadi, A. and Jafarabadi, H. 2004. Black cumin seed essential oil, as a potent analgesic and antiinflammatory drug. *Phytother*. *Res.*, 18: 195-199.

Hafez, M., M.M., Matter and Younes, A.A. 2003. Entomological effects of Sorghum seedlings extract on the cotton leafworm and its parasitoid, Microplitis rufiventris. *Pakistan Journal of Biological Sciences*. 6(19), 1649-1654.

Hassan F. D., Aida, S.K., Nehad, M. E. B. and Mona, F. A. E. A. 2011. Pyridalyl Effectiveness on Some Biological and Physiological Parameters of Cotton Leafworm Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae) *Journal of American Scienc*e, 2011; 7 (12).

Hatice, B.A.S. and Yusuf, K. 2011. Chlorpyrifos induced cardiotoxicity in Rats and the Protective Role of Quercetin and Catechin. *Gazi University Journal of Science GU J Sci* 24(3):387-395 .

Khan, I., Qamar,A.,Mehdi S.H. and Shahid, M. 2011. Histopathological effects of Datura alba leaf extract on the midgut of Periplaneta Americana. *Biology and Medicine*, 3 (2) Special Issue: 260-264.

Khawas, M.A.M. and Abd El-Gawad, H.A.S. 2002. The efficiency of two plant extracts (Fenugreek and Lupine) and commercial biofungicide (Biofly) on the cotton leaf worm, *Spodoptera littoralis* (Boisd) (Lepidoptera:Noctuidae) larvae as a new approach of control. *J. Egypt. Ger. Soc. Zool.* 37: 39-57.

Kulkarni, A.P., Fabacher, D.L., Hodgson, E. 1980. Pesticides as inducers of hepatic drug-metabolizing enzymes - II. Glutathione S-transferases. *Gen Pharmacol*. 11: 437-441.

Mansour, N.A., El-Defrawi, M.E., Toppozada, A. and Zeid, M. 1966. Toxicological studies on the Egyptian cotton leaf-worm, Prodenia litura. VI. Potentiation and antagonism of organophosphorus and carbamate insecticides. *J. Econ. Entomol*., 59(2): 307-311.

Marie, S.S, Amr ,E.M. and Salem.N.Y. 2009. Effect of some plant oil on biological, physiological and biochemical aspects of *Spodoptera littoralis*. *Res. J. Agric. and Biochem. Sci*., 2(1): 103-107.

Maroni, M., Colosio, C., Ferioli, A. and Fait, A. 2000. Biological monitoring of pesticide exposure: a review. Introduction. *Toxicol*. Vol. 143, 2000, p. 1-118.

Meddah, B., Ducroc, R., El Abbes, F. M, Eto, B., Mahraoui, L., Benhaddou,A. A., Martineau, L.C., Cherrah, Y. and Haddad, P.S. 2009. *Nigella sativa* inhibits intestinal glucose absorption and improves glucose tolerance in rats, *J. Ethnopharmacol*., 121: 419-424.

Mesbah, H.A., Mourad, A.K. and Rokaia, A.Z. 2006. Efficacy of some plant oils alone and/or combined with different insecticides on the cotton leaf-worm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) in Egypt. *Commun Agric Appl Biol Sci*. 71(2 Pt B):305-28.

Mozaffarian V. 1998. A Dictionary of Iranian Plants Names. Farhang Moaser Publishers, Tehran, pp. 365

Mukesh, K. C. 2007. Insecticidal activity of Trachyspermum ammi (Umbelliferae), Anethum graveolens (Umbelliferae) and Nigella sativa (Ranunculaceae) essential oils against stored-product beetle Tribolium castaneum Herbst (Coleoptera: Tenebrionidae) *African Journal of Agricultural Research* Vol. 2 (11), pp. 596-600.

Mushtaq, A. 2004. Potentiation/antagonism of deltamethrin and cypermethrins with organophosphate insecticides in the cotton bollworm, Helicoverpa armigera (Lepidoptera: Noctuidae). *Pesticide Biochemistry and Physiology*, 80: 31–42.

Pavela, R., Vrchotova, N. and Sera, B. 2008. Growth inhibitory effect of extracts from Reynoutria sp. Plants against *Spodoptera littoralis*. *Agrociencia*. 42(5): 1405-1413.

Rawi, S.M., Bakry, F.A. and Al-Hazm, M.A. 2011. Biochemical and histopathological effect of formulated and non formulated plant extract on *Spodoptera littoralis*. *International Research J. Plant Sci*. 2(4): 107-118.

Rattan, R.S. 2010. Mechanism of action of insecticidal secondary metabolites of plant origin. *Crop Protec.* 2010; 29: 913-20.

Salem, M.L. and Hossain, M.S. 2000. Protective effect of black seed oil from *Nigella sativa* against murine cytomegalovirus. *Int. J. Immunopharmacol.,* 22: 729-740.

Sameeh A. M.,Asmaa, Z. E. S. and Amina. R. A. 2004. Botanical biocides .12. Mosquitocidal Activity of citrus peel oils with respect To their limonene content. *Egyptian Journal of Natural Toxins*, Vol. 1: 111-134.

Sayed, M.R., Fayey, A. B. and Mansour A.A.H. 2011. Biochemical and histopathological effect of formulated and non formulated plant extracts on *S. littoralis* larvae, *International Research Journal of Plant Science* (ISSN: 2141-5447) Vol. 2(4) pp. 107-118.

Schmidt, G.H. and Assembe, T.S. 2002. Effect of oral application of Melia fruit extract on growth, development and gregarization of the desert locust Schistocerca gregaria (Forsk) (Caelifera, Acridiae). *Zeitschrift fur Pflanzenkrankheiten und Pflanzenachutz*.109:38-56.

Sharma, A. P., Kaushal,K.C. and Kumar, R. 2006. Bioefficacy of some plant products against Diamondback moth Plutella xylostella L. (Lepidoptera: Yponomeutidae). *J. Entomo. Res. Soc*., 30: 213–217.

Shonoda, M.L., Farrag, R.M. and Salama, O.M. 2012. Efficacy of the Botanical extract (myrrh), chemical insecticides and their combination on the cotton leaf worm *Spodoptera littoralis*. *Published on Alexandria University* (http://www.alexu.edu.eg).

Simpson, D. R., Bull, L.D. and Lidquist, A. D. 1964. A semi-microtechnique for estimation of cholinestrase activity in boll weevil. *Ann. Ent. Soc. Am*. 57 (3), 367-377.

Tarek M. H., Abdel-Tawab, H. M., Gehan. K.H. M. and Mona, A.A.R. 2012. Cyromazine and Chlorpyrifos Induced Renal Toxicity in Rats: The Ameliorating Effects of Green Tea Extract. *J. Environ. Anal Toxicol.*, 2(5). 1-7

Tekeoglu, I., Dogan,A. L. ,Ediz, M. B. and Demirel, A. 2007. Effects of thymoquinone (volatile oil of black cumin) on rheumatoid arthritis in rat models. *Phytother. Res*., 21: 895-897.

Tariq, M. 2008. Nigella sativa seeds: folklore treatment in modern day medicine. *Saudi J. Gastroenterol*., 14: 105-106.

Weil, C.S. 1952."Tables for convenient calculation of medium effective dose (LD50) or ED50) and instruction in their use" *Biometrics*, 8:263 -294.

Wilkinson, F. 1976. Insecticide Biochemistry and Physiology. Plenum Press, New York. World Health Organization/Vector Biology and Control/Insect Genetic and resistance. 74: 27.

Manal, M. Y. and Ola, M. Y. 2008. Pathological and Biochemical Studies of Profenofos Toxicity on Rats. *Egypt. J. Comp. Path. and Clinic. Path*. Vol. 21 No. 4; 75 - 92

Young, I. S. and Woodside, J. V. 2001. Antioxidants in health and disease. *J. Clin. Pathol.* 54:176-186.

Zama, D., Meraihi, Z., Tebibel, S., Benayssa, W., Benayache, F., Benayache, S. and Demirel, Vlietinck A.J. 2007. Chlorpyrifos-induced oxidative stress and tissue damage in the liver, kidney, brain and fetus in pregnant rats: The protective role of the butanolic extract of Paronychia argentea L, *Indian J. Pharmacol.* Vol 39 (3) 145-150.

Zaoui, A., Cherrah, Y., Mahassini, N., Alaoui, K., Amarouch, H. and Hassar, M. 2002. Acute and chronic toxicity of *Nigella sativa* fixed oil., *Phytomedicine*. ,9 (1): 69-74

Zohra, G., Khaled, H., Mongi, S., Zouheir, S., Khaled, M. Z., Abdelfattah E.F. and Ahmed H. 2012. Effect of *Nigella sativa* seeds on reproductive system of male diabetic rats, *African Journal of Pharmacy and Pharmacology.* Vol. 6 (20), pp. 1444-1450, 29.

Corresponding author: Dr. Hosam H. Osman, **Anatomy Department, College of Medicine and Medical Science, Taif University, KSA**, **Anatomy Department, College of Medicine, Al Azhar Universty,Cairo, Egypt. Email:** semsemcairo61@yahoo.com