

**The Effect of Water Hyacinth, *Eichhornia Crassipes*, and King-Bo Compound  
on Some Biological and Biochemical Aspects of Cotton Leaf Worm  
*Spodoptera littoralis* (Boisd).**

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**ABSTRACT**

Water hyacinth, *Eichhornia crassipes*, is considered as the worst aquatic plant in the world that causes a serious hazard to nation's developmental activities. The present study was planned to explore the potential of *E. crassipes* by making its positive attributes outweigh the negative ones. Thus, the present study was carried out to disclose to the effect of *E. crassipes* extract "extracted by different solvents petroleum ether, acetone, ethanol and water" and kingbo commercial formulation of plant extract on 4<sup>th</sup> larval instar of *Spodoptera littoralis*. Some biological aspects were recorded. Also, the phytochemical screening was carried out to *E. crassipes* extracts. In addition, the efficiency and residual effects of kingbo against *S. littoralis* under field conditions were also investigated.

Obtained results revealed that the percentage of larval mortality was increased with increasing the concentrations of all tested compounds. It also showed that all tested compounds caused reduction in pupal weight compared with control. Results showed that, sterols/terpenoid and flavonoids were the most constitute of *E. crassipes*. In addition, the present findings showed inhibition effect on total protein content except petroleum ether extract which clarify non-significant effect on 6<sup>th</sup> instar larval haemolymph. Also, in Esterases pattern were differed than the normal pattern.

**Keywords:** *Spodoptera littoralis* - Water hyacinth - Plant extracts – kingbo - Toxicological - Biological activities - physiochemical scanning – Total protein –Esterases pattern.

**INTRODUCTION**

The Egyptian cotton leaf worm *Spodoptera littoralis* (Boisd.) plays an important role in cotton production in Egypt, so the success in controlling such insect is considered to be of great economic importance. Modern insect control is shifting from using synthetic organic insecticides to more reliance on an integrated approach for pest management (IPM). Among the reasons for increased interest in IPM are the problems resulting from chemical pesticides such as, inducing pest resistance, residual toxicity in environment and adversely affecting the non-target organisms. Therefore, there is always a need for finding out new

material having specific modes of action to replace the conventional insecticides.

The endeavors have been made by several scientists for finding out many safe and naturally occurring chemicals such as botanical extracts, which contain a very diverse range of compounds such as sterols/terpenoids, alkaloids, flavonoids and unusual amino acids. These compounds may act as insecticides (Mogahed *et al.*, 1997) antifeedant (Zhang and Chiu, 1985) and insect growth regulators (Banerj, 1988) against a variety of insect species.

Kingbo is a new type of botanical pesticide with wide pest killing spectrum. The active ingredients are oxymatrine. Oxymatrine is a major alkaloid found in *Sophora flavescens* Aiton (Fabaceae), an

ancient Chinese herb whose dry roots („Ku Shen”) have long been used in traditional medicine. Ku Shen extracts have been formulated lately as pesticides and recommended to be used in commercial products to manage populations of various plant pests and diseases (Zheng et al., 2000 and Fu et al., 2005).

*Eichhornia crassipes* commonly known as Water hyacinth is a warm water aquatic plant belonging to the family Pontederaceae. It is listed as one of the most productive plant on earth and it considered the world worst aquatic weed (Grodowitz, 1998). It can be used as compost, paper, fuel, and animal feed and water purification (Kristie, 2012). It is also found to be effective in controlling microorganisms such as fungi and bacterial diseases in plants and humans because of its phytochemical compounds (Lata and Venapani, 2010). This work was designed to achieve the following purposes:

- 1- To evaluate the toxicological and biological activities of Kingbo compound and *Eichhornia crassipes* extract by using different solvents against 4<sup>th</sup> larval instare of *S.littoralis* and their latent effect on the different stages.
- 2- To identification and isolation of some plant constituents.
- 3- To clarify the effect of these compounds on biochemical and physiological activities after studying total protein and esterase in order to determine their effect on the vital system of *S. littoralis*.

#### MATERIALS AND METHODS

The cultural of *S. littoralis* used in this study originated from eggs obtained from a susceptible strain established in the cotton leaf worm Department, Plant Protection Research Institute, Dokki, Giza. This strain was reared in the

laboratory under constant laboratory conditions of 27±2 °C and 65±5 % RH (EL- Defrawy *et al.*, 1964).

#### The tested compounds:

*Eichhornia crassipes* extract"extracted by different solvents petroleum ether, acetone, ethanol and water”

#### Extraction method:

*Eichhornia crassipes* was extracted by different solvents (petroleum ether, acetone, ethanol and water). Briefly, each solvent was used at a rate of 500 ml/50gm. Plant material was soaked in large flask (1-litter) for 72 h. The flask was then shaken for one hour, and its contents were filtered through whatman no.1 filter paper. Each solvent extract was evaporated to dryness by a rotary evaporator. The residues were weighed and kept in deep freezer till used.

The commercial product Kingbo, aqueous solution; active ingredients: 0.2% oxymatrine + 0.4% psoralen (or prosuler; natural regulator); manufacturer Beijing Kingbo Biotech Co. Ltd., Beijing, China.

#### Insecticidal and biological studies:

To assess the activity of the tested compounds, different concentrations were prepared ranged from 5x10<sup>-1</sup> to 2x10<sup>4</sup> ppm. The larvae used in the experiments were freshly moulted 4<sup>th</sup> instar larvae within 6 h after ecdysis. The leaf dipping technique was adopted, where freshly castor oil beans leaves were dipped for 15 second in one of the prepared concentrations. The treated leaves were left to dry for approximately 30 minutes at room temperature before being offer to *S. littoralis* larvae. Three replicates contained 20 larvae / jar were used for each treatment and also for the control experiments. These tests were carried out to define the larval mortality, percentage of pupation, pupal weight, adult emergence, sex ratio. The mortality percentages of treated larvae were corrected against those of the control by using Abbotts formula, (Abbott, 1925).

Probit analysis was determined to calculate LC<sub>50</sub> and slope values of the tested compounds (Finney, 1971), through software computer program. Statistical analysis of results according to (SAS, 1996).

#### **Evaluation of residual effect.**

The purpose was to evaluate the efficiency and residual effects of Kingbo against the 4<sup>th</sup> instar larvae of the cotton leafworm *S. littoralis* (Boisd.) under field conditions, Experiment was conducted at Kaha Research Station, Toukh district, Qalyobia Governorate, Egypt. The experiment area was divided into plots, each plot 1/100feddan in cultivation season 2012 - 2013 complete randomized design was applied and each treatment was replicated four times together with control plots. A motor sprayer was used. The volume of spray solution was 200 liters/feddan. After one hour from spraying the treated clover, plant samples were collected (zero time) Also, after 2, 4 and 6 days from application. Then, it transfer directly to the laboratory for feeding 1<sup>st</sup> and 3<sup>rd</sup> larval instars of *S. littoralis* four experiment was applied to estimate the mortality percent at different time intervals Exp.(1) at zero time, Exp.(2) at 3<sup>rd</sup> day from application, Exp.(3) at 5<sup>th</sup> day from application and Exp.(4) at 7<sup>th</sup> day from application.

#### **Phytochemical screening of crude plant extracts.**

*Eichhornia crassipes* extract was analyzed to detect the presence of sterol and/or terpenoids, flavonoids, saponins, alkaloids, phenolic glucosides, resins and tannins.

#### **A-Test for sterols and/or terpenoids**

Sterols and/or terpenoids in the investigated samples detected according to (Schmidt, 1964).

#### **B- Test of phenolic glucosides**

Phenolic glucosides were detected by the method described by Balbaa (1981).

#### **C- Test of flavonoids**

Flavonoids were detected according to (Moftah, 2001).

#### **D- Test of saponins**

Saponins were detected according to (Moftah, 2001).

#### **E-Test of alkaloids**

Alkaloids were detected by the method described by Farnsworth, (1966).

#### **F- Test of tannins**

Tannins were estimated by the method described by (Moftah, 2001).

#### **G- Test of resine**

Resins were detected according to the method described by (Moftah, 2001).

#### **Biochemical studies:**

##### **Haemolymph collection.**

Haemolymph samples from 6<sup>th</sup> instar larvae were collected by puncturing a proleg and drawing the exuded haemolymph into 10µl capillary tube. The haemolymph was placed in 1.5ml ice -cold microcentrifuge tubes that contained few crystals of phenylthiourea (PTU). The haemolymph was centrifuged at 10000 rpm for 5min at 4°C.

##### **Determination of total protein**

Total protein reagent was determined by the method of Brad Ford (1976), using coomasie brilliant blue G-250 reagent.

##### **Esterases analysis:**

Esterase bands were separated by Polyacrylamide gel electrophoresis (PAGE) according to the method of (Salama *et al.*, 1992).

## **RESULTS**

### **Insecticidal and biological studies:**

To study the toxicological and biological effects of *Eichhornia crassipes* extract and Kingbo on *Spodoptera littoralis*, the experiments were carried out on the 4<sup>th</sup> instar larvae. Castor bean leaves, *Ricinus communis*, were dipped in tested compounds at different concs, on which starveling larvae fed for 48 hours.

### Effect of water extract of *Eichhornia crassipes*.

Results presented in Table (4) show that the corrected percentages of larval mortality were increased with increasing the concentration of water extract. The percentages of the corrected larval mortality were 14.28, 28.57, 46.43, and 71.42 % at the concentrations of 0.25, 0.5, 1 and 2% respectively. The pupation percentage was decreased with an increasing in the concentration, and the most effective concentration was 2 % as expressed by the lowest pupation

percentage (26.67 %). Also, the weight of the resulted pupae showed significant effect compared with the control. For example, the pupal weight was 0.306, 0.299, 0.296 and 0.297 g compared with 0.351 g in the check experiment. Water extract at different concentrations was clearly affected the adult emergence. For instance, it is clear from results that the percentage of the adult emergence was decreased gradually with an increase in the concentrations till reaching 23.33 at the concentration 2 %.

Table 1: Biological influences of water extract of *Eichhornia crassipes* on 4<sup>th</sup> instar larvae of *S. littoralis*.

Conc. %	Corrected Larval mortality %	Pupation %	Pupal weight gm Mean±SE	Pupal duration	Adult Emergence %	Total inhibition of adult Emergence %	Sex ratio%	
							male	Female
Cont	0.0	93.33±3.33	0.351±0.005	9.88±0.32	86.67±3.33	0.0	51.92	48.08
0.25	14.28	80±5.77	0.306±0.008	8.92±0.42	68.33±4.41	21.16	58.82	41.18
0.5	28.57	66.67±3.33	0.299±0.013	8.60±0.53	63.33±3.33	26.93	42.11	57.89
1	46.43	50±0.0	0.296±0.011	8.08±0.08	46.67±3.33	46.15	50	50
2	71.42	26.67±3.33	0.297±0.01	8.33±0.33	23.33±3.33	73.08	42.86	57.14

### Effect of alcohol extract of *Eichhornia crassipes*

The data given in Table (2) declare that the larval mortality increased with the increase in concentration as

expressed in corrected percentages of 17.85, 42.85, 71.42, and 94.64 % at the concentrations of 0.25, 0.5, 1 and 2 % respectively.

Table 2: Biological influences of alcohol extract of *Eichhornia crassipes* on 4<sup>th</sup> instar larvae of *S. littoralis*.

Conc. %	Corrected Larval mortality %	Pupation %	Pupal Weight gm Mean±SE	Pupal duration	Adult Emergence %	Total inhibition of adult Emergence %	Sex ratio %	
							male	Female
Cont	0	93.33±3.33	0.351±0.005	9.88±0.32	86.67±3.33	0.0	51.92	48.08
0.25	17.85	76.67±6.009	0.284±0.015	9.5±0.10	73.33±8.82	15.39	48.8	51.2
0.5	42.85	53.33±3.33	0.300±0.016	9.5±0.10	43.33±3.33	50.01	35.29	64.71
1	71.42	26.67±3.33	0.278±0.001	9.08±0.3	23.33±4.41	73.08	45.45	54.55
2	94.64	5±2.887	0.263±0.015	8.92±0.08	3.33±1.667	96.16	0.0	100

The pupation percent was obviously declined as expressed by 76.67, 53.33, 26.67 and 5.0 %. As well as, the weights of the resulted pupae were clearly affected as appeared in Table (2). Results clearly indicated that the reduction in the adult emergence was increased gradually with an increasing in

the concentration. The percentages of the adult emergence were 73.33, 43.33, 23.33 and 3.33 % respectively at ascending concentrations as compared with 86.67 %, in the check test. Also, it was remarkable that sex ratio directed to the female side.

**Effect of acetone extract of *Eichhornia crassipes*.**

Results presented in Table (3) reveals that the corrected percentage of larval mortality had a positive relationship with different concentrations of acetone extract. The percentages of larval mortality were 24.99, 32.14, 49.99 and 67.86 at the concentrations of 0.25, 0.5, 1 and 2 %, respectively. On the other hand, there was an inverse relationship between acetone extract concentrations and the pupation percentage, (70, 63.33,

46.67 and 30 % at conc. of 0.25, 0.5, 1 and 2%), respectively, as compared with 93.33 in the control. The weight of the resulted pupae was affected as show in the Table (3). With regard to adult emergence, there was an increasing in the reduction percentage of adult emergence as expressed by 21.16, 34.61, 51.92 and 71.15 %, respectively, at the concentrations of 0.25, 0.5, 1 and 2%. While, the sex ratio was nearly remained unaffected except at higher concentrations.

Table 3: Biological influences of acetone extract of *Eichhornia crassipes* on 4<sup>th</sup> instar larvae of *S. littoralis*.

Conc. %	Corrected Larval mortality %	Pupation %	Pupal Weight gm Mean± SE	Pupal duration	Adult Emergence %	Total inhibition of adult Emergence %	Sex ratio %	
							male	Female
Cont	0.0	93.33±3.33	0.351±0.005	9.88±0.32	86.67±3.33	0.0	51.92	48.08
0.25	24.99	70±5.77	0.293 ±0.011	9.98±0.09	68.33±3.33	21.16	48.8	51.2
0.5	32.14	63.33±4.41	0.296± 0.011	9.5±0.10	56.67±1.67	34.61	46.67	53.33
1	49.99	46.67±3.33	0.297±0.01	8.92±0.08	41.67±1.67	51.92	46.15	53.85
2	67.86	30±5.7	0.274±0.015	9.08±0.3	25±7.64	71.15	25	75

**Effect of acetone extract of *Eichhornia crassipes*.**

It is evident from results presented in Table (4) that there was an effect induced by Hexane extract on the larval mortality as expressed in corrected percentages by 17.85, 24.99, 49.99 and 60.71 % at subsequent ascending concentration. The pupation percent was obviously declined as expressed by 76.67, 70, 46.67 and 36.67 % as well as

the pupal weight was also affected as shown in the Table (4). The percentage of adult emergence was calculated and it is clear at the higher concentration, the less percentage of adult emergences. For example, the percentage of adult emergence was 70, 66.67, 43.33 and 33.33 % as compared with 86.67 % in the control. It was noticeable that, the sex ratio took a tendency to female side at higher concentration only.

Table 4: Biological influences of petroleum ether extract of *Eichhornia crassipes* on 4<sup>th</sup> instar larvae of *S. littoralis*.

Conc. %	Corrected Larval mortality %	Pupation %	Pupal Weight gm Mean± SE	Pupal duration	Adult Emergence %	Total inhibition of adult Emergence %	Sex ratio %	
							male	Female
Cont	0.0	93.33±3.33	0.351±0.005	9.88±0.32	86.67±3.33	0.0	51.92	48.08
0.25	17.85	76.67±3.33	0.299± 0.013	9.98±0.27	70±2.87	19.23	45.24	54.76
0.5	24.99	70±5.77	0.308 ±0.016	9.98±0.09	66.67±8.81	23.08	42.5	57.5
1	49.99	46.67±3.33	0.293 ±0.011	8.92±0.08	43.33±3.33	50.01	46.15	53.85
2	60.71	36.67±3.33	0.278 ±0.001	7.67±0.33	33.33±3.33	61.54	40	60

### Effect of Kingbo Compound.

Results recorded in the Table (5) declare that there was a significant effect on the larval mortality that given in corrected percentages by 32.14, 44.64, 71.42 and 83.93 at the concentrations of 0.05, 0.1, 0.25 and 0.5 ppm, respectively. Also, the pupation percentage was greatly reduced to 63.33, 51.67, 26.67, 15 as compared with 93.33 % in the control.

The weights of the resulted pupae clearly affected when treatments carried out with different concentrations. The percentage of the adult emergence was decreased with an increase in the concentrations as indicated by 56.67, 41.67, 18.33 and 13.33%, respectively, at ascending successive concentration. Observation showed that sex ratio directed to female side.

Table 5: Biological influences of Kingbo compound on 4<sup>th</sup> instar larvae of *S. littoralis*.

Conc. ppm	Corrected Larval mortality %	Pupation %	Pupal Weight gm Mean± SE	Pupal duration	Adult Emergence %	Total inhibition of adult Emergence %	Sex ratio %	
							male	Female
Cont	0.0	93.33±3.33	0.351±0.005	9.88±0.32	86.67±3.33	0.0	51.92	48.08
0.05	32.14	63.33±4.41	0.289±0.008	9.98±0.09	56.67±1.67	34.61	47.06	52.94
0.1	44.64	51.67±4.409	0.283±0.004	8.60±0.53	41.67±4.41	51.92	48	52
0.25	71.42	26.67±3.33	0.263±0.015	8.92±0.08	18.33±6.009	78.85	45.45	54.55
0.5	83.93	15±2.887	0.221±0.004	8.33±0.33	13.33±1.67	84.61	28.57	71.43

### Residual Effect of Kingbo Compound.

At Qalyobia Governorate, data in Table (6) show the efficiency of recommended rates of tested compound against 2<sup>nd</sup> and 4<sup>th</sup> larval instars of *S. littoralis* under field- lab. condition. Experiment (1) reveals the efficiency of tested compound at zero time from application and the mortality percentage recorded after 48h. from experiment. The effect of kingbo recorded 66.7 and 56.67

% for 2<sup>nd</sup> and 4<sup>th</sup> larval instars of *S. littoralis*. The highest mortality recorded at Exp.(1) zero time and decreased gradually through (Exp. (2) at 3<sup>rd</sup> day from application, Exp.(3) at 5<sup>th</sup> day from application and Exp.(4) at 7<sup>th</sup> day from application.) to reach 13.33 and 6.67% for 2<sup>nd</sup> and 4<sup>th</sup> larval instars kingbo treatment at Exp.(4). This result indicates that, the persistence of this compound is very short.

Table 6: Corrected mortality % for 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of *S. littoralis* after treated *T. alexandrium* field by recommended rate of Kingbo compound

Compound	Recommended rate/feddan	Corrected larval mortality % at different time intervals									
		Initial		Residual						Residual Toxicity mean	
		Exp.(1) Zero time		Exp.(2) 3 <sup>rd</sup> day from application		Exp.(3) 5 <sup>th</sup> day from application		Exp.(4) 7 <sup>th</sup> day from application			
		2 <sup>nd</sup>	4 <sup>th</sup>	2 <sup>nd</sup>	4 <sup>th</sup>	2 <sup>nd</sup>	4 <sup>th</sup>	2 <sup>nd</sup>	4 <sup>th</sup>	2 <sup>nd</sup>	4 <sup>th</sup>
Kingbo 0.6%	600ml/feddan	100	83.33	63.33	50.0	23.33	16.67	13.33	6.67	33.3	24.45

### Phytochemical scanning in different extracts of *Eichhornia crassipes*.

Data in Table (7) show that, sterols, terpenoids and flavonoids in *Eichhornia crassipes* were detected in different

extracts. Also, phenol found in all extracts except in the case of pet. ether extract. Tannins appeared only in water extract. While resins and saponins were absent from all extracts.

Table 7: Phytochemicals scanning in different extracts of *Eichhornia crassipes*

Extracts Phytochemicals	Water	Ethyle alcohol	Acetone	Pet. ether
Sterol/terpenoids	++	++	++	+
Phenols	++	+	+	-
Flavonoids	++	++	+	++
Alkaloids	++	+	--	-
Tannins	++	--	--	--
Resins	--	--	--	--
Saponins	--	--	--	--

**Biochemical studies:**

**Changes in protein contents of *S. littoralis* haemolymph after treatment with the tested compounds.**

Result given in the Table (8) show that all tested compounds (water, Alcohol, Acetone and Pet.ether extract of *E. crassipe* and Kingbo) have mostly induced reduction in haemolymph

protein of 6<sup>th</sup> larval instar of *S. littoralis* treated as 4<sup>th</sup> larval instar with LC<sub>50</sub> except in the case of larvae treated with Pet.ether extract which showed non-significant effect compared with the control that is declared by their percentage changes:-21.03,-32.01,-16.12,-3.04 and -29.67 at the previous arrangement of tested compounds.

Table 8: Changes in Haemolymph protein contents of *S.littoralis* after treated 4<sup>th</sup> larval instar with tested compounds.

Compounds	Control	Water extract	Alcohol extract	Acetonextract	Pet. Ether extract	Kingbo
Total Protein mg protein/ml Mean±SE	4.28±0.098	3.38±0.02	2.91±0.146	3.59±0.133	4.15±0.05	3.01±0.209
% changes	--	-21.03	-32.01	-16.12	-3.04	-29.67

**Isozymes patterns**

**Esterase of larval haemolymph detected by using α-naphthyl acetate as substrate.**

The larval haemolymph of untreated and treated samples of *S. littoralis* had three bands of esterolytic activity capable of hydrolysing α-naphthyl acetate as substrate. The esterase patterns of larval haemolymph

had R<sub>f</sub> ranged from 0.26 to 0.40 as revealed in Table (9) and Figure (1). The band no. 2 and 3 were common bands for control and all tested samples with R<sub>f</sub> values (0.35and 0.4), respectively. While the bands no. 1 detected in control, water *E. crassipe* extract and alcohol *E. crassipe* extract samples with R<sub>f</sub> values 0.26.

Table (9): Relative fragmentation R<sub>f</sub> and amount percentage of *S. littoralis* haemolymph α-esterase pattern after treated the 4<sup>th</sup> instar larvae with *Eichhornia crassipes* extracts and Kingbo compound.

Band No.	Samples	Cont.	Amount %	w. ex.	Amount %	Alc. ex.	Amount %	Ac. ex.	Amount %	Pet. ex.	Amount %	K.	Amount %
	R <sub>f</sub>												
1	0.26	+	15.26	+	20.14	+	17.08	-	-	-	-	-	-
2*	0.35	+	49.25	+	50.26	+	53.07	+	59.91	+	67.41	+	61.93
3*	0.40	+	35.49	+	29.6	+	29.85	+	40.09	+	32.59	+	38.07

\* Asterisks indicator to common bands

(+) = Present band  
(-) = Absent band

Cont. = control

W.ex. = Water extract

Alc. ex. = Alcohol extract

Ac. = Aceton extract

Pet. ex. Pet. Ether extract

K. = Kingbo

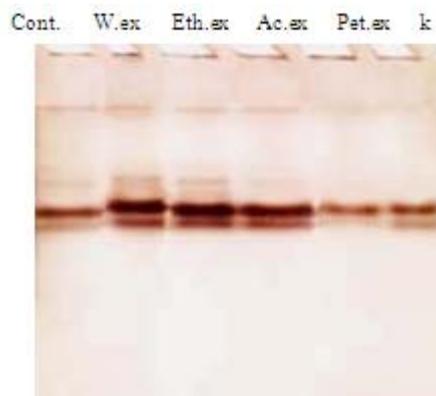


Fig. 1: Esterase pattern of larval haemolymph detected by using  $\alpha$ -naphthyl acetate as substrate

### Esterase of larval haemolymph detected by using $\beta$ -naphthyl acetate as substrate

The electrophoretic pattern of esterase was obvious through 4 electrophoretic bands with  $R_f$  ranged from 0.24 to 0.45 as shown in Table (10) and Figure (2). Total number of bands in untreated and treated samples water,

Alcohol, Acetone and Pet.ether extract of *E. crassipe* and Kingbo were 3, 4, 4, 3, 3 and 3, respectively. The band no. 2, 3 and 4 were common bands for control and all tested samples with  $R_f$  values (0.29 and 0.45), respectively. While the bands no. 1 detected in water *E. crassipe* extract and alcohol *E. crassipe* extract samples with  $R_f$  values 0.24.

Table 10: Relative fragmentation  $R_f$  and amount percentage of *S. littoralis* haemolymph  $\beta$ -esterase pattern after treated the 4<sup>th</sup> instar larvae with *Eichhornia crassipes* extracts and Kingbo compound.

Band No.	Samples $R_f$	Cont.	Amount %	w. ex.	Amount %	Alc. ex.	Amount %	Ac. ex.	Amount %	Pet. ex.	Amount %	Ki. ex.	Amount %
1	0.24	-	-	+	9.75	+	10.9	-	-	-	-	-	-
2*	0.29	0.29	11.53	+	17.36	+	14.16	0.29	11.43	0.29	18.41	0.29	16.2
3*	0.39	0.38	47.54	+	46.18	+	48.53	0.39	50.22	0.39	48.9	0.39	43.71
4*	0.45	0.43	40.94	+	26.71	+	26.41	0.45	38.35	0.45	32.69	0.45	40.09

For legends and abbreviations, see the foot note of table (9)

\* Asterisks indicator to common bands

(+) = Present band

(-) = Absent band

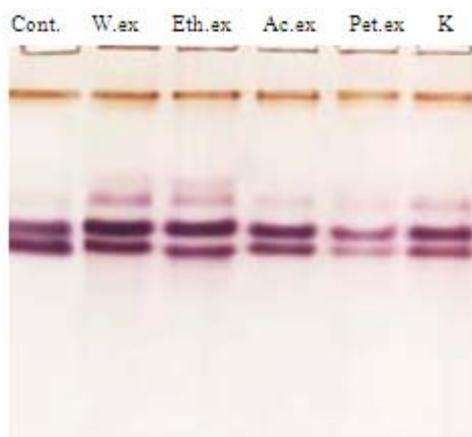


Fig. 2: Esterase pattern of larval haemolymph detected by using  $\beta$ -naphthyl acetate as substrate

### DISCUSSION

In order to avoid pesticides hazards, there is a great need to develop alternative safe control agents with new

modes of action. Among these agents are the plant extracts. Several plant extracts or isolated active compounds have been shown to act as potential acute or chronic

insecticides (Abdalla and Smmour, 1991), antifeedant (Dimetry and Abdalla, 1991) and insect growth regulators (Abo El-Ghar *et al.* 1994) against a variety of insect species including *Spodoptera littoralis*.

In the present study, Commercial plant extract named kingbo and *Eichhornia crassipes* extract extracted by different solvents, (Pet.ether, Acetone, Ethanol and Water), were tested against 4<sup>th</sup> larval instar of *S. littoralis*.

*Eichhornia crassipes* was extracted by different solvents, the most effective one on biological and biochemical parameters is ethanol extract followed by water, acetone and pet. ether extract, respectively. Therefore, the difference in their potency may refer to the quantity of extracted material rather than to the quality of such material (Mansour *et al.*, 1996). The insecticidal, antifeedant and growth inhibitor action of plant extracts to several insect species may be due to the presence of substance such as sterols, terpenoids, flavonoids, alkaloids, Tannins, saponins, ...etc. This observation was noticed by many authors. Banerji, (1988) found that leaves extract of *Abies balsamca* induce strong juvenile hormone mimic activity against insects due to presence of juvabione (steroid group). From the leaf extract of *Ajuga remota*, 3 terpenoids componets, Ajugarins I, II, III were isolated. Ajugorins 1 showed toxicity to *S. littoralis*, whereas all the three components exhibited antifeedant property to *S. exempta*. (Kubo and Nakanishi, 1978). Srinmannarayan and Rao (1985) and Geetanjali *et al.* (1987) also isolated a flavone, an active component, from *Pongamia glabra* seed extract and found it to show antifeedant activity to *S. litura*. The flavonoid isolated from the dodonae (leaves) had a toxic effect on 2<sup>nd</sup> larval instar of *S. littoralis* (Mogahed *et al.* 1997). Simmonds (2003) found that flavonoids affect the feeding behavior of a range of

noctuidae larvae. Torres *et al.*, (2009) suggests that the various Alkaloid extractes from *Lupinus* spp. act differently on caterpillars, and could be used to control *Spodoptera* populations. Also, Kingbo is a new type of botanical pesticide. The active ingredients are oxymatrine. Oxymatrine is a major alkaloid found in *Sophora flavescens* Aiton (Fabaceae). Mao and Henderson (2007) stated that matrine and oxymatrine (two major alkaloids) had a strong antifeedant and toxic effects against Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). At the present study, the use of *E. crassipes* extracts and Kingbo compound for treatment 4<sup>th</sup> larval instar of *S. littoralis* caused higher larval mortality, decrease pupation, pupal weight and adult emergence. These aspects may be referring to the presence of sterol, terpenoids, phenol, flavonoid, alkaloid and tannins in the tested compounds.

In addition, the present findings showed inhibition effect on total protein content in 6<sup>th</sup> larval instar haemolymph except pet. ether extract clarify non significant effect. Changes in the protein content of the haemolymph may reflect specialization and adaptation in the organisms. It is worthy to note that each protein is considered as reflect to the activity of specific gene through the production of enzyme, which act as catalyst to produced the demanded protein; this type of produced protein is responsible for a specific biological character. Thus, the inhibition of protein can explain some other experimental results such as reduction in pupal weight, tissue degeneration and preventing adult emergence. These results are confirmed with that obtained by Schoter (1985) who found that the storage of proteins in fat bodies, which is necessary for pupation, did not occur when treated the last larval instar of *E. varivestis* with higher doses of azadirachtin.

Also, esterase activity examined because of the importance of carboxylesterases in the catabolism of juvenile hormone (Slade and Zibitt, (1972); Whitmore *et al.*, (1972); Ajami and Riddiford, (1973)). JH esterase appears to be an important mode of regulation of JH titers in Lepidoptera (Venkatesh *et al.*, 1987). The change in haemolymph JH metabolic activity appears to result from changes in JH specific esterases as 1 - naphthyl acetate esterase activity, follows a different pattern during the reproductive /parental cycle (Scott *et al.*, 2001). In addition AchE serve as specific proteases involved in the breakdown of bioactive peptides or their precursors (Small *et al.*, 1987; Sikorov *et al.*, 1988). In general, there are several indications that AchE activity may be correlated with development in vertebrates and insects (Lenoir-Rousseaux and Gautron, 1987). Therefore, electrophoresis plays a major role in identifying esterases (Grafton – Cardwell *et al.*, 1998). In the present work polyacrylamide gel electrophoresis were used for separation of different enzymes which help in explanation of different biological processes that occur inside the living organisms due to use of tested compound. The results showed great differences in number of zones of esterase activity and in substrate specificity between treated and untreated samples. These events would finally lead to the observed failure in metamorphosis, which is characterizing the evaluation of unhealthy adults. These findings are in full agreement with El-Bermawy, (2004) who analyzed esterases from body extracts of 6<sup>th</sup> larval instar and newly formed pupa of *S. littoralis* produced from treated 2<sup>nd</sup> larval instar by four botanicals using polyacrylamide gel electrophoresis and two substrates.

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## ARABIC SUMMARY

تأثير مستخلص ورد النيل و مركب الكينج بو على بعض المظاهر البيولوجية والكيموحياتية على دودة ورق القطن اسويدبتر ليتورالز

هبة عبد الوهاب حسن

معهد بحوث وقاية النباتات- مركز البحوث الزراعية – الدقى- جيزة.

ويعتبر نبات ورد النيل من أسوأ النباتات المائية في العالم حيث انه يسبب خطرا كبيرا للأنشطة التنموية للبلاد. ولذلك كان الهدف الرئيسي من هذه الدراسة هو تحويل هذا النبات الى منتجات اقتصادية كالمبيدات الحشرية التي تستخدم على نطاق واسع في مكافحة الافات في العالم بأسرة ولقد تضمنت هذه الدراسة بعض التجارب العملية لتقييم تأثير مستخلص نبات ورد النيل و الذى استخلص بأربعة مذيبات عضوية متزايدة القطبية و مركب الكينج بو على بعض المظاهر البيولوجية لدودة ورق القطن الكبرى من حيث نسبة الموت فى اليرقات و نسبة التعذر و وزن العذارى و النسبة الجنسية و نسبة خروج الطور اليافع الناتج عن كل معاملة. كما شخصت المكونات الفعالة فى نبات ورد النيل. كما تم بحث الكفاءة والآثار المتبقية لمركب الكينج بو على دودة ورق القطن الكبرى تحت ظروف الحقل. إضافة إلى ذلك اشتملت الدراسة على التغيرات البيوكيميائية الحادثة للبروتين الكلى ومستوى الكربوهيدرات و الدهون الكلية للحشرة موضع الدراسة وكذلك التغير فى خمائل الاستيريز. وقد كشفت النتائج أنه كلما زاد تركيز المركبات المختبرة كلما زادت نسبة الموت ونسبة خروج الفراشات كما ادت جميع المركبات التى خضعت للاختبار الى انخفاض وزن العذارى مقارنة بالكنترول. وقد أثبت التحليل الكيميائى لوجود كل من مجموعات الأستيرولات والتربينات فى المستخلصات المختلفة لنبات ورد النيل وكذلك مجموعة الفلافونيد وجدت بجميع المستخلصات بينما اختفت تماماً مجموعات الصابونين والراتجات من جميع المستخلصات المختلفة لنبات ورد النيل. كما اظهرت النتائج انخفاض المحتوى البروتينى الكلى فى دم الطور اليرقى السادس الناتج من معاملة العمر اليرقى الرابع بالمركبات المختبرة وكذلك ادت المعاملة بالمستخلص الاسيتونى والاثير البترولى لنبات ورد النيل ومركب الكينج بو الى اختفاء بعض خمائل الاستيريز التى لها القدرة على هضم الالفا نفثيل اسيتات بينما ادت المعاملة بالمستخلص المائى والمستخلص الكحولى لنبات ورد النيل الى ظهور بعض خمائل الاستيريز التى لها القدرة على هضم البيتا نفثيل اسيتات .