

LABORATORY STUDIES ON CINNAMON, CINNAMOMUM ZEYLANICUM NEES BARK EXTRACTS AGAINST GRANARY WEEVIL, SITOPHILUS GRANARIUS LINNAEUS (COLEOPTERA : CURCULIOIDAE)

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ABSTRACT: *The toxic effect of wheat grains treated with cinnamon, Cinnamomum zeylanicum bark using different extracts offered to granary weevil, Sitophilus granarius L. was determined. Petroleum ether extract was found to be the most effective. Reproductive potential of treated weevils was strongly affected as the number of egg laid reduction. Progeny were obtained when adults were fed on wheat grains treated with either LC₅₀ or LC₉₅ of tested extracts. Treatment with LC₉₅ of extracts protected wheat grains up to seven weeks of storage. All tested extracts caused a few reduction in germination at the initial time, while it was increased at the end of storage period. Treatment with cinnamon reduced the weight loss of insect infestation grains with the granary weevil. Biochemical studies showed that protein and carbohydrate contents were decreased, while total lipids content was increased. Furthermore, the tested extracts caused a decrease in GOT and GPT as compared with control. Petroleum ether extract caused an inhibition in the activity of cholinesterase, while chloroform and acetone extracts increased such activity.*

Key words: *Cinnamon extract, Granary weevil, reduction.*

INTRODUCTION

The control of pest infestation in stored grains is mainly depending on the use of conventional insecticides which leads to several problems such as toxic residues and environmental contamination (Zettler and Cuperus, 1990 and White 1995). Indigenous materials of botanical origin are an important source of grain protectants, because they have been found to exhibit toxic effects against insects (Arroyo, 1995). The use of plant or their extracts exhibiting an insecticidal activity or insecticidal synergistic activity against several insect species (Afifi *et al.* 1989, Salwa 2001 and Salwa, *et al.* 2006).

The granary weevil, *Sitophilus granarius* L. is one of the most important insect pests, as it infests stored grains and consumed it. Furthermore, it contaminate the grains with insect fragments, faeces, webbing, ill-smelling metabolic products and with a variety of microflora. Therefore, it causes a high damage, major sanitation and quality control problem.

The present work was conducted to evaluate the bioactivity of cinnamon, *Cinnamomum zeylanicum* bark extracts on the granary weevil, *Sitophilus*

granarius L. Also, several researchers showed that some enzymes and some contents in several insect species are affecting by feeding them on a diet treated with the plant extract (Mitlin *et al.*; 1977 Ahmad *et al.*, 2000 and Abd El-Wahab, 2002). For this reason the main total contents (protein, carbohydrate and lipid) and several enzymes in weevil adults were estimated to clarify if they were affected in treated insects.

MATERIALS AND METHODS

A- Rearing technique:

The stock culture of *Sitophilus granarius* L. was obtained from a laboratory strain maintained in the Stored Grains Pest Research Dept, Plant Protection Research Institute, Agricultural Research Centre, Dokki, Giza. The weevil were maintained on wheat grains for several generations under laboratory conditions of $27 \pm 1^\circ\text{C}$ and 65 ± 5 R.H., without any insecticidal pressure adults, two weeks old, were used in the current study.

B-Preparation of cinnamon and the extraction:-

Cinnamon, *Cinnamomum zeylanicum* Nees (Family Lauraceae) bark was purchased from local market and grinding into a fine powder by electrical home mill. The grinded powder was extracted first with petroleum ether (with ratio of 1:3 powder/solvent) in a flask and left for 48hr. Then, the extract was filtered and the solvent was evaporated under reduced pressure using a rotary evaporator. The detained powder was thoroughly dried before being extracted next with chloroform, then acetone solvent as adopted from Afifi *et al.* (1989).

C-Evaluation of extracts toxicity:-

Toxicity of cinnamon as extracted by the three organic solvents was determined by adding different concentrations ranging from 0.1 up 0.45 mg/kg for petroleum ether, 0.4 up to 5.0 mg/kg for chloroform and 0.5 up to 4.0 mg/kg for acetone to wheat grains.

Twenty five *S. granarius* adults, two weeks old, were obtained from the maintained stock culture and placed on the treated wheat grains which were then placed in glass tubes. The tubes were covered with muslin fixed with rubber bands. Control was prepared containing untreated grains. After three, five, seven and 14 days, the tubes were investigated and the number of dead weevils was recorded. Accumulated mortality percentage (LC_{50} and LC_{95}) and regression lines slope were determined and corrected using Abbott's formula (1925) and computed mortality percentages were conducted after 72 hrs. exposure according to Finney (1952).

D-Effect of cinnamon extracts on fecundity and F1 progeny of *Sitophilus granarius*:-

Eighteen weights of 10 gm. of wheat grains were prepared and each 10 gm of grains were treated with the determined LC_{50} or LC_{95} of each of cinnamon

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as extracted by the three organic solvents. The treated wheat grains were placed in glass tubes (1x 3 inches). Subsequently, five couples of *S. granarius*, two weeks old, were introduced into each tube. After two weeks, the insects were removed and the number of deposited eggs on the grains was counted according to the method described by Frankenfeld (1948) and Howe (1952).

The same previous experiment was repeated, but laid eggs were left undisturbed until hatching. After two weeks, adults were removed and the tubes were left for seven weeks, up to progeny emergence and the number of adult emerged was recorded. All of the aforementioned experiments and untreated wheat were replicated three times.

E-Assessment of residual efficiency of cinnamon organic extracts:-

Tubes containing 10 gm of wheat grains were treated with LC₉₅ of each extract. Then, divided into several groups and stored. Three tubes were selected every week and twenty five adults of *S. granarius* were introduced into each tube. This process was repeated weekly for ten weeks. Mortality counts were carried out following the 3rd day of introducing the weevils. Insect mortality percentages were calculated and corrected according to Abbott's Formula (1925). Similarly, three replicates of untreated wheat were used as control for each week.

F-The effect of cinnamon extract on grain germination:-

Germination of the wheat grains treated with LC₉₅ of cinnamon extracts was determined according to the international rules for seed testing (Anonymous, 1966) at the initial time and the end of the considered storage period (10 weeks).

G- Weight loss of treated wheat grains with cinnamon extracts:-

Percentage moisture content of wheat grains treated with cinnamon bark organic extracts was determined according to the equation reported by Khare and Johari (1984) equation:

$$\text{Weight loss \%} = \frac{\text{Initial dry weight} - \text{Final dry weight}}{\text{Initial dry weight}}$$

H-Biochemical determinations:

1 - Preparation of samples for biochemical analysis:

Homogenate for biochemical assay was obtained from the two weeks old of *S. granarius* weevil adults when it offered to wheat grains treated with LC₅₀ of cinnamon extracts. After 48 hrs. of feeding, the insects were removed. Weights of 0.2 gm of these weevils were homogenized in distilled water using

a Teflon homogenizer surrounded with a jacket of crushed ice for three minutes. Homogenates were centrifuged at 6000 r.p.m. for 10 minutes at 5°C. After centrifugation, the supernatant fluid was divided into small aliquots (0.5 ml) and stored at -20 °C until analysis. Three replicates were carried out for each biochemical determination.

2- Determination of total protein content:-

The protein content of the homogenate was determined using folin phenol reagent according to the method of Lowry *et al.* (1951).

3 - Determination of total carbohydrates content:-

The total carbohydrates content of the homogenate was determined according to Singh and Sinha (1977).

4- Determination of total lipids content:-

The total lipids content of the homogenate was determined by the phosphovallin method of Baronos and Blackstock (1973).

5- Determination of transaminases activity:-

The level of both transaminases (GOT and GPT) was determined calorimetrically according to Reitman and Frankel (1957).

6- Acetylcholine esterase (AChE):-

This enzyme was determined according to method described by Simpson *et al.* (1964).

RESULTS AND DISCUSSION

Toxicity of cinnamon bark extracts on *Sitophilus granarius*:-

Data in Table (1) show the susceptibility of *Sitophilus granarius* weevil adults, two weeks old, towards the three extracts (petroleum ether, chloroform and acetone) by feeding. The data indicated that the mortality percentages were increased by increasing the concentrations of used solvents. A high concentration induced 100% mortality after seven days of treatment. On the other hand, a low concentration induced 62%, 64% and 59% mortalities for petroleum ether, chloroform and acetone, respectively. Based on LC₅₀ values of the three tested extracts (Table 2) the toxicity could be arranged in descending order as follow: petroleum ether 0.17 ml/kg > acetone 0.9 ml/kg > chloroform 1.22 ml/kg. This indicated that petroleum ether was the most effective extracts against the tested insect. The difference in toxicity was significant. The obtained data is similar to Ahmed *et al.* (2006) who mentioned that cinnamon bark extracts has an effect on *Rhizopertha dominca* (Fab).

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Table (1): Effect of cinnamon bark extracts against adults of *Sitophilus granarius* L.

Extracts	Concentration ml/kg	Mean mortality % ± S.E. after indicated days				
		One	Three	Five	Seven	Fourteen
Petroleum ether	0.1	± 0.6 2	0.0 ± 25	1.73 ± 33	0.0 ± 62	2.0 ± 79
	0.2	0.0 ± 0	7.6 ± 60	0.0 ± 78	±0.0 100	0.0 ± 100
	0.3	1.5 ± 16	2.9 ± 75	2.52 ± 90	±0.0 100	0.0 ± 100
	0.4	1.9 ± 20	5.5 ± 85	0.0 ± 100	±0.0 100	0.0 ± 100
	0.45	0.0 ± 20	4.15 ± 90	0.0 ± 100	±0.0 100	0.0 ± 100
Chloroform	0.4	0.0 ± 0	2.5 ± 20	0.0 ± 39	0.0 ± 64	1.7 ± 72
	0.8	0.0 ± 0	3.5 ± 35	1.0 ± 72	±0.0 100	0.0 ± 100
	1.0	0.0 ± 0	0.0 ± 45	1.73 ± 83	±0.0 100	0.0 ± 100
	2.0	2.6 ± 10	1.15 ± 60	0.0 ± 100	±0.0 100	0.0 ± 100
	4.0	2.4 ± 14	2.5 ± 80	0.0 ± 100	±0.0 100	0.0 ± 100
	5.0	2.5 ± 26	0.0 ± 94	0.0 ± 100	±0.0 100	0.0 ± 100
Acetone	0.5	0.67 ± 4	4.0 ± 30	0.57 ± 45	±2.0 59	2.4 ± 83
	1.0	0.88 ± 4	4.17 ± 60	0.0 ± 83	100±0.0	0.0 ± 100
	3.0	0.0 ± 12	1.5 ± 70	0.0 ± 100	100±0.0	0.0 ± 100
	4.0	2.6 ± 29	0.58 ± 90	0.0 ± 100	100±0.0	0.0 ± 100

Table (2): Values of LC₅₀, LC₉₅ and slopes of regression lines for the three different extracts of cinnamon bark against *Sitophilus granarius* L.

Extracts	LC ₅₀ ml/kg	LC ₉₅ ml/kg	Slope
Petroleum ether	0.17	0.63	2.89
Chloroform	1.22	9.0	1.90
Acetone	0.9	7.8	1.79

Effect of cinnamon bark extracts on fecundity and F₁ progeny of *Sitophilus granarius*:-

Wheat grains treated with cinnamon extracts and offered to *S. granarius* weevils reduced their fecundity as well as the number of F₁ adults emerged progeny (Table 3). At LC₅₀ level, cinnamon chloroform extract proved to be the most effective in this respect as the number of laid eggs by 5 couples was the lowest as compared to control (a reduction equal to 91%). This was followed by 87% and 80% reduction for acetone and petroleum ether extracts, respectively. At all cases, no progeny produced or eggs were laid when the extracts were used at LC₉₅ levels. No progeny appeared in all treatments with organic extracts at LC₅₀ or LC₉₅, (Table 3).

Table (3): Effect of cinnamon bark extracts on fecundity and F₁ progeny of *Sitophilus granarius* L.

Extracts	Concentration ml/kg	Mean No. of eggs/5 pairs ± S.E.	Reduction %	Mean No. of emerged progeny ± S.E.	Reduction %
Petroleum ether	LC ₅₀ 0.17	3.7 ± 1.2 a	80	0 a	100
	LC ₉₅ 0.63	0 ± 0.0 b	100	0 a	100
	Control	19 ± 1.74 c		7 ± 2.0 b	
Chloroform	LC ₅₀ 1.22	1.7 ± 0.33 a	91	0 a	100
	LC ₉₅ 9.0	0 ± 0.0 b	100	0 a	100
	Control	20.3 ± 1.77 c		7.66 ± 0.88 b	
Acetone	LC ₅₀ 0.9	2.3 ± 0.33 0a	87	0 a	100
	LC ₉₅ 7.8	0.33 ± 0.33 0b	98	0 a	100
	Control	18.3 ± 1.2 c		7.66 ± 0.88 b	

Residual effect of cinnamon bark extracts on wheat grains offered to *Sitophilus granarius* weevil:-

The residual toxic effect of tested extracts at LC₉₅ level (Table 4) showed that the effect of these extracts was relatively stable up to ten weeks of storage. At all treatments extracts were persistent up to seven weeks of storage at which the mortality of *S. granarius* weevil adults two weeks old, ranged from 92% to 94% meanwhile, at the 8 week, the persistence of the three tested extracts was slightly deteriorated to 61, 72 and 75% for petroleum ether, chloroform and acetone, respectively. Acetone extract was the most effective one at which it can remain persistent for longer period.

Table (4): Corrected mortality percentages (Mean ± S.E.) of *Sitophilus granarius* L. exposed to wheat grains treated with LC₉₅ of tested extracts after different post treatment periods

Period after treatment (weeks)	Petroleum ether	Chloroform	Acetone
Initial *	95 ± 5.2 a	96 ± 0.0 a	95 ± 1.16 a
1	96 ± 0.0 a	96 ± 0.58 a	95 ± 0.0 a
2	95 ± 0.58 a	95 ± 1.16 a	94 ± 0.57 a
3	95 ± 1.16 a	95 ± 1.43 a	95 ± 1.6 a
4	95 ± 0.0 a	94 ± 1.0 a	95 ± 0.0 a
5	94 ± 0.0 a	95 ± 0.0 a	95 ± 2.52 a
6	93 ± 1.53 a	95 ± 0.0 a	93 ± 0.0 ab
7	94 ± 0.0 a	94 ± 1.53 a	92 ± 1.16 ab
8	88 ± 1.16 b	90 ± 1.16 b	90 ± 0.0 b
9	70 ± 2.52 c	84 ± 1.16 c	86 ± 1.16 c
10	61 ± 0.58 d	72 ± 1.53 d	75 ± 1.16 d
L.S.D.	3.98	3.11	3.42

* Initial = at the beginning of the treatment.

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Effect of tested extracts on germination of wheat grains:-

The germination of wheat grains was slightly reduced following treatments with LC₉₅ levels of cinnamon bark extracts. This effect was more apparent when acetone was used as a solvent for extraction, followed by chloroform and petroleum ether i.e, 88, 90 and 91%, respectively. Meanwhile, after ten weeks of storage of treated wheat grains, germination was 83, 89 and 86% for acetone, chloroform and petroleum ether, respectively, (Table 5). These results are in agreement with Khair *et al.* (1992) and Pacheco *et al.* (1995) who reported adverse effects of plant extract treatment on the germination percent of grains.

Table (5): Germination of wheat grains stored for ten weeks after treatment with LC₉₅ of cinnamon bark extracts.

Extracts	LC ₉₅ ml/kg	Initial (zero time)		After ten weeks of storage	
		Mean germination% ± S.E.	Reduction%	Mean germination% ± S.E.	Reduction%
Petroleum ether	0.63	91 ± 0.58 a	7.14	86 ± 3.06 a	11.34
Chloroform	9.0	90 ± 1.16 a	8.16	89 ± 2.3 a	8.25
Acetone	7.8	88 ± 1.73 a	10.20	83 ± 3.0 ab	14.43
Control	-	98 ± 0.0 c	-	97 ± 1.0 d	-
L.S.D.		3.82			

* Initial = at the beging of the treatment.

Weight loss of treated wheat grains with cinnamon bark extracts:-

Wheat grains treated with LC₅₀ levels of the tested extracts showed a reduction in weight loss ranging between 80.40 and 86.59% in wheat grains as compared to control. Treatment with LC₉₅ levels reduced this loss to 93.25-97.49% than the control. Acetone extract resulted a most efficiency, while, chloroform extract showed the lowest effect (Table 6). These results agree with Al-Moajel (2004) who found that the treatment of wheat grains with mesquite plant extracts (petroleum ether, chloroform and acetone), reduced the weight loss in the grains. The same results were obtained by Salwa *et al.* (2006) who reported that the use of cinnamon extracts reduced the weight loss in the grains.

Effect of tested extracts on the main homogenate contents (total protein, carbohydrate and lipid) of *Sitophilus granarius*:-

Results in Table (7) showed that treatment of *S. granarius* weevil adults with LC₅₀ of the three tested solvents induced a decrease in whole body protein and carbohydrates contents of treated insects. The highest reduction

in the whole body protein content was recorded after treatment with acetone extract, where it was 13.94 mg/ml, followed by petroleum ether and chloroform extracts with values of 14.87 and 15.96 mg/ml, respectively, compared to 18.59 mg/ml for untreated ones. Similar reduction in total protein content of *Sitophilus oryzae* (L.) after treatment with *Acorus calamus* extracts Ahmad *et al.*, (2000). There was a direct correlation between developmental retardation and protein reduction. This may explain the delayed metamorphosis and death of the treated insects.

Table (6) Effect of the three tested extracts on the weight loss of wheat grains

Extracts	Concentrations ml/kg	Dry weight loss %	Reduction%
Petroleum ether	LC ₅₀ 0.17	3.48 a	84.94
	LC ₉₅ 0.63	1.56 b	93.25
Chloroform	LC ₅₀ 1.22	4.53 a	80.40
	LC ₉₅ 9.0	1.45 b	93.73
Acetone	LC ₅₀ 0.9	3.10 ab	86.59
	LC ₉₅ 7.8	0.58 c	97.49
Control		23.11 d	
L.S.D.		1.74	

Table (7): Effect of LC₅₀ of the three tested extracts of cinnamon bark on the main total contents (protein, carbohydrate and lipid) of *Sitophilus granarius* L. adults

Extracts	Mean total protein mg/ml ± S.E.	Mean total carbohydrate mg/ml ± S.E.	Mean total lipid mg/ml ± S.E.
Petroleum ether	14.87 ± 0.56 a	8.75 ± 0.82 a	22.06 ± 0.83 a
Chloroform	15.96 ± 0.51 a	11.19 ± 0.31 b	31.55 ± 0.90b b
Acetone	13.94 ± 0.56 a	8.54 ± 0.50 a	26.85 ± 1.36 c
Control	18.59 ± 0.57 b	11.93 ± 0.87 b	18.43 ± 0.12 d
L.S.D.	2.43	1.85	2.17

The decrease in the protein content in treated adults in the present study might be due to inhibition of DNA and RNA synthesis as suggested by Mitlin *et al.* (1977). Or it may be due to decrease in activity of GPT known to be related to protein synthesis as obtained in the present study.

Acetone and petroleum ether extracts caused a significant decrease in total content of carbohydrates. The highest reduction was noticed after treatment with acetone extract, where it was 8.54 mg/ml compared to 11.93 mg/ml for untreated ones. On the other hand, chloroform extract induced an insignificant decrease. Similar results were obtained for *Spodoptera littoralis* treated with acetone extracts of *Melia azadiracht* (Abd El-Wahab, 2002). Decreasing the total carbohydrate content may be attributed to a decrease in

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the activity of trehalose. Moreover, such decrease may be attributed to the antifeeding action of these compounds (Salem, 1994). Compared with protein and carbohydrate contents all the tested solvents caused a significant increase in the total lipid content of body of treated adults. Chloroform extract caused a highest increase in the contents of total lipid, where it was 31.55 mg/ml compared to 18.43 mg/ml for control.

Data obtained in Table (8) revealed that chloroform extract caused a significant increase in the activity of Aspartate Glutamate Aminotransferase (GOT) in the body of treated individuals, where it was 6.66 μ g oxaloacetate min/ml compared to 4.34 μ g oxaloacetate min/ml for untreated ones. On the other hand, petroleum ether caused a significant decrease in the activity of the enzyme.

Also, the results indicated that all tested solvents induced significant decrease in the activity of Glutamic Pyruvic Transaminase (GPT) of treated adults. The highest significant decrease was noticed after treatment with Petroleum ether extract, where it was 8.34 μ g pyruvate / min/ml compared to 27.61 μ g pyruvate /min/ml for untreated adults, followed by chloroform and acetone extracts, respectively.

Maintenance of the balanced amino acid pool in insects is the results of various biochemical reactions carried out by a group of enzymes called amino acid transaminase (Meister, 1957). The transaminase enzymes have been correlated with protein synthesis in many tissues of insects and the changes in their levels are used as indicators of the metabolism of protein and amino acids (Sutholt, 1976). This finding was confirmed by the reduction in protein content caused by cinnamon bark in the present study. The amino acid transferase especially alanine amino transferase (GPT) is one of the components of oxidative metabolism of protein which in certain insects is utilized during the initiated periods of flight (Bursell, 1963), it also acts as a catalytic factor in the metabolism of carbohydrate (Katunuma *et al.*, 1968). Thus, a decrease in the activity of GPT in the present study may be the cause of the decrease in carbohydrate content detected in the treated insects.

Data obtained in Table (8) revealed that acetone extract caused a significant increase in cholinesterase activity in treated adults of *S. granarius*, where it was 1479.05 μ g acetylcholine Br. / min/ml compared to 1230.83 μ g acetylcholine Br. / min/ml for untreated ones. Petroleum ether caused an insignificant decrease in the enzyme activity, while, chloroform extract caused an insignificant increase. From all the previous results, it could be concluded that acetone extract of cinnamon, *Cinnamomum zeylanicum* bark caused a highest decrease in protein and carbohydrates content and a highest increase in cholinesterase activity in treated individuals. In other words, it disrupts many physiological process which leads to death of insects than the other two tested solvents.

Table (8): Effect of LC₅₀ of the three tested extracts of cinnamon bark on the mean activities of aminotransferases and acetylcholinesterase of *Sitophilus granarius* L. adults

Extracts	Mean activity of GOT (µg oxaloacetate/min/ml) ± S.E.	Mean activity of GPT (µg pyrovate/min/ml) ± S.E.	Mean activity of Acetyly - cholinesterase (µg A.ch.Br /min/ml) ± S.E.
Petroleum ether	3.65 ± 0.37 a	8.34 ± 0.23 a	1126.42 ± 56.18 a
Chloroform	6.66 ± 0.17 b	19.37 ± 0.72 b	1245.64 ± 35.72 a
Acetone	4.54 ± 0.08 c	20.88 ± 0.60 b	1479.05 ± 6097 b
Control	4.34 ± 0.12 c	27.61 ± 0.96 c	1230.83 ± 46.54 ac
L.S.D.	1.63	4.18	123.77

A.ch.Br : Acetyly-Cholin Bromide

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دراسات معملية علي مستخلصات قلف القرقة ضد سوسة الحبوب

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الملخص العربي

- ١- تم تقدير تأثير معاملة حبوب القمح بمستخلصات قلف القرقة علي سوسة الحبوب وتم تحديد التركيز القاتل لـ ٥٠% وكذا القاتل لـ ٩٥%.
- ٢- وجد أن مستخلص الايثيرالبترولي كان أكثر كفاءة عند استخدامه للمستويين ٥٠% ، ٩٥%.
- ٣- تأثرت الكفاءة التناسلية للحشرة بالتركيز القاتل لـ ٥٠% وكذا التركيز القاتل لـ ٩٥% حيث حدث إنخفاضاً شديداً في عدد البيض ولم تنتج أي خلفة من الحشرات المعاملة.
- ٤- وجد أن معاملة الحبوب بالتركيز القاتل لـ ٩٥% أعطي حماية من الإصابة لفترة سبعة أسابيع.
- ٥- تأثر الإنبات في بداية التخزين تأثيراً طفيفاً وزاد هذا التأثير قليلاً عند نهاية فترة التخزين.
- ٦- الحبوب المعاملة بالتركيزات القاتلة لـ ٥٠% ، ٩٥% للمستخلصات المختبرة أدي إلي إنخفاض الفقد في الوزن مقارنة بالغير معاملة.
- ٧- أوضحت الدراسات البيوكيميائية أن محتويات أجسام الحشرات المغذاة علي حبوب معاملة بالمستخلصات المختبرة أن البروتين والكربوهيدرات والدهون قد نقصت كميتها.
- ٨- أظهرت النتائج إنخفاضاً واضحاً في نشاط الإنزيمات الناقلة للأمينات مثل GOT, GPT في أجسام الحشرات المعاملة.
- ٩- تسبب مستخلص الإيثيرالبترولي في تثبيط أنزيم الكولين استريزأما الكلوروفورم والاسيتون فتسببا في زيادة نشاطه.