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# HISTOPATHOLOGICAL EFFECTS OF SOME BIOCIDES ON THE FOURTH ABDOMEN SEGMENT OF *Pectinophora gossypiella* (SAUND.)

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## ABSTRACT

The newly hatched larvae of pink bollworm (PBW), *Pectinophora gossypiella* (Saunders) were fed for two days on artificial diet treated with the $LC_{50}$ concentration of some biocides *i.e*., Agrin (*Bt*), Ecotech-pro (*Btk* x *Bta*) and Bio-clean (*Btk* x *Beauveria. bassiana*). The survived 2nd and 4th instar larvae of normal appearance fed on treatments and control diets were used to study the histopathological changes of the aforementioned biocides on the fourth abdomen segment. Examination of the microtomical transverse sections of this segment showed abnormal effects in the histopathology of some organs and tissues (midgut, Malpighian tubules, fat bodies, body muscles & cuticle) of the treated larvae. Generally, the pathological effects of the tested biocides were more serious against the 2nd instar larvae than against the 4th one. The type and degree of these effects differed according to the tested biocide, whereas Bio-clean was the most potent one.

## INTRODUCTION

Traditionally, chemical control is the major method for protecting cotton from pink bollworm (PBW) damage, but the efficacy of these insecticides usually declines due to the evolution of resistance to insecticides in pink bollworm (Yang *et al*., 2000). Recently, attempts have been done to replace conventional pesticides by biological agents. It has been proven that the commercial products based on entomopathogenes (bacteria or fungi) provide an effective microbial control agents against pink bollworm larvae (Rashad & Ali, 1997 and El-Lebody *et al*., 2003).

*Bacillus thuringiensis* (*Bt*) is the most widely applied biological insecticide. Most of the current bacterial insecticides products registered for Lepidoptera control based on *Bt kurstaki* (*Btk*) strains HD-1 (Rechcigl & Rechcigl, 2000). Later, strains of *Bt azawia* (*Bta*) were commercialized as they are effective against *Spodoptera* sp. and certain key pests that had developed resistance of *Bt k.* In addition, some other products were developed based on the conjugation of two distinct strains (i.e., *kurstaki* and *azawia* or *tenebrionis*). These strains have a wide host range and were registered as new strains: EG 2348, EG 2349, EG 2371(Copping, 1998 & Tabashink *et al*., 1998). Moreover, *Bt* might be applied in concert with other insect pathogens, including entomopathogenic fungi (Wraigh & Ramos, 2005). However, the most widely used fungal insecticide is *Beauveria bassiana* (Copping, 1998). In this respect, Ahmed (2001) reveled that entomopathogen commercial products (i.e. Agrin, Ecotech-pro & Bio-clean) have latent adverse effects on the biology and biochemistry of pink bollworm larvae.

Histopathological examination is a part of the study of the mechanism by which *Bt* insecticidal crystal protein CIYA(a) (toxin specific for lepidopterans) acts on target insects (Nagamatsu *et al*., 1998 and Rausell *et al.,* 2000). For decades, the mechanism of insect killing by *Bt* has been assumed to be toximediated lyses of the gut epithelial cells, which leads to starvation or *Bt* septicemia. Partially, the toxic effects of *Bt* on lepidopterous insects can be investigated and described by histological studies (Sheikh *et al*., 1990; Zidan *et al*., 1998 and Moustafa, 2005). The present work aims to investigate the latent histopatholigical effects of the $LC_{50}$ concentration of some biocide formulations against pink bollworm larvae.

## MATERIALS AND METHODS

**Rearing of *P. gossypiella***

Newly hatched larvae of *P. gossypiella* were obtained from a colony maintained in the laboratory of Bollworms Department, Plant Protection Research Institute, ARC, Dokki, Giza, for several generations at 27 ± 1°C and 75 ± 5% relative humidity (RH). Larvae were reared on a modified artificial diet as described previously by Abd El-Hafez *et al.* (1982).

**Biocides**

The following three biocide formulations based on *Bt*, differing in their international units (IU), were used:

**Agerin®.** Commercial product of *Bt,* at 38000 IU/g.

**Ecotech-pro®.** Commercial product of *Bt* subspecies *kurstaki* strain EG 2348 (transconjugant *Btk* X *Bta*) contains endotoxins coded by genes Cry IA (a), Cry IA (c) (2 x) and Cry IIA, at 16300 IU/mg (IU *Trichoplusia ni*).

**Bio-clean®.** A commercial microbial formulation containing a mixture of 300 g of the bacterium, *Btk*, serotype 3b, at 32000 IU/g mixed with 400 g of the fungus *B. bassiana* at 2.3 X $10^7$ spores/g.

**Histological study**

Detection of the chronic effects of the tested biocides against either $2^{nd}$ or $4^{th}$ instar larvae of pink bollworm treated as newly hatched larvae was as follows:

The newly hatched larvae of PBW were fed on diets containing $LC_{50}$ values of the tested biocides and held at 27 ± 1°C and 75 ± 5% RH%. The $LC_{50}$ values were statistically calculated through a Proban software computer program. These values were 0.128, 0.158 & 0.174 g/L for Agrin, Ecotech-pro & Bio-clean, respectively. Two days after treatment, the survivors were transferred on untreated diet and incubated at the same previous conditions. The survived $2^{nd}$ and $4^{th}$ instar larvae (7 and 14 days old), which have normal appearance were taken and frozen for the histological sudies. Microtomical sections of the $4^{th}$ abdomen segment were done according to Gad (1951). The same procedure was adopted on untreated larvae as comparison.

The microtomical sections of both treatments and control were observed under light microscope. The latent histopathological effects on the midgut (Mg), fat bodies [inner (Ifb) and outer (Ofb) layers, body muscles (Bms), Malpighian tubules (M), silk gland (Sg) and cuticle (C) were recorded.

A Lucida camera was used to measure the thickness of cuticle, as well as the diameter of Malpighian tubules of the 4th instar. The silk gland shrinkage percentage for each treatment was calculated. Figures of the microtomical sections of both treatments and control were taken by digital camera for comparison.

## RESULTS AND DISCUSSION

**The light microscope examination of transversal section of the untreated PBW larvae**

Figure (1a) exhibits the various organs and tissues of fourth abdomen segment of the untreated 2nd instar larvae of PBW i.e. midgut [gut lumen (Gl), peritrophic membrane (Pm), epithelial cells layer (Ep), basment membrane (Bm) and gut muscles (i.e. circular muscle (Cm) & longitudinal muscles (Lm)]. The same Figure shows the two layers of fat bodies; an outer fat bodies layer (Ofb) underlying the cuticle and an inner fat bodies layer (Ifb), which surrounds and enters between the various organs. Also, silk gland (Sg), cuticle [epicuticle (EpC), endocuticle (EnC), Epidermis (E) & Basement membrane (Bm) were obvious. When the larvae reached the 4th instar, Ifb occupied the most of fourth abdomen segment (Figure 1b). Also, the body muscles (Bms) became more obvious. In addition, the Malpighian tubules were observed. Meanwhile, the thickness of EnC layer increased while the thickness of epidermal layer decreased, when compared with that of 2nd instar larvae (Figure 1a).

**The latent histopathological effects of Agrin**

Figure (2a) shows the latent histopathological effects of Agrin against 2nd instar larvae of PBW. These effects are: complete destruction of midgut; vigorous degeneration of fat bodies (Ifb & Ofb); sometimes reduction of the silk gland width (compared with the control) and degeneration of some epidermal cells. The measurement of the cuticle thickness was so difficult to be measured because it is mixed with the internal materials.

Otherwise, there was a partial degeneration of midgut of the 4th instar larvae of PBW as a result of Agrin treatment (Figure 2b). The obtained data showed an elongation of Ep and separation of from Bm which was separated from coated Cm. However, some of Ep follow into Gl in groups form and destruction of Pm. Moreover, degeneration of Ofb and Ifb, shrinkage of body muscles (Bms), decrease of the Sg width and cuticle thickness by 18.2 % (but without deformation) appeared when compared with the control.

**The latent histopathological effects of Ecotech-pro**

The latent histopathological effects produced by Ecotech-pro against the 2nd instar larvae of PBW are illustrated graphically in Figure (3a). These effects are as follows:

Vigorous enlargement of midgut size, destruction of the Pm, Shrinkage of Ep, destruction of some Ep, partial separation between Ep and Cm, destruction of Ofb & Ifb and Shrinkage of Sg by 55.64% than in control. The gut muscles (i.e. Cm & Lm) were not affected when compared with control (Figure 1a). On the other hand, there was a decrease of Ec thickness and degeneration of some E cells of the cuticle, so the reduction of the cuticle thickness reached 63.6% when compared with the control.

**Figure ($1^{a\ \&\ b}$). Tissues and cells of various organs in the fourth abdomen segment of the $2^{nd}$ and $4^{th}$ instars of *P. gossypiella* untreated larvae.**

**Pm: Peritrophic membrane; Gl: Gut lumen; Ep: Epithelium cells; Ifb: Inner fat bodies; Ofb: outer fat bodies; Sg: Silk gland; C: Cuticle.**

**Figure ($2^{a\ \&\ b}$). Various organs and tissues in the fourth abdomen segment of the $2^{nd}$ and $4^{th}$ instars of *P. gossypiella* larvae treated with Agrin.**

**Pm: Peritrophic membrane; Ep: Epithelium cells; Ifb: Inner fat bodies; Sg: Silk gland; C: Cuticle.**

As for the latent effects of Ecotech-pro against the $4^{th}$ instar larvae, there was a reduction in midgut size, shrinkage and degeneration of Ep, serious degeneration of the nucleus of Ep cells, partially separation between Ep cells and Cm (Figure $3^{b}$). In addition, shrinkage of the Sg by 55.2% less than the control, degeneration of Ifb, destruction of cell content inside the Ofb, while the membranous sheath was not rupture, swelling of the Ofb, shrinkage of the body muscles and the cuticle was not affected.

**Figure ($3^{a\ \&\ b}$). Various organs and tissues in the fourth abdomen segment of the $2^{nd}$ and $4^{th}$ instars of *P. gossypiella* larvae treated with Echo-tech- Pro.**

**The latent histopathological effects of Bio-clean**

Bio-clean caused the following adverse effects against $2^{nd}$ instar larvae of PBW: destruction of the midgut, Ifb & Ofb, shrinkage of Sg by 33.3 % less than the control (Figure $4^{a}$) and severe degeneration of the Bm, E and Enc layers of the cuticle. As larvae became older ($4^{th}$ instar), swelling and degeneration of the midgut, destruction of Pm, shrinkage and degeneration of Ep cells were appeared. Also, there were degenerations in Ofb and Ifb, shrinkage of body muscles, shrinkage of Sg by 34.5% than the control, shrinkage of the Enc and E layers of the cuticle, since, the cuticle thickness was reduced by 27.3 % than control (Figure, $4^{b}$).

**Figure ($4^{a\ \&\ b}$). Various organs and tissues in the fourth abdomen segment of the $2^{nd}$ and $4^{th}$ instars of *P. gossypiella* larvae treated with Bioclean.**

In the present study, the latent histopathological effects of the three tested biocides on the midgut of both $2^{nd}$ & $4^{th}$ instar larvae of PBW is in agreement with those reported by Sheikh *et al* (1990) who revealed that, endotoxin of *Bt* (Sotto) killed the PBW larvae as it caused rupture of the Ep cells. They added that, the muscles around the Ep seem to be relaxed. Also, Zidan *et al*., (1998) indicated that, "MVPII" caused morphological changes to the midgut Ep of treated larvae of *P. gossypiella* and *Earias insulana*. Moreover Rashmi & Singh (2004) studied the histological effects of *Btk* against the midgut of fourth instar larvae of *Helicoverpa armigera.* They found that, there was a hypertrophied columnar cells in the epithelium, Pm pushing into the sloughing cells, resulting in the complete degeneration of Ep cell leading to loss of structural organization. Also, the entomopathogenic fungi caused histopathological effects against the midgut of target insect (Ramlee *et al*., 1996; Moraga *et al*., 2006 and El- Sinary & Rizk, 2007).

The present results showed adverse effects of the tested biocides against the fat bodies (Ofb & Ifb), Bms) and Sg of PBW. These findings are in agree meat with those reported by Ramlee *et al* (1996) as they found that, the fungal hyphae of *B. bassiana* damaged the fat tissues of *Psychid metisa* larvae and invaded the muscle tissues, Malpighian tubules, gut musculature, Ep cells at 72 hours post infection. Furthermore, the tested biocides may caused complete destruction of Malpighian tubules of PBW larvae. In this regard, Ogutcu *et al*. (2005) noted that, *Btk* not only affects the midgut epithelium of insects, but also causes pathological changes in cells of Malpighian tubule cells of *Thaumetopoea pityocampa*. Where it disturbs the mechanism of oxidative phosphorilation and causes the dissolution of nucleoplasm, disordering of microvilli, swelling of mitochondria and vacuolization. Generally, the pathological effects of the tested biocides were more serious against the 2$^{nd}$ instar larvae than it against the 4$^{th}$ instar one. In this regard Zangerl *et al*., (2001) and Dutton *et al*., (2002) reported that, the young actively feeding larvae are most affected by *Bt* products than the old ones.

Finally, Bio-clean was the most effective biocide against PBW larvae. This result agrees with that reported by Ahmed (2001) who studied the latent effects of Agrin, Ecotech-Pro and Bio-clean on the biology and biochemistry of PBW and concluded that, Bio-clean was the most effective pathogen.

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## التـاثيرات الهستولوجية لبعض المبيدات الحيوية على الحلقة البطنيـة الرابعـة لـدودة اللوز القرنفلية

**علية عبد الحافظ ، كريمة اللبودي ، كارم أبو زيد حسن و دينا عبد الظاهر أحمد**
**معهد بحوث وقاية النباتات- مركز البحوث الزراعية- الدقي- جيزة**

تمت تغذية يرقات دودة اللوز القرنفلية حديثة الفقس لمدة يومين على بيئة صناعية معاملة بـ $Lc_{50}$(التركيز القاتل لـ 50%) لبعض المبيدات الحيوية وهي الأجرين، الإيكوتك برو، البيوكلين. وتم نقل اليرقات الحية بعد ذلك لاستكمال نموها على بيئة غير معاملة. واستخدمت يرقات العمر الثاني والرابع في كل من المعاملة والمقارنة والتي تبدو في صورة طبيعية لإجراءالدراسات الهستولوجية على الحلقة البطنية الرابعة.

أظهر الفحص الميكروسكوبي لقطاعات تلك الحلقة وجود تغيرات غير طبيعية في أنسجة بعض أعضاء اليرقات المعاملة مثل المعدة الوسطى- أنابيب ملبيجي- الأجسام الدهنية- العضلات والجلد. وبصفة عامة كانت التأثيرات الباثولوجية للمبيدات المختبرة أكثر خطورة على يرقات العمر الثاني منها علي يرقات العمر الرابع. هذا وقد اختلف نوع ودرجة التـأثير حسب المبيد المختبر، فقد كان مبيد البيوكلين أقوى المبيدات المختبرة.