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# LATENT EFFECT OF DIFFERENT COMPOUNDS ON *Pectinophora gossypiella* (SAUNDERS)

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## ABSTRACT

In the present work newly hatched larvae of Pink bollworm, *Pectinophora gossypiella* (Saund.) were fed on diet treated with $LC_{50}$ of four compounds two IGR's, chromafonozide 80% & diflubenzeroun48% and two oils super misrona (mineral oil) & linseed oil( botanical oil) to investigate their toxicity, biological aspects and the biochemical impacts in full grown larvae**.**

Obtained results show that the tested compounds affected the PBW larvae by increasing larval and pupal duration, total immature stages, pre oviposition, oviposition and post oviposition period, on contrary these compounds decrease weight of larvae and pupae, longevity of females and males, fecundity of females (total laying eggs/♀) and the percentages of egg hatchability than untreated ones. In addition ,the biochemical analysis revealed that the tested compounds, Chromafonozide, Diflubenzeroun, mineral oil and linseed oil when used at $LC_{50}$ values caused significant reduction in soluble protein ,total lipids and ALT and AST content in adult which caused inhibition and/or reduced the reproductive potentiality compared to control.

**Keywords:** Toxicity, biological aspects ,biochemical analysis, *P. gossypiella*, PBW
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## INTRODUCTION

The pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) is considered one of the most injurious cotton pests. Larvae feed on flower buds, flowers, bolls and the juicy seeds causing damage to developing seeds, and the termination of growth results in boll rotting, premature or partial boll opening, reduction of staple length, strength and increases trash content in the lint (Lykouressis *et al*., 2005). Noble (1969) mentioned that newly hatched larvae of PBW entered fruiting forms within 30 minutes. For this reason it is difficult to control it. In Egypt, cotton control programs including different sprays with conventional insecticides are recommended by Egyptian Ministry of Agriculture to combat this pest. So, many investigators studied the effect of different insecticides in rotations against bollworms infesting cotton plants during successive seasons under different levels of infestation to determine the best sequence for pests' control Hegab (2002), Al – Shannaf (2002); and Zaki, (2006). Insect growth regulators (IGRs) have a more specific mode of action on pests and are not highly toxic to non- target organisms when compared to many conventional insecticides. These characteristics, the use of IGRs appears promising in Integrated Pest Management (IPM). However, there are few studies on the affects of these types of compounds on the Lepidopterous insects, especially, *P. gossypiella*. used $LC_{50}$ or sub lethal-doses of deferent IGRs compounds applied topically to eggs and/or newly hatched larvae of *P. gossypiella*. These compounds caused significant mortality in subsequent developmental stages

prolonged larval, pupal periods and the latent effect appears on the longevity, fecundity of adult stage & fertility of eggs, it was effective with a relatively slow but strong action, ( Tasei 2001,Toscano 2007, Kandil, 2005, El-Shennawy 2009 and Kandil *et al.* 2012). Also, some authors are interested in the application of natural products as a factor to complement chemical control or as component of Pest management. The botanical oils and mineral oils are considered important compounds play this role in the last two decade.

The present study was carried out to determine the toxicity of Chromafonozide (Virtu (80%), Diflubenzuron (Dimilin (48%), Mineral oil Super Misrona and linseed oil (Flaxseed) against newly hatched larvae of *Pectinophora gossypiella* (Saunders). The study was extended to investigate the effect of these compounds on some biological aspects for immature and adult stages of PBW resulted from treated newly hatched larvae. In addition, the main metabolites levels of total proteins, total lipids and some vital enzyme activities of transaminases; Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were studied in the full grown larvae resulted from treated newly hatched larvae.

## MATERIALS AND METHODS

**Insect used:**

A laboratory strain of the pink bollworm, ***P. gossypiella*** was reared at Bollworms Research Department, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt, on semi artificial diet as described by **Abd El-Hafez *et al.* (1982)**. Rearing conditions were adjusted at 27±1$^{o}$C and 70-75% RH.

**Pesticides used:**

Two insect growth regulators ($IGR_S$) and two oils were experimentally used in this study:

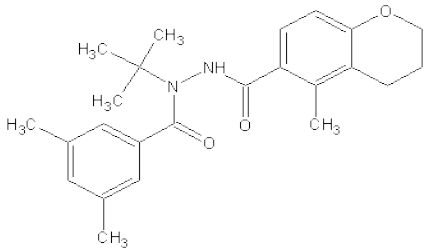
**Insect growth regulators**

1**- Common name:** Chromafonozide

**Trade name:** Virtu (80%)

**Chemical name:** 3,4 DIHYDRO-5-METHYL-2H-1-benzopyran -6-Carboxylic acid 2-(3-5-dimethyl benzoyl)-2-(1,1-dimethyl) hydrozide.

**Structural formula:**

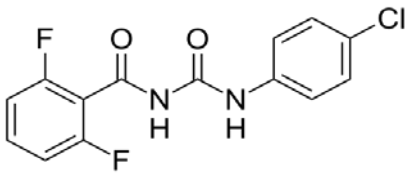



**2- Common name: Diflubenzuron.**

**Trade name :** Dimilin (48%)

**Chemical name:**N- [(4-chlorophenyl) amino]-carbonyl]-2,6-diflorobenzamide]

**Structural formula:**




**Oils:**

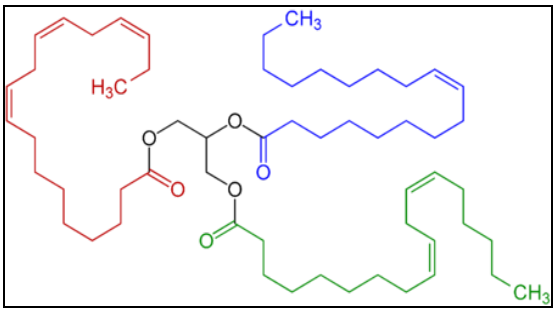
**1- Common name:** Mineral oil

**Trade name:** Super Misrona 95% EC (a local mineral oil, containing 95% paraffinic oil w/w and 5% inert ingredients, unsulfonated residue content reached 92).

**2- Common name:** linseed oil

**Trade name:** Flaxseed

**Structural formula:**




**- Toxicity of the tested compounds against larval stage**

Thin film technique was used as a method of application in the present work against the newly hatched larvae of *P. gossypiella*. Each Petri-dish was treated with 1.0 ml of the tested concentration; five concentrations were used: (12.5, 25, 50, 100 & 200 ppm) for chromafonozide; (50, 25, 12.5, 6.25, 3.625 & 1.812 ppm) for diflubenzeroun ; (50, 100, 150, 200 & 250 ppm) for misrona oil and (6, 8, 10, 12 & 14 % ) for linseed oil. Three replicates for each concentration were used. The Petri-dish which used as control was treated with water only. Thirty of newly hatched larvae/replicate was exposed for one hour to the tested compounds film in each Petri-dish.

The alive larvae of each replicate/ concentration were transferred individually into clean glass vials (2 X 7 cm) containing small piece of normal diet. Vials were plugged with absorbent cotton and incubated at the same conditions. The acute toxicity of the tested bioinsecticide was assessed after 1 day. Latent or chronic toxicity was determined by inspecting all the tubes for mortality after 7 days post treatment. Percentages of mortalities were corrected according to Abbott's formula (Abbott, 1925). The data were then subjected to probit analysis (Finney, 1971) through software Computer

program (Propane) to obtain the $LC_{50}$ & $LC_{90}$ and slope values.

**- Biological studies.**

Newly hatched larvae of the pink bollworm, *P. gossypiella* was treated with $LC_{50}$'s of Chromafonozide, diflubenzeroun ,misrona oil and linseed oil, to investigate some biological aspects of the pest affected by the tested compounds. Larval stage as well as pupation and adult emergence were estimated. After moth emergence, three replicates each contained 5-pairs/cage of emerged moths was used to measure the reproductive potential of the insects /each tested compound. Pre-oviposition, oviposition and Post-oviposition periods were estimated in days. Laid eggs were counted daily and kept under the same conditions and the percentage of hatchability was estimated as follows:

- Reduction in hatchability percentage was calculated according to **Zidan and Abdel-Megeed (1987)**.

$$\% \text{ Egg hatchability} = \frac{\text{No. hatched eggs}}{\text{No. deposited eggs}} \text{ X } 100$$

- Fecundity percentage was calculated according to **Crystal and Lachance (1963)** as follows:

$$\% \text{ Fecundity} = \frac{\text{No. eggs/ treated female}}{\text{No. eggs/ untreated female}} \text{ X } 100$$

The obtained data of PBW biological aspects were analyzed using Costat statistical program software, 1990 and Duncan's multiple range test (Duncan, 1955) at 5% probability level to compare the differences among time means.

**Biochemical analysis:**

To study the effect of Chromafonozide, diflubenzeroun, misrona oil and linseed oil on some biochemical parameters, of ***P. gossypiella*** moth which resulted from treating larvae with oils and IGRS, samples of PBW moth were collected after one day of each treatment and kept in clean tubes at 4 ºC for chemical analysis.

Total protein, total lipids, glutamic oxaloacetic transaminase (GOT = ALT) and glutamic pyruvic transaminase (GPT = AST) enzyme activities were determined colorimetrically according to Koller (1984), Drevon and Schmitt (1964) and Trinder (1969), respectively.

## RESULTS AND DISCUSSION

**Toxicity of four compounds on *P. gossypiella* larvae:**

Data in Table (1) shows the $LC_{50}$ & $LC_{90}$ values for newly hatched larvae of *P. gossypiella* treated with chromafonozide, diflubenzeroun, super misrona and linseed oil.

**Table (1): Comparative toxicity of 4 compounds against newly hatched larvae of *Pectinophora gossypiella* (S.)**

| Pesticides used | $LC_{50}$ | $LC_{90}$ | Confidence limits for $LC_{50}$ | | Slope ± S.D. | Toxicity Index* | |
|---|---|---|---|---|---|---|---|
| | | | Upper | Lower | | $LC_{50}$ | $LC_{90}$ |
| Chromofenozide | 139.47 | 1706.03 | 169.98 | 87.96 | 1.29 ± 0.027 | 35.11 | 66.56 |
| Diflubenzeron | 48.98 | 1135.56 | 68.73 | 26.48 | 0.78 ± 0.25 | 100 | 100 |
| Mineral oil | 159.82 | 1463.14 | 189.54 | 94.24 | 1.01 ± 0.15 | 30.64 | 77.61 |
| Linseed oil | 170.51 | 1685.02 | 249.86 | 103.27 | 1.29 ± 0.27 | 28.72 | 67.39 |

$$\text{Toxicity index (Sun, 1950)} = \frac{LC_{50} \text{ or } LC_{90} \text{ of the efficient compound}}{LC_{50} \text{ or } LC_{90} \text{ of other compound}} \text{ X } 100$$

**Effect of $LC_{50}$ of the four compounds on some biological aspects of PBW:**

Data in Table (2) clearly show the effect of $LC_{50}$ of chromafonozide, diflubenzeroun, super misron and linseed oil on the development of pink bollworm when the newly hatched larvae were fed on diet treated by the $LC_{50}$. All the tested compounds were adversely affected on the development of larval stage of PBW in comparison to control. The statistical analysis show highly significant difference in percentage of mortality of treated neonate when fed on chromafonozide, diflubenzeroun, super misrona oiland linseed oil. The percentages were increased to 63.0, 59.93, 65.3 and 59.0 % in the tested compounds, respectively, compared with 7.66 % in the control. In the same respect a prolongation in larval duration also were increased to 21.0, 19.17, 24.56 and 19.9 days compared with 15.03 days for control. Rashad *et al*., (2006) recorded that the IGRs compounds increase developmental period of *P. gossypiella* larvae. Also, pupal duration were increased when the new hatched larvae fed on chromafonozide IGR, super misrona oil, diflubenzeroun IGR and linseed oil to 10.5, 10.1, 9.63 and 9.03 days compared with 8.1 days in control. The total immature stages increased to 34.6 days in case of super misrona oil, 31.5 days in chromafonozide IGR, 29.24 days in 28.8 days in diflubenzeroun IGR compared with 23.0 days in the control In contrary, the larval and pupal weight resulted from treated neonate were decreased than control as shown in table (2). (Kandil *et al.* 2005) found that chlorfluazuron prolonged the pupal period and reduced the size.

**Table (2): Effect of $LC_{50}$ of four tested compounds on larval and pupal aspects of *P. gossypiella* under controlled conditions (26±1˚c & 75±5 % R.H.).**

| Treatments | Parameter of different immature stages | | | | | |
|---|---|---|---|---|---|---|
| | % mortality | Larval duration (days)* | Weight of larvae (gm)* | Pupal duration (days)* | Weight of pupae(gm)* | Total immature stages(days)* |
| Chromafonozide | 63.0 | $21.0^{B}$ | $0.0202^{E}$ | $10.5^{A}$ | $0.0163^{E}$ | $31.5^{B}$ |
| Diflubenzeroun | $59.0^{D}$ | $19.17^{D}$ | $0.0226^{D}$ | $9.63^{C}$ | $0.0206^{D}$ | $28.8^{D}$ |
| Super misrona | $65.30^{AB}$ | $24.56^{A}$ | $0.0229^{C}$ | $10.1^{B}$ | $0.0211^{C}$ | $34.6^{A}$ |
| Linseed | $59.93^{CD}$ | $19.9^{C}$ | $0.0268^{B}$ | $9.03^{D}$ | $0.0246^{B}$ | $29.24^{C}$ |
| Control | $7.66^{E}$ | $15.03^{E}$ | $0.0347^{A}$ | $8.1^{E}$ | $0.0301^{A}$ | $23.0^{E}$ |

*** Means followed by the same letter at the same column are not significantly different at P= 0.05.**

**Toxicity of four compounds on adult *P. gossypiella* stages:**

**Oviposition period:**

Pre-ovipostion, ovipostion and post-ovipostion periods, adult longevity, total number of deposited eggs (fecundity) and the total number of hatching larvae from the eggs (fertility) for the four tested compounds chromafonozide, diflubenzeroun, super misrona and linseed oil in comparison to the control were recorded in Table (3). It is obvious that the pre-ovipostion period was highly significant increased by four tested compounds. This period were 4.4, 3.97, 3.63 and 2.98 days for females resulted from larvae treated with linseed oils, diflubenzeroun IGR, super misrona and chromafonozide, respectively, while it was 2.73days in control.

Also, oviposition period recorded significant increase in new hatched larvae fed in diflubenzeroun, chromafonozide IGR's, super misrona and linseed oil that the values reached to 18.06, 16.9, 15.6 and 15.17 days compared with 13.8 days in the control.

**Table (3): Effect of treating newly hatched larvae of *P.gossypiella* with $LC_{50}$ concentration of tested compounds on oviposition period, fecundity, fertility and longevity**.

| Treatments | Adult stages | | | | | | |
|---|---|---|---|---|---|---|---|
| | Oviposition period(days)* | | | Fecundity | fertility | Longevity | |
| | Pre oviposition period (days) | oviposition period (days)* | Post oviposition period (days)* | No. eggs/♀ | Hatchability % | ♀ (days)* | ♂ (days)* |
| Chromafonozide | $2.98^{D}$ | $16.90^{B}$ | $4.22^{A}$ | $179^{C}$ | 68.3 | $19.87^{C}$ | $18.17^{C}$ |
| Diflubenzeroun | $3.97^{B}$ | $18.06^{A}$ | $3.23^{B}$ | $159.0^{E}$ | 62.0 | $20.66^{BC}$ | $19.66^{BC}$ |
| Super misrona | $3.63^{C}$ | $15.6\pm^{C}$ | $3.0^{B}$ | $169.4^{D}$ | 65.2 | $17.9^{E}$ | $17.57^{E}$ |
| Linseed | $4.4^{A}$ | $15.17^{D}$ | $3.53^{B}$ | $191.3^{B}$ | 70.0 | $18.1^{DE}$ | $17.9^{DE}$ |
| Control | $2.73^{E}$ | $13.8^{E}$ | $2.83^{B}$ | $232.0^{A}$ | 91.33 | $25.37^{A}$ | $22.66^{A}$ |

***Means followed by the same letter at the same column are not significantly different at P= 0.05.**

The same trend of elongation was occurred in the post oviposition period. This period were increased to reach 4.22, 3.53, 3.23 and 3.0 days when the new hatched larvae fed on chromafonozide IGR, linseed oil, super misrona and diflubenzeroun IGR compared with 2.83 day in the control. (Rashad, *et al.*, 2006) recorded that the $IGR_s$ compounds increase developmental period of *P. gossypiella* larvae.

**Reproductive potential:**

Data presented in (Table, 3) Show high reduction in numbers of eggs laid by females resulted from PBW treated newly hatched larvae with **$LC_{50}$** of the 4 tested compounds. The mean numbers of laid eggs value were 191.3, 179.3, 169.0 and 159.0 eggs/ female in linseed oils, chromafonozide IGR, super misrona and diflubenzeroun IGR compounds, respectively, compared to 232.0 eggs/ female in control.

As shown in Table (3) the percentage of eggs hatchability were 70.0, 68.3 65.2and 62.0 % in case of treatments with linseed oils, chromafonozide

IGR, super misrona oil and diflubenzeroun IGR respectively, compared with 91.33 % in control. Yasir *et al.* (2012) recorded that the fecundity and egg hatchability were reduced at all concentrations of Lufenuron used against *T. castaneum* larvae.

**Adult longevity:**

Present results in table 3 show that female's longevity of PBW decreased to 20.66, 19.87, 18.1 and 17.9 days/♀ in diflubenzeroun, chromafonozide, linseed oils and super misrona oil, respectively, compared to 25.37 days/ female in control. Also, the males' longevity resulted from PBW treated larvae were decreased than the control, the recorded means were 19.66, 18.17, 17.9 and 17.57 days/♂ from larvae treated with diflubenzeroun , chromafonozide, linseed oils and super misrona oil, respectively, compared with 22.66 days/ ♂ in control (Table, 3). These results are in agreement with Abd El-Ghani *et al.*, (1985) who found that treatment of both larval and pupal stages of *S. littoralis* with low concentration of IGRs reduced the fecundity and egg hatching and increased the sterility of adults.

**Adult emergence and sex ratios:**

Results in Table (4) show high reduction in the moth emergence percentage compared with control. The percentages of adult emergence were 76.2, 72.0, 66.81 and 64.33% adults resulted from treated larvae with diflubenzeroun , super misrona oil, chromafonozide IGR and linseed oils, respectively compared with 97.2 % in the control.

Sex ratios were affected by the tested compounds as 62:38 %♀:♂ in case of chromafonozide IGR, 60:40 % ♀:♂ with super misrona oil, 56:44 % ♀:♂ treated by linseed oil and 55:45 % ♀:♂ in adult resulted from larva fed on diflubenzeroun IGR compared with 52:48 % ♀:♂ in the control.

**Table (4):Effect of treating newly hatched larvae of *Pectinophora gossypiella* by four tested compounds on adult emergence % and Sex ratio.**

| Treatments | Adult emergence % | Sex ratio ♀:♂ |
|---|---|---|
| Chromafonozide | 66.81 | 62:38 |
| Diflubenzeroun | 76.2 | 55:45 |
| Super misrona | 72.0 | 60:40 |
| Linseed | 64.33 | 56:44 |
| Control | 97.2 | 52:48 |

**Biochemical analysis:**

**Total soluble protein:**

Data in Table (5) revealed that the tested compounds, Chromafonozide, Diflubenzeroun, mineral oil and linseed oil when used at $LC_{50}$ values caused significant reduction in soluble protein content in adult compared with control, the total soluble proteins were 5.01, 6.3, 4.1 and 6.1 mM/gm body weight, respectively, compared with 10.1 in the control.

The present result is in agreement with Assar *et al.* (2010) who found that the total protein content and total concentration of amino acids decreased in the house fly treated with match and consult. Also, Ghoneim *et*

*al.* (2012) found that proteins in treated *Schistocerca gregaria* by insect growth regulators (IGRS) were generally exhibited.

**Total lipid:**

Data in Table (5) indicated that the tested compounds, Chromafonozide, Diflubenzeroun, mineral oil and linseed oil when used at $LC_{50}$ values caused significant reduction in total lipids , the values reached 9.4, 11.01, 8.5 and 11.0 mM/gm body weight, respectively, compared with 17.2 in the control. Hamadah *et al.* (2012) found a predominant inhibitory in lipid content of *S. gregaria* nymphs that treated with pyriproxyfen, tebufenozide or lufenuron. On contrary to the present result, Keeley (1985) and Kunkle and Nordin (1985) recorded that the increase in total lipid caused increase on the mean number of eggs and hatchability percentage. In insect adult females, the major function of the fat body is the synthesis and release of proteins and lipids for yolk formation during oocyte maturation.

**Transaminase enzymes (GOT and GPT or ALT and AST).**

Data in Table (5) showed a significant reduction in the ALT and AST enzymes activity of PBW adults treated with $LC_{50}$ of Chromafonozide, Diflubenzeroun, mineral oil and linseed oil. The levels of ALT after treatment were 23.0, 26.0, 20.0 and 26.0 mM/gm body weight, respectively, compared with 40.0 mM/gm body weight in the control. The same trend of the result in ALT enzyme was occurred in case of AST enzyme that the $LC_{50}$ of tested compounds Chromafonozide, Diflubenzeroun, mineral oil and linseed oil were significant reduced in AST enzyme that the values were 25.0, 33.0, 22.0 and 32.0 mM/gm body weight compared with 43.0 mM/gm body weight. Assar *et al.* (2010 & 2012) found that match induced inhibitory effect on the house fly, *Musca domesticaat* 1000 ppm. Consult had no effect on the total activity of AST. With respect to the total ALT activity, match and consult elicited inhibitory effect on the total ALT activity.

**Table (5): Effect of tested compounds on total protein, lipid, ALT and AST of *P. gossypiella* adult.**

| Compounds | Protein (mM/gm body weight)* | Lipid (mM/gm body weight)* | ALT (mM/gm body weight)* | AST (mM/gm body weight)* |
|---|---|---|---|---|
| Chromofenozide | $5.01^{D}$ | $9.4^{D}$ | $23.0^{C}$ | $25.0^{D}$ |
| Diflubenzeron | $6.3^{BC}$ | $11.01^{C}$ | $26.0^{B}$ | $33.0^{B}$ |
| Mineral oil | $4.1^{E}$ | $8.5^{E}$ | $20.0^{D}$ | $22.0^{E}$ |
| Linseed oil | $6.1^{C}$ | $11.0^{BC}$ | $26.0^{B}$ | $32.0^{C}$ |
| Control | $10.1^{A}$ | $17.2^{A}$ | $40.0^{A}$ | $43.0^{A}$ |

***Means followed by the same letter at the same column are not significantly different at P= 0.05.**

**In conclusion**, the chemical changes and the reduction in adults enzymes explain the relationship between prolonged duration with less weight in treated PBW with $LC_{50}$ of the tested compounds on immature stages. On the other hand, the reduction in protein, lipid, ALT and AST caused inhibition and/or reduced the main metabolites in larvae as well as the reduction in reproductive potentiality of PBW resulted from treating larvae with oils and IGRS.

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# التأثير الباقى لمركبات مختلفة على دودة اللوز القرنفلية

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تمت معاملة يرقات حديثة الفقس لدودة اللوز القرنفلية بالتركيز النصف مميت لأربعة مركبات لكلا من مثبطات النمو الفيترو والديملين وزيت مصرونا ومستخلص زيت بذرة الكتان وذلك لدراسة تأثيرهم على جميع اطوار دودة اللوز القرنفلية .

واظهرت النتائج ان المركبات الأربعة عملت على زيادة فترة الطور اليرقى وطور العذراء وكذلك اطالة مدة فترة ما قبل وضع البيض وفترة وضع البيض وفترة ما بعد وضع البيض وذلك مقارنة بالفقس الحديث الغير معامل. وعلى العكس من ذلك أدت المعاملة بهذة المركبات الى نقص فى وزن اليرقات والعذارى وكذلك نقص كمية البيض الذى تضعة الاناث ونسبة الفقس وايضا نقص فى عمر الاناث والذكور لحشرة اللوز القرنفلية مقارنا بالفقس الحديث الغير معامل .

وأبرزت نتائج التقييم البيوكيميائي لليرقات الناتجة من معاملة الفقس الحديث بالجلرعة نصف المميت للمركبات المختبرة من مثباطات الكيتين والزيت المعدني وزيت بذرة الكتان أنها خفضت المحتوي البروتيني والدهون وإنزيمات AST & ALT . مما أدي لخفض الكفاءة التناسلية للحشرات المعاملة مقارنة بالكنترول .