

EVALUATION OF PHOTSENSITIZER TOXICITY AND BIOCHEMICAL EFFECTS ON METABOLITES LEVEL TOTAL CARBOHYDRATES, PROTEINS, TOTAL LIPIDS OF PEACH FRIUTE FLY, *BACTROCERA ZONATA* (DIPTERA: TEPHRITIDAE)

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Abstract

Evaluation the effect of biochemistry and toxicity by different concentrations of photosensitizer compound against full grown larvae of *B. zonata*. Treatments were evaluated under laboratory conditions. The results revealed that, chlorophyllin taken by *Bactrocera zonata*, showed low toxicity under severe dark and light-based toxic conditions when insects were exposed to light. Fruit fly after processing by photosensitizer compound (magnesium chlorophyllin) and exposure to sunlight for two hour was more effective than photosensitizer (copper chlorophyllin) at same conditions, Sub-lethal concentrations (LC₅₀) (0.00002 M, 0.008 M), respectively. Bioassays show significant differences and good correlation found between the toxicity results and biochemical assays in our study give highest increase of activity level in total carbohydrate and on the other hand, total lipid and total Protein show variation reduction in most treatments.

Key words: *Bactrocera zonata*, photo insecticidal, photosensitizer, Copper chlorophyllin, Magnesium Chlorophyllin, total proteins, total carbohydrate and total lipid.

INTRODUCTION

Fruit flies are most destructive pest in the world of fruit and vegetables, causing millions of dollars worth of damage. The peach fruit fly, *Bactrocera zonata*, is an economically significant pest. In the African region, it has been reported in Egypt (Hashem *et al.*, 2001), The peach fruit fly, *Bactrocera zonata* has many host plants but prefers peach, mango, guava, apple, papaya, quince, phalsa, bitter gourd, dates, pomegranate, almond and sweet orange. In recent years the focus of increased attention on the photo-compound insecticide as a new type of pesticides high-friendly and efficient environment. (Ben Amor and Jori 2000). At present, a light-sensitive material used as insecticides has been discovered to control several insect species *Colpoda inflate*, *Ceratitis capitata* and *Stomoxys calcitrans* (Lukšienė *et al.* 2007). For control fruit fly photocompound, recommend as replacement for Malathion insecticide (Heitz 1995). in United States, control of corn rootworms and reduced doses of toxins has been a special potential to exchange foliar and soil insecticides with phloxine B - cucurbitacin bait (Schroder *et al.* 2001). Photosensitization involves the activation of photosensitive compounds and the production of chemical reactions that harm or

destroy cells; in some cases the excited Photo compound is converted into a noxious product (Spikes, 1985). In the development process, different agents such as protein for synthesis of ATP, proteins is the most vital organic and play an important role in energy production in animal tissues. (Taşkın and Aksoylar, 2011). In insect, fat is a source of energy, structural organs and hormone precursors in many insects It is accumulate in different areas of the insect body. Fat in the egg also plays an vital role in meeting the energy necessarily of fetal development (Boz and Gülel, 2012). The main organ responsible for metabolism and also the organ of conversion and storage of fat, carbohydrates, and proteins it's fatty body (Arrese and Soulages 2010). Thiruvassagam (1994), reveal that an important decrease of total lipid of *Homarus americanus* when exposed to toxicants, the similar trend has been notes in lipids, protein and carbohydrate by *Aspongopus janus* (Hemiptera) when treated with nimbeciline including 0.03% Azadirachtin. The fatty body is responsible for the metabolism of carbohydrates and expands particularly in insect larvae. Kobozo and Lancaster (1981) showed an important reduction in fat content in the fatty body of *Homarus americanus* when exposed to toxic substances.

MATERIALS AND METHODS

Biological study:

Insects:

The laboratory susceptible colony of the peach fruit fly, *Bactrocera zonata* was established from a strain continuously reared in the laboratory of Horticultural Insects Department, Plant Protection Research Institute, Agricultural Research, Center, Dokki, Adults were reared in wooden cages capacity (30 x 30 x 30 cm). The flies were fed with sugar and hydrolysis protein at ratio of 3:1. Adults were provided with a small plastic bottle filled with water as drinking sites, until mating took place then females started oviposition. The cage was supplied with false plastic fruits that had many fine pores (as oviposition receptacles). These plastic fruits are filled with 3ml water to receive and prevent drying of the eggs. Also, at the top of these plastic fruits, small plastic vials containing cotton wicks saturated with guava juice were put to enhance egg lying within these false fruits. Larvae were reared on an artificial diet consisted of 500 ml distilled water, 3.00 g citric acid, 3.00g sodium benzoate, 84.50 g sugar, 84.50 g brewer's yeast and 330gwheat bran. These ingredients carefully mixed in large plastic container scattered on the surface of the diet which was placed in plastic trays of 20 x 10 x 8 cm that were tightly covered with muslin clothes using rubber bands. The trays placed in a wooden cage with sand at the bottom to allow the jumping larvae to pupate. All pupae were sieving the sand.

Laboratory Bioassay: The bioassay tests were performed on Adult larvae and 1-days pupae. Concentrations of photosensitizer were prepared by dissolving in water with sugar and hydrolysis protein hydrolysis at ratio of 3:1 respectively. The treated Adults put in dark for 24 hr. and exposure to sunlight to different time (30 min, 60 min. and 120min.). Using Abbott's formula (1925) to corrected mortality percentage. The statistically computed according to Finney (1971) used to corrected mortality percentage of each compound.

Abbott's formula

$$\text{Corrected \%} = \left(1 - \frac{\text{n in T after treatment}}{\text{n in Co after treatment}} \right) * 100$$

Where : n = Insect population , T = treated , Co = control

✚ Direct Sunlight :

The treated larvae were exposed to the sunlight for 30min, 60 and 120 mins. The fluency rate measured by the dosimeter taken as the average of intensities during exposure time.

✚ Dark experiment :

The larvae treated with Copper chlorophyllin, Magnesium Chlorophyllin left in the dark until the end of larval life.

✚ Biochemical study:

Preparation of samples for biochemical studies:

The biochemical assay was done after *Bactrocera zonata* treated and exposed to different interval of sunlight. The larvae homogenates and after centrifugation the supernatant was used directly for enzyme assay.

✚ A-Determination of total proteins:

Total proteins were determined according Bradford (1976).

✚ B- Determination of total carbohydrates:

Total carbohydrates were determined according Singh and Sinha (1977).

✚ C- Determination Total soluble lipids (TSL):

Total lipids were estimated according to Knight *et al.* (1972).

RESULTS AND DISCUSSION

Toxicological Study

Table (1) and fig. (1), indicate that the tested laboratory strain treated with photosensitizer (magnesium chlorophyllin) and exposed to sunlight for two hour was the most susceptible ($LC_{50} = 0.00002$ M) and has resistance ratio (RR = 1), where the tested laboratory strain *Bactrocera zonata* tested with photosensitizer (copper

chlorophyllin) and exposed to sunlight for two hour show ($LC_{50} = 0.008$ M) and resistance ratio ($RR = 400$). On the other hand the dark treatment by magnesium chlorophyllin show ($LC_{50} = 0.417$) and resistance ratio ($RR = 20850$). The dark treatment by magnesium chlorophyllin show ($LC_{50} = 1908.947$) and resistance ratio ($RR = 9.54E+07$).

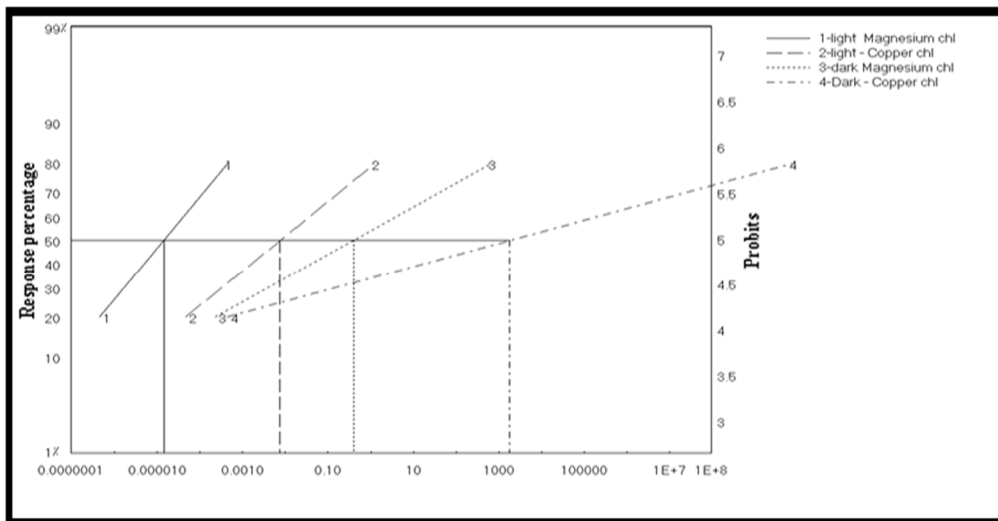
Table 1. Toxicity and resistance ratios of copper chlorophyllin and magnesium chlorophyllin against susceptible strains of *Bactrocera zonata* after treated and exposure for 2hour of sunlight (355 w/m^2).

| No | Line name | LC_{50} | LC_{90} | Upper limit | Lower limit | Slope | RR | Index |
|----|-------------------------------|-----------|-----------|-------------|-------------|-------|----------|----------|
| 1 | light Magnesium chlorophyllin | 0.00002 | 0.0028 | 0.00003 | 4.32E-06 | 0.564 | 1 | 100 |
| 2 | light Copper chlorophyllin | 0.008 | 18.446 | 1.848 | 0.0015 | 0.381 | 400 | 0.25 |
| 3 | dark Magnesium chlorophyllin | 0.417 | 34204.36 | ---- | ---- | 0.261 | 20850 | 0.0048 |
| | Dark Copper chlorophyllin | 1908.947 | 2.16E+13 | ---- | ---- | 0.127 | 9.54E+07 | 1.05E-06 |

* Copper chlorophyllin = CUCH

* Resistance Ratio (RR) compared with lower LC_{50}

Fig. 1. Log-probit concentration lines of copper chlorophyllin and magnesium chlorophyllin on the laboratory strains of *Bactrocera zonata* after treated and exposure to sunlight for 2 hour.



Index compared with light Magnesium chlorophyllin

Resistance Ratio (RR) compared with light Magnesium chlorophyllin

BIOCHEMICAL STUDY:

The data summarized in table (2) represented total Carbohydrate changes in the homogenate of adult *Bactrocera zonata*, and tested by compound Copper chlorophyllin ($Cu 10^{-2}$) or Magnesium Chlorophyllin ($Mg 10^{-2}$) showed increase in total carbohydrates at most treated *Bactrocera zonata*, the data in table (2) represented,

after *Bactrocera zonata* exposed to sunlight for two hour after treated by compound Copper chlorophyllin or Magnesium Chlorophyllin showed significant increase (47.87%, 131.38%) respectively,. Also after *Bactrocera zonata* treated by compound Copper chlorophyllin or Magnesium Chlorophyllin showed significant increase (73.87% & 102.72%) respectively, relative to control in dark experimental, that mean Copper chlorophyllin (Cu 10⁻²) or Magnesium Chlorophyllin (Cu 10⁻²) have toxicity on adult *Bactrocera zonata* before exposed to Sun light. that mean Phosensitizer accumulates in the body of insects and have toxicity and the like exposure to visible light, induces deadly photochemical reactions and death.

Table 2. Percentage of total Carbohydrates on *Bactrocera zonata* homogenate after treated with LC₅₀ of Photosensitizer (Copper Chlorophyllin-Magnesium Chlorophyllin) and exposed to Sun light for two hour.

| Total Carbohydrates (mg glucose/g body weight) | | | | | | | | | | | | |
|--|----------|-------|---|------|--------|-------|----------|-------|---|------|--------|--------|
| | Compound | Adult | | | % | * | Compound | Adult | | | % | * |
| 1 | Cu Light | 45.10 | ± | 1.51 | 147.87 | 47.87 | Mg Light | 70.57 | ± | 2.76 | 231.38 | 131.38 |
| 2 | Cu Dark | 53.03 | ± | 1.61 | 173.87 | 73.87 | Mg Dark | 61.83 | ± | 2.13 | 202.72 | 102.72 |
| 3 | Control | 30.50 | ± | 1.06 | 100.00 | 00.00 | Control | 30.50 | ± | 1.06 | 100.00 | 00.00 |

Cu Copper Chlorophyllin Mg Magnesium Chlorophyllin % = percentage relative to control

* = increase percentage relative to control

In table (3) Data show that, *Bactrocera zonata* after treated with LC₅₀ of Copper chlorophyllin compound or Magnesium Chlorophyllin compound and exposure to sunlight for 2hr showed variation reduction in total Protein level all light or dark experimental relative to control.

Table 3. Percentage of total Protein on *Bactrocera zonata* homogenate after treated with LC₅₀ of Photosensitizer (Copper Chlorophyllin-Magnesium Chlorophyllin) and exposed to Sun light for different time.

| Total Protein (mg protein /g body weight) | | | | | | | | | | | | |
|---|----------|-------|---|------|--------|---------|----------|-------|---|------|--------|---------|
| | Compound | Adult | | | % | * | Compound | Adult | | | % | * |
| 1 | Cu Light | 17.23 | ± | 0.22 | 70.13 | - 29.87 | Mg Light | 17.95 | ± | 0.22 | 73.06 | - 26.94 |
| 2 | Cu Dark | 17.47 | ± | 0.28 | 71.10 | - 28.90 | Mg Dark | 17.79 | ± | 0.58 | 72.41 | - 27.59 |
| 3 | Control | 24.57 | ± | 0.72 | 100.00 | 00.00 | Control | 32.80 | ± | 0.95 | 100.00 | 00.00 |

Cu Copper Chlorophyllin Mg Magnesium Chlorophyllin % = percentage relative to control

* = decrease percentage relative to control

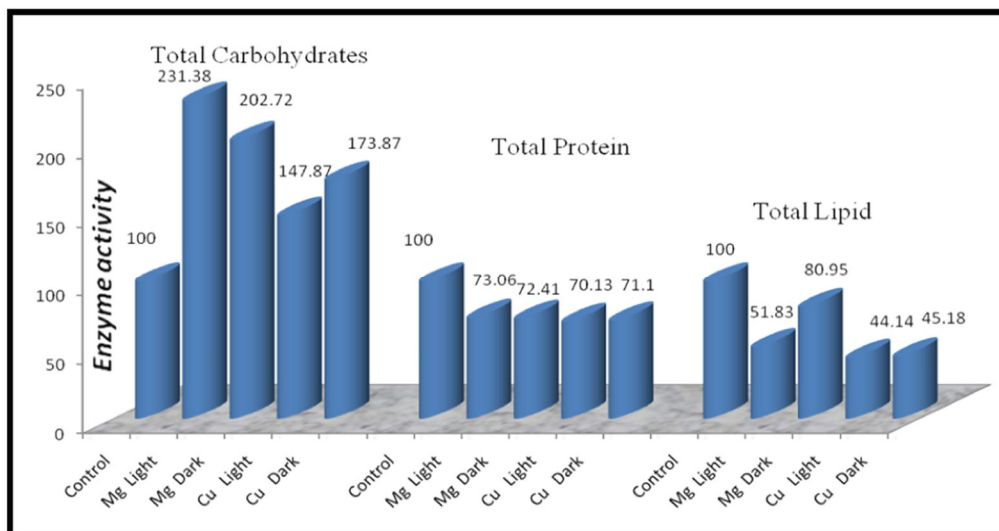
Table 4. Percentage of total Lipid on *Bactrocera zonata* homogenate after treated with LC₅₀ of Photosensitizer (Copper Chlorophyllin-Magnesium Chlorophyllin) and exposed to Sun light for different time.

| Total Lipid (mg /g body weight) | | | | | | | | | | | | |
|---------------------------------|----------|-------|---|------|--------|---------|----------|-------|---|------|--------|---------|
| | Compound | Adult | | | % | * | Compound | Adult | | | % | * |
| 1 | Cu Light | 2.467 | ± | 0.13 | 45.18 | - 54.82 | Mg Light | 2.83 | ± | 0.13 | 51.83 | - 48.17 |
| 2 | Cu Dark | 2.41 | ± | 0.12 | 44.14 | - 55.86 | Mg Dark | 4.42 | ± | 0.11 | 80.95 | - 19.05 |
| 3 | Control | 5.46 | ± | 0.18 | 100.00 | 00.00 | Control | 5.46 | ± | 0.18 | 100.00 | 00.00 |

Cu Copper Chlorophyllin Mg Magnesium Chlorophyllin % = percentage relative to control

* = decrease percentage relative to control

Fig. 2. Percentage of total Carbohydrates, total Protein and total Lipid on *Bactrocera zonata* treated by Photosensitizer (Copper Chlorophyllin - Magnesium Chlorophyllin) and exposed to Sun light for two hour



The data summarized in table (4) reveal the changes in total Lipid in the homogenate of *Bactrocera zonata*, After exposure to sunlight for two hour after treated by compound Copper chlorophyllin or Magnesium Chlorophyllin showed high reduction (-54.82%, 48.17%) respectively relative to control, *Bactrocera zonata*, treated by compound Copper chlorophyllin showed high reduction in dark experimental (-55.86%), On the other hand after treated by compound Magnesium Chlorophyllin showed low reduction in dark experimental (-19.05%), relative to control.

DISCUSSION

From bioassays result found that, there was significant correlation found between the results of bioassays and biochemical assays in this study, highest decrease of activity level in total carbohydrate and on the other hand, total lipid and total Protein show lower reduction in most treatments. The copper chlorophyllin or magnesium Chlorophyllin is effective in activity level of all experimental of *Bactrocera zonata*. Light, photosensitizer and oxygen is potentially damaging, Singlet oxygen one of the main responsible for caused harmful in biological systems (Weishaput *et al.*, 1976). The size of damaged tissue can be modify by selecting a light wave length with specific absorption properties (Svaasand *et al.*, 1990). This means that light plays an important role in the completion of the *Bactrocera zonata* photosensitivity reaction after treated by copper chlorophyllin or magnesium Chlorophyllin, and increased exposure to sunlight may have a significant effect on enzyme level activity. It is importance to develop new, technologies that are environmentally safe to control the number of insect pests. photocompound can be used as pesticide agents because, it limited effect in environmental, non-toxic and no significant mutagenic. Inside the body of insect photo-compound accumulates and after exposure to light, it stimulates deadly photochemical reactions and it stimulates deadly photochemical reactions and

death. Porphyrin inactivated catalase through producing $^1\text{O}_2$ caused damage in amino acid (Hirakawa *et al.* 2013). Proteins consider most significant components of animal tissues, including insects, and responsible for energy production. In the development process, different factors such as protein is required for the synthesis of ATP (Taşkın and Aksoylar, 2011). In insect, fat is a source of energy, structural organs and hormone precursors in many insects It is accumulate in different areas of the insect body. Fat in the egg also plays an vital role in meeting the energy necessarily of fetal development (Boz and Gülel, 2012). Fat content fluctuation in different types of insects has been reported to be treated with a lot of toxic substances by many researchers. of photovoltaic protection in insects gives a special advantage to photosensitive compounds as insecticides that are largely irresistible. The primary photo-damage site leading to feeding inhibition it's midgut wall (Ben Amor *et al.* 1998).

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تقييم تأثيرات السمية الضوئية والبيوكيميائية على مستوى التمثيل الكلى
للكربوهيدرات، البروتينات الكلية و الدهون الكلية لذبابة الخوخ
***Bactrocera zonata* (Diptera: Tephritidae)**

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ذبابة الخوخ *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) هي آفة خطيرة تهاجم مجموعة واسعة من الفواكه في مصر. حيث تم تقييم التأثيرات السمية والبيوكيميائية للتركيزات المختلفة لمركبات الحس الضوئي بتركيزات مختلفة ضد الحشرة الكاملة لذبابة الخوخ ، وتمت المعاملة تحت ظروف المختبر. وأظهرت النتائج لذبابة الخوخ بعد المعاملة انخفاض السمية تحت الظروف المظلمة والسمية الحادة عند التعرض للضوء، معاملة ذبابة الفاكهة بمركب كلوروفيلين ماغنسيوم التعرض لضوء الشمس لمدة ساعتين كان أكثر فعالية من المعاملة بمركب كلوروفيلين النحاس ، وكانت قيم الجرعة النصفية (LC₅₀) (0.00002 ، 0.008) على التوالي. ولقد أظهرت الاختبارات الحيوية اختلافات معنوية ووجدت علاقة جيدة بين قيم التأثيرات السمية ونتائج الاختبارات لبيوكيميائية في هذه الدراسة ، حيث أظهرت النتائج إرتفاع في مستوى الكربوهيدرات الكلية وعلى الجانب الآخر فإن إجمالي مستوى الدهون الكلية والبروتين الكلية اظهرت تذبذب في الانخفاض في معظم المعاملات.

