EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
F
TOXICOLOGY & PEST CONTROL

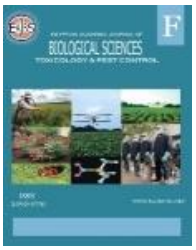
ISSN
2090-0791

WWW.EAJBS.EG.NET

Vol. 12 No. 2 (2020)

Egypt. Acad. J. Biolog. Sci., 12(2): 9-17 (2020)

Egyptian Academic Journal of Biological Sciences
F. Toxicology & Pest Control
ISSN: 2090 - 0791
http://eajbsf.journals.ekb.eg/



Follow Up Effectiveness for A Field Application of a Chlorfluazuron Compound (Topron) On Long-Term on *Earias insulana* (Boisd.) Through the Histological Examination of The Integument.

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ARTICLE INFO
Article History
Received:2/6/2020
Accepted:10/8/2020
Key words:
Spiny Bollworm.
chitin synthesis inhibitors,
integument,
histo-microscopic examination

ABSTRACT

Under a single field application, the long-term effect of a chitin synthesis inhibitor, Chlorfluazuron (Topron 5% EC) on spiny bollworm, *Earias insulana* (Boisd.) was evaluated through histological investigation on the integument as it an essential background to the action of that insecticide. After two weeks off use, samples of large-sized larvae (12 - 15 days age-old approximately) under the effect of the procedure were to represent the criterion of the long-term for the tested compound. The microscopic examination of the treated sections showed distinct changes in the tissue components of integument compared to the untreated specimens. The treated sections showed an increase in the thickness of the epidermis layer with noting that there is no new layer of cuticle or show any other signs of further development. Various specimens of the treated larval sections revealed the presence of an individual layer of cuticle, which looked thick, and the spines were rough, extra-long, and tanned. Also, in many sections, the cuticle had been seen shrinkage from the epidermis. The current study confirms and suggests that the long-term impact must be considered for such compounds when evaluated under field conditions.

INTRODUCTION

Given the importance of the cotton crop in Egypt, the control of the significant cotton pests is of considerable significance in terms of implementing integrated control programs, the quality of appropriate pesticide groups, and the timing of their use. Insect Growth Regulators (IGRs) seem promising because of their a more specific mode of action towards pest insects and their lower toxicity to non-target organisms (Tongxian and Philip 2004; Nedjoua and Noureddine 2011; Yu *et al.*, 2014). IGRs can be grouped according to their mode of action, as follows: chitin synthesis inhibitors (i.e. of cuticle formation) and substances that interfere with the action of insect hormones (i.e. JHs, ecdysteroids) (Smet *et al*., 1990; Oberlander *et al*., 1991 and Oberlander *et al.*, 1997, Tunaz and Uygun, 2004.). Chlorfluazuron is one of the leading benzoylphenylurea IGR insecticides and acts as a chitin synthesis inhibitor (CSI) (Ishaaya and Casida 1974; Post et al. 1974, Bakr and Hussein 1988, Shim *et al.*, 2007; Sammour *et al.*, 2008). Topron (Chlorfluazuron) compound recommended for use in Egyptian fields cotton for controlling the cotton leafworm, *Spodoptera littoralis* populations. As the level of boll

formation increases, the bollworm complex of lepidopteran pests begins to appear, pink bollworm (PBW), *Pectinophora gossypiella* (Saund.), Gelechiidae, and spiny bollworm (SBW), *Earias insulana* (Boisd.), Noctuidae. Bollworms complex are controlled through programs of recommended pesticides once they reach the economic threshold level. IGR compounds as larvicide active upon ingestion, do not penetrate leaves, and had no characterized of contact impact (Mulder and Gijswijt, 1973; Elings and Dieperink, 1974; Wright and Harris, 1976; Ascher and Nemny, 1976). These compounds are stomach poisons and many sucking insects and hidden feeders, e.g. pink bollworm (PBW), cannot be adequately controlled by these compounds. So recommended managing bollworms control tactics not include IGR compounds where pink bollworm behaviour, not compatible and limits the success of exposure to these pesticides.

On the other hand, spiny bollworm (SBW) causes significant damage to cotton fields in Egypt and middle eastern countries (Rashad and Ammar, 1984; Al-Mosa, 1986; Hiremath, 1987, Mirmoayedi and Maniee, 2009). Spiny bollworm is distinguished from pink bollworms by its marked propensity in moves some distance on its host plant before settling down to feed (Pearson, 1958). A single larva moves about considerably and can destroy several buds and bolls in its life. Pearson (1958), reported that the damage caused by spiny bollworm larvae might seem disproportionate to their number owing to their habit of moving to different parts of the plant. Spiny bollworm is predominant until the middle of the cotton season in many areas in Egypt. Application, an insect growth regulator, compounds against spiny bollworm located in these areas during one part of the season at the beginning of the formation of cotton bolls may be favourable. It may affect future spiny bollworm populations and the ability to control this pest at the following points during the season. Several previous studies have shown by means laboratory experiments, the chronic and slow toxic effect of Chlorfluazuron as an inhibiting chitin synthesis against bollworm (Mossan *et al.*, 1995; Kandil *et al.*, 2005; El Shennawy, 2009). Under field application, this effect means that it is a relatively slow procedure for evaluating its results on pests. Slow action means that the appropriate practical judgment of these compounds requires more time between treatment and evaluation. Therefore, the evaluation design based on the short-term effect is insufficient. In this regard, the current study used the insecticide, Tobron (Chlorfluazuron) to assess its long-term impact on the spiny bollworm under field conditions through a single application. The treatment evaluated through histological investigation for the integument as it an essential background to the action of that insecticide.

MATERIALS AND METHODS

The experiment carried out in Desouk district, Kafr El-Sheikh Governorate, in a cotton field that not previously treated with insecticides. Before insecticidal application checking samples of green bolls inspected to confirm the presence of spiny bollworms infestation. The experimental field had divided into six plots, each 7 x 6m. Three plots receiving a single foliar spraying application of Topron (Chlorfluazuron) as a chitin synthesis inhibitor insecticide on cotton plants at the same recommended dose on cotton leafworm (400 ml / Feddan), on July 8, 2019, the spray had applied by using a backpack motor sprayer. The other three plots allocated without treatment as a comparison. After two weeks off application, the long-term effect of the tested pesticide had evaluated by targeting large-size spiny bollworm larvae as the duration of their life (12 - 15 days old, approximately) under the impact of the procedure represents the criterion of the long-term effect of the tested compound. To determine the insecticidal effect, samples of 25 green boll had taken from each treated plot and as well as the untreated plot (five balls

from each quadrant and centre/plot). The samples were laboratory inspected by boll dissect. Dead larvae obtained were not taken into consideration. From treated and, untreated large-size larvae obtained, specimens were selected for this study and had prepared for histological microscopy. Treated and untreated larvae were fixed (for six h) in Bouin's fluid after cutting off their heads and their ends for fixation, dehydrated in series ethyl alcohol, and cleared in xylene. The specimens were then embedded in soft paraffin and finally transferred to hard paraffin, and cut into 6µ thick slices before staining. Sections were stained with Ehrlich's hematoxylin-eosin and observed under a light microscope at 400x magnification and photomicrographs were taken.

RESULTS AND DISCUSSION

By comparison to the untreated live larvae obtained from the boll samples, the large sizes of the treated larvae appeared very sluggish, curved, dark in colour, and the spines seemed stiffness and longer. The various sections of the integument of large sizes untreated spiny bollworm (plate 1 Fig. u1- u3 & plate 2 Fig. u1-u3) showed a single layer of epidermal cells (ep) and both old and new cuticles were continuously observed to co-exist. The old cuticle(oc) layer appeared separate from the epiderm. The new cuticle (nc) also appeared along with the epidermis layer. By comparison, the treated sections, Plate 1 (Fig. t1- t3) & plate 2 (Fig. t1-t3) showed a noticeable increase in the thickness of the epidermis layer and no indication of a new cuticle formation or any other signs of further development. Various specimens of the treated larval sections revealed the presence of an individual layer of cuticle, which looked thick and sclerotized, and the spines were rough, extra-long, and tanned. Also, in many sections, the cuticle had been seen shrinkage from the epidermis. The current results showed tissue changes in the integument of the large size larvae located in the treated plots, just like many researches on the effect of other chitin synthesis inhibitors (Kitahara *et al.,* 1983; Nishioka *et al.,* 1979; Rehimi and Soltani, 1999; Soltani, 1996; Vinuela and Budia 1994). IGR's are often active at one particular stage during the lifespan of susceptible insects. Occurrence an imbalance in the components of larva's integument at an age prevents it from developing to the next age (Unsal *et al.,* 2004). The Large-size of spiny bollworms expresses a lifespan of 12-15 days. The occurrence of tissues changes in their integument despite they had developed generally over several instars under the influence of treatment indicates the acting ability of the compound in the long-term. The microscopic observations showed apparent histological changes represented in a thick and sclerotized cuticle with noting the absence of a new cuticle and, irregular shrinkage the epidermis layer from the overlying cuticle. The larval integument is an essential background to the action, CSI compounds interfere with the moulting process and chitin formation (Sun et al. .2015; Harðardóttir, Hulda, *et al.,* 2019). In this context, many histological sections have shown growth abnormal length spines and apparent rigidity. Glenn and Richards (1979)defined the spines as a multicellular with cells similar to those of the general epidermis. These microstructure observations are consistent with many studies that have reported that the chitin synthesis inhibitor compounds (CSIs) caused abnormal epidermal cells in the treated insect (Del Bene and Porcinai, 1981, Hassan. and Charnley1987, Ren et al. .1988, Unsal *et al.,* 2004). Studies of both Ker (1978) and Unsal *et al.* (2004) on the effect of diflubenzuron as chitin synthesis inhibitor (CSI) showed many vacuoles of various sizes in epidermal cells of the histological sections of the treated *G. mellonella* larvae. Their findings were consistent that the change in cell morphology of epidermis cells was due to their affected by the compound. The present results showed an increase in the thickness of the epidermis and cuticle compared to the untreated. Many insects are protected against the

penetration of contact insecticides by a thick and sclerotized cuticle. (Ebeling, 1974). The increase in the thickness of the cuticle expresses the reaction of the large size larvae towards the toxicity of Topron (Chlorfluazuron), which indicates the effectiveness of the compound until the advanced stage of larval ages. Current microscopic observations indicate that, under field application, Topron (Chlorfluazuron) compound was able to cause an effect on the fully developed larval and blocked the cuticle secretion. The relation between the effect of CSI compounds and the disruptions they cause to a healthy balance of both the juvenile hormone and ecdysone in the stages of insect development was the target of many several reports published during the past decades and, indicate the possibility that benzoylphenyl ureas might affect the hormonal balance in insects through action on the insect endocrine system (Mitlin et al. 1977; Soltani *et al.,* 1984; Dhadialla *et al.* 1998). Madhavan (1968) reported that some species of lepidopteran pupae, and adults, retract the epidermis from the cuticle when injected with ecdysone, but do not secret a new cuticle or show any other signs of further development. Wolfgang and Riddiford (1981), and Riddiford (1981) mentioned that these hormones need to be present to ensure the formation of a morphologically normal cuticle. The exposure to these hormones results in a series of cellular events leading to the synthesis of the new cuticle. The study refers that follow up on the efficacy of CSI compounds based on short-term effects proves to be too short to discover several specific aspects of the impact of IGRs. The results obtained from follow up the long-term effect of Tobron (Chlorfluazuron) on spiny bollworm larvae confirm and suggest that, the long-term effect must be considered for such compounds when evaluated under field conditions.

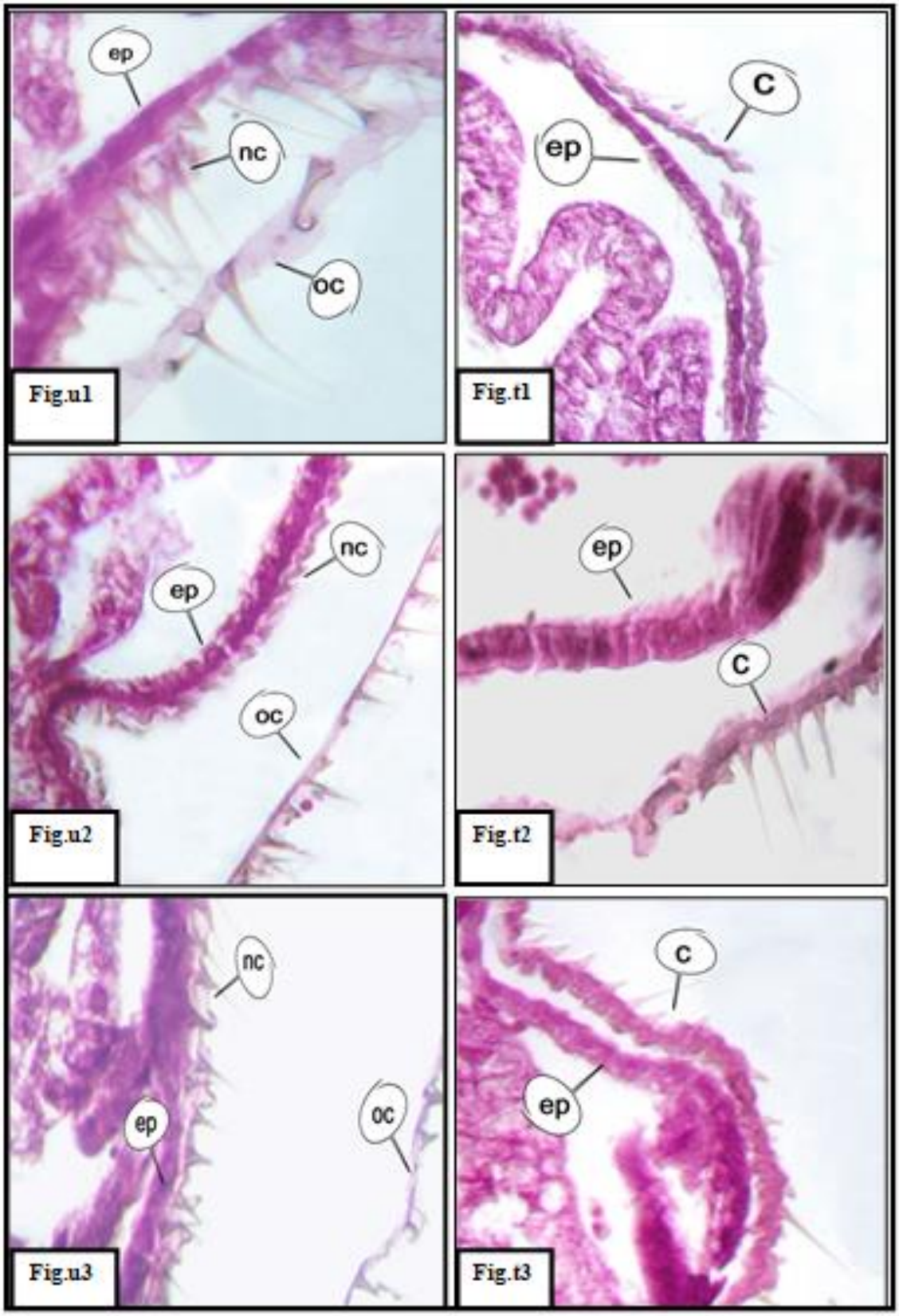

Plate 1: shows the histological changes in the treated sections (Fig.t1 – Fig.t3) of large-size spiny bollworms induced by the long-term effect of Topron (Chlorfluazuron) under field condition compare to the untreated specimens (Fig.u1 – Fig.u3). (c=cuticle, ep= epidermis, oc= old cuticle, nc= new cuticle)

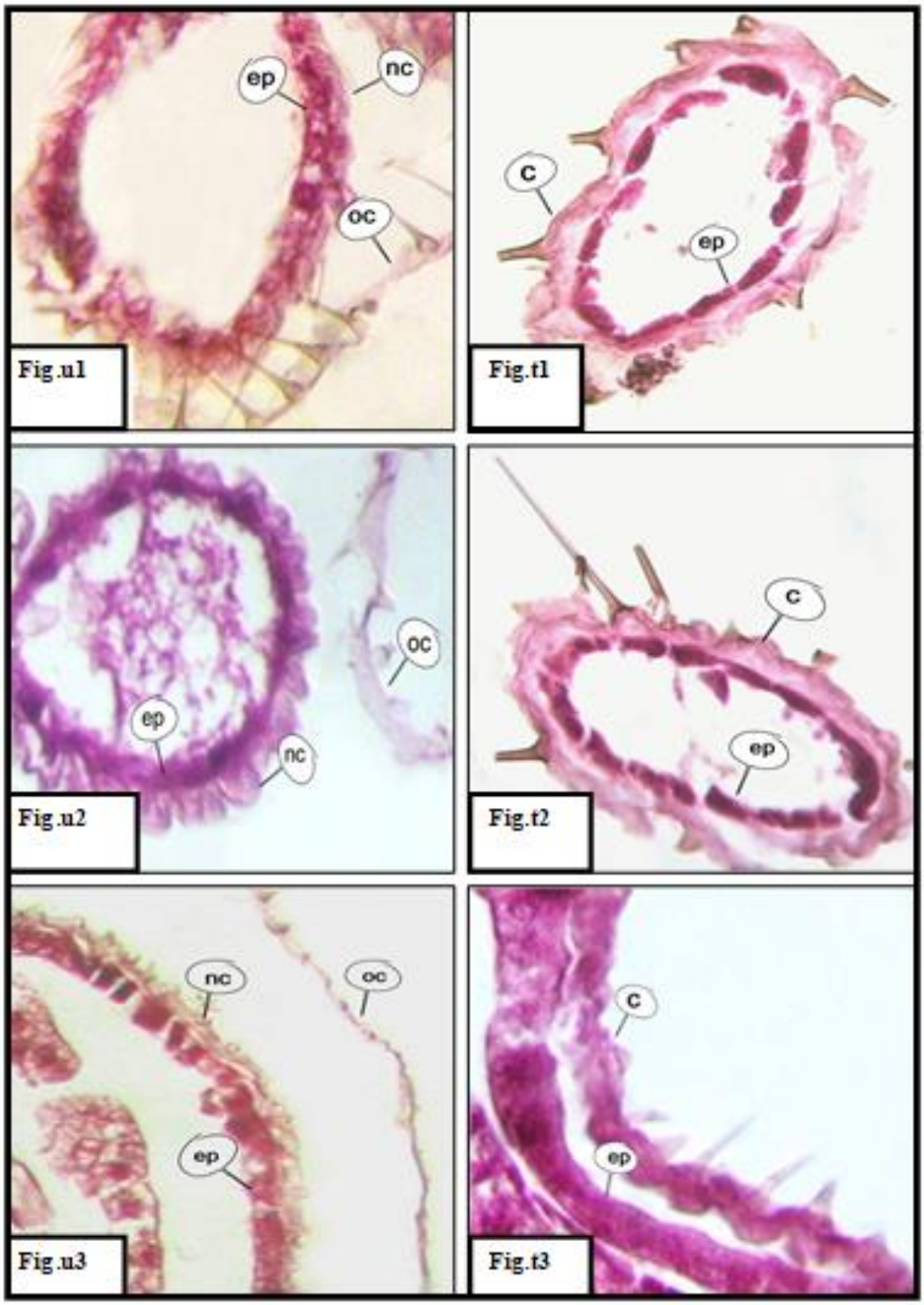

Plate 2: shows the histological changes in the treated sections (Fig.t1 – Fig.t3) of large-size spiny bollworms induced by the long-term effect of Topron (Chlorfluazuron) under field condition compare to the untreated specimens (Fig.u1 – Fig.u3). (c=cuticle, ep= epidermis, oc= old cuticle, nc= new cuticle)

Acknowledgement

The author offers his sincere appreciation to Prof. Dr. Mostafa A. M. Shalaby, Bollworms Research Depart., for the valuable assistance in conducting the research.

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ARABIC SUMMARY

متابعة الفعالية للتطبيق الحقلى لمركب كلورفلازرون (توبرون) فى الامد البعيد على دودة اللوز الشوكية من خلال الفحص النسيجى للجلد

أيمن محمد محى الدين عدلى

معهد بحوث وقاية النباتات – مركز البحوث الزراعية

استخدمت الدراسة الحالية مركب توبرون (5 % EC)كأحد مثبطات تخليق الكيتين لتقييم تأثيره فى المدى البعيد على دودة اللوز الشوكية. بعد أسبوعين من تطبيقه واحدة فى الحقل، تم تقييم المعاملة من خلال فحص انسجة الجلداليرقى عن طريق اختيار عينات من اليرقات كبيرة الحجم حيث تمثل مدة حياتها (١٢ - ١٥ يومًا تقريباً) تحت تأثير الإجراء معياراً للمدى البعيد للمركب المختبر. أظهر الفحص المجهري للقطاعات النسيجيه المعامله تغيرات واضحة في مكونات الأنسجة مقارنة بالعينات غير المعامله. أظهرت القطاعات المعاملة زيادة في سمك طبقة الابيدرمس مع ملاحظة أنه لا توجد طبقة جديدة من الكوتيكل أو تظهر أي علامات أخرى لمزيد من التطور. كشفت عينات مختلفة من قطاعات اليرقات المعاملة عن وجود طبقة فردية من الكيوتيكل، والتي بدت سميكة، وكانت الأشواك تبدو صلبة، طويلة جدًا، ومدبوغة. أيضا، في العديد من القطاعات، شوهدت طبقة الابيدرمس تنكمش عن الكيوتيكل. تشير الدراسة إلى أن متابعة فعالية مركبات مثبطات تخليق الكيتين بناءً على الآثارالسريعة الظهور بعد مدة قصيرة المدى من التطبيق يثبت أنها قصيرة لاكتشاف العديد من الجوانب المحددة لتأثير هذه المركبات كما تؤكد الدراسة الحالية وتقترح أنه يجب مراعاة التأثير على المدى البعيد لمثل هذه المركبات عند تقييمها في الظروف الحقلية.