

**Efficacy of Lufenuron (CGA-184699) and Diofenolan (CGA-59205)  
on survival, growth and development of the red palm weevil,  
*Rhynchophorus ferrugineus* (Coleoptera: Curculionidae)**

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**ABSTRACT**

Seven doses (500, 100, 50, 10, 1.0, 0.1 and 0.01 µg/insect) of Lufenuron (CGA-184699) and Diofenolan (CGA-59205) were topically applied onto the prepupae of *Rhynchophorus ferrugineus*. Survival of the prepupae had not been affected except at the higher two doses of Lufenuron or only at the highest dose of Diofenolan. Increasing water loss may explain the increasing mortality % in pupae. Depleting effect of both IGRs had been exhibited on the prepupal maximal body weights especially at the higher two dose-levels. Pupal development was hastened and duration was shortened as the dose-level of each IGR increased. Reduction of the body weights in pupae were observed by the action of each IGR, irrespective of the age. The higher two doses of Lufenuron, but only the highest dose of Diofenolan, remarkably reduced the pupation percent. Also, the pupation program was impaired variously by different dose-levels. The adult eclosion was completely blocked by increasing the dose-level of Lufenuron and by the higher two doses of Diofenolan. Different dose-levels of Lufenuron affected the adult morphogenesis but only the lower two doses of Diofenolan deranged it in 12%.

Keywords: *Rhynchophorus ferrugineus*, Lufenuron, Diofenolan, mortality, growth, development, morphogenesis, pupation, emergence, deformation.

**INTRODUCTION**

Insect growth regulators (IGRs) have received a great deal of attention as so-called "Third-generation insecticides" (Williams, 1976). These compounds including insect juvenile hormone mimics and other compounds controlling the insect development have mode of action disports from other insecticides and low toxicity against non-target organisms. The use of IGRs is increasing of controlling various insects of

agricultural, horticultural, stored product and public health pests (Retnakarn *et al.*, 1985).

On the other hand, diflubenzuron (with its commercial name: Dimilin) was the pioneer of benzoylphenyl urea exhibiting a chitin synthesis inhibition (Verloop and Ferrell, 1977) in various insect species (Hajjar and Casida, 1978, 1979, Mitsui *et al.*, 1984; Neumann and Guyer, 1983). In addition to this group, chitin synthesis inhibition have been caused by several groups, extracts and compounds such as polyoxins, nikkomycines, avermectin, ... etc. (*cf.* Cohen, 1987).

Lufenuron (Match or CGA -184699) and Diofenolan (Aware or CGA-59205) are assorted in a group among chitin biosynthesis inhibitors, or IGR, in general. The present study extends our previous studies (Bream *et al.* 2001) assessing some extracts and IGRs on the red palm weevil *Rhynchophorus ferrugineus* which was recorded at 1992 in Egypt as a destructive pest for the date palms *Phoenix dactylifera* (Cox, 1993). This paper deals with the toxicological, developmental and morphogenic effects of Lufenuron and Diofenolan on this weevil.

## MATERIALS AND METHODS

### 1) The Experimental Insect:

The red palm weevil *Rhynchophorus ferrugineus* is a serious pest of coconut causing damage and often killing the palm in its prime of life. The hatched grubs burrow into the trunk and feed on tissue of the stem. The pupation and adult emergence within the same stem allow successive generations within the same stem. In the present study, prepupae were collected for every experiment from large cavities of infested date trees specialized for this purpose, i.e. received no chemicals such as insecticides. No laboratory culture of *Rh. ferrugineus* could be established because of the legislative regulation preventing the transfer of it outside the infestation region (Ismailia and Sharqia Governorates, during the period of the practical work of the present study, 2000).

### 2) Administration of Insect Growth Regulators:

Two acylureas were used in the present study. Lufenuron (CGA 184699) and Diofenolan (CGA 59205). The first compound has the chemical name: N {[2,5-dichloro-4-(1, 1, 2, 3, 3, 3 - hexafluoropropoxy)phenyl]amino]carbonyl}-2,6-difluoro-benzamide (CA). The second compound has the chemical name: cis, trans-(±)-2-Ethyl-(4-

phenoxy-methyl,1,3-dioxolone(mixture of the four configurationally isomers).

Seven dose-levels of each compound were prepared: 500.00, 100.00, 50.00, 1.00, 0.10, 0.01 µg/insect and topically applied onto the pronotum of prepupae in 1 µl acetone. Eight replicates for each treatment were treated. Twelve replicates of controls were topically applied with acetone only. All treated and control insects were kept at  $27 \pm 2$  °C and  $70 \pm 5$  % RH.

### **3) Criteria and calculations:**

Pupal mortalities were observed during the pupal period, especially of the early-, mid- and late-aged pupae. Also, adult mortalities were calculated basing on the successfully emerged individuals All mortalities were counted and expressed in percentages.

In addition, pupation and adult emergence percentages were calculated as suggested by Jimenez-Peydro *et al.* (1995). Morphogenic aberrations were recorded and expressed in %s. For calculating the developmental duration Dempster's equation (1957) was used, and for calculating the developmental rate, Richard's equation (1957) was used. Growth index was determined according to Saxena and Sumittra (1985). Water loss % was calculated basing on the data of initial and final weights of pupae.

### **4) Statistical analysis of data:**

Data obtained were analyzed by the Student's *t*-distribution and refined by Bessel correction (Moroney, 1956).

## **RESULTS**

The two IGRs, Lufenuron (CGA-184699) and Diofenolan (CGA-59205), were bioassayed against the red palm weevil, *Rh. ferrugineus* and the obtained results can be assorted as follows.

### **1) Lethal effects:**

Survival potential of the prepupae did not affect except by the higher two doses of Lufenuron (Table 1). In the light of data in the same table, it is easily seen that the insecticidal action of this compound run parallel to the dose level. Moreover, the lowest dose level could not cause a pupal mortality, irrespective of the pupal age. Total mortality consecutively correlated to the dose value. The increasing water loss may

explain the increasing mortality % with ascending level of dose (38.17% at the highest level vs. 13.52% of controls, Table 1). As similar trend of the effect on the pupal survival, the adult had been undergone to the action of Lufenuron (Table 1).

Depending on the data of Table (2), the survival potential of prepupae had not been affected except by the highest dose of Diofenolan. Otherwise, pupal survival was remarkably affected. This adverse effect run parallel to the rising level of the compound. As mentioned in Table (1), Table (2) shows a dose - depending water loss %. This course of drought may interpret the increasing deaths of pupae.

## **2) Effects on growth and development:**

The data presented in Table (3) revealed the reducing effect of Lufenuron on the prepupal body weights. This effect elegantly observed after the use of two higher dose-levels ( $3.38 \pm 0.37$  and  $3.45 \pm 0.50$  mg by using 500.0 and 100.0  $\mu\text{g}/\text{insect}$ , respectively, vs.  $4.54 \pm 0.90$  mg of control congeners). This effect was substantiated by the calculation of growth index, which decreases in no certain trend (Table 3).

The development was hastened and the durations were shortened. This shortening was more exiguously detected by increasing dose-level. The results of the same Table (3) revealed that this shortness was statistically significant by the higher four doses of Lufenuron ( $5.55 \pm 0.45$ ,  $5.88 \pm 0.87$ ,  $6.00 \pm 0.67$  and  $6.15 \pm 1.28$  days vs  $7.45 \pm 0.93$  days of control corresponding).

Dealing with the pupae, data arranged in the same table showed the shortening action of Lufenuron on the pupal stage in the meaning of accelerated development of pupae. This effect appeared significantly by the use of two higher doses ( $4.31 \pm 0.46$ ,  $4.57 \pm 0.81$  days vs  $6.16 \pm 1.58$  days of controls). This effect was explored by the great values of the developmental rate (23.20 at 500.00  $\mu\text{g}/\text{insect}$  vs. 16.23 of controls).

On the other hand, the topical application of Lufenuron in its higher four doses considerably suppressed the body weights of the pupae (both the newly- and late-aged, for more details, see Table 3). It is noteworthy to mention that the mean body weights of the newly formed pupae were 0.64 of the control body weight (after treatment of the prepupae with the highest dose 500.00  $\mu\text{g}/\text{insect}$ ). Also, the late-aged pupae weighed 0.53 of the control weight (after treatment with the same dose-level).

As it is distributed in Table (4), only the higher two doses of Diofenolan caused significant depletion of prepupal body weights ( $3.38\pm 0.37$  and  $3.45\pm 0.50$  mg vs.  $4.54\pm 0.90$  mg of controls). This compound, in other dose-levels, did not stimulate the prepupae to attain similar significantly recorded body weights or growth index.

Results of the same table indicated the shortening effect of Diofenolan on prepupae before the transformation into pupae. Pronouncedly shortened durations were measured for the prepupae after treatment with the higher four doses ( $5.55\pm 0.45$ ,  $5.88\pm 0.87$ ,  $6.00\pm 0.67$  and  $6.15\pm 1.28$  days vs  $7.45\pm 0.93$  days of control congeners). In other words, they had fast developmental rates as a response to the action of Diofenolan at higher dose levels. Moving to the right half of the same Table (4), it is quite clear that the pupal durations were shortened and the developmental rate increased consecutively to the dose level. This compound at its three high dose-levels, led to the faster developmental rates (23.81, 20.49 and 19.96 vs. 16.23 of controls).

In view of data presented at the same half of the table, different degrees of the reduction in pupal body weights were observed, irrespective of the age. This decreasing effect was a dose-dependent. Also, it is noticed that the resulted pupae from the treated prepupae with the highest dose weighed 0.75 of their controls at the beginning and weighed 0.53 of their controls at the end of the stage.

### **3) Metamorphic and morphogenic effects:**

Data given in Table (5) revealed the metamorphosing action of Lufenuron. The topical application onto prepupae with 500.00 and 100.00  $\mu\text{g}$  Lufenuron/insect resulted in the hindering of pupation process. On the other hand, the pupation program was impaired in different degrees by various dose levels. This effect was approximately a dose-dependent, with few exceptions. In addition, the capability of pupae to metamorphose into adults was deranged in different degrees. The rate of adult emergence decreased by increasing the dose-level, which could not be observed for the adult morphogenesis. Unexpectedly, the present IGR, at its higher two dose levels did not induce adult deformities but caused only 87.5 and 63.0% adult blockage (at 500 and 100  $\mu\text{g}$ /insect, respectively).

Only the highest dose level of Diofenolan caused a decreasing of pupation, but slightly, and defused the pupation program in only 25% (see Table 6). Some degrees of such effect could be appreciated except at the two lower dose levels which could not disturb the adult morphogenesis.

The same table showed a complete blockage of adult eclosion by Diofenolan at its two higher dose-levels. Such effect gradually decreased as the dose-level decreased (75 and 12% emergence blockage by 50.00 and 0.01 µg/insect, respectively). Table (6), also, indicated that only the two lower dose-levels deranged the adult morphogenesis in 12%.

The majority of pupal deformities produced by the action of Lufenuron can easily be observed in Fig. (1). These deformities varied between charred body, collapsed appendages and atrophied elytron pads.

Diofenolan treatments resulted in similar degrees of pupal deformation in addition to dorso-ventrally compressed body, failure of complete escape from the prepupal skin, tubercle thorax and dwarf wing pad (Fig. 3).

Lufenuron exerted some action on adult morphogenesis. Fig. (2) demonstrates some photos of pupal-adult intermediates, adults with remains of pupal skin, adult with atrophied wings and legs. Diofenolan caused similar adult malformations beside to some other features such as permanently expanded membranous wings, collapsed appendages and evaginated elytre (Fig. 4).

## DISCUSSION

Benzoylphenyl urea are known to be highly effective IGRs against many agricultural pests with a relatively low toxicity to mammals and natural enemies (Degheele, 1990; Ishaaya, 1990). Diflubenzuron (Dimilin), the most thoroughly investigated compound of this group, has been reported to have no appreciable effect on hymenopterous and dipterous parasites; (Granett and Weseloh, 1975; Ravensberg, 1981). On the other hand, Westigard (1979) found that application of Dimilin was harmful to natural enemies of *Laspeyresia pomonella*, although only at relatively high concentrations. Also, detrimental effects of Dimilin on beneficial insects were reported by (McWhorther and Shepard, (1977) and Zungoli, *et al.*, (1983). Thus, it was necessary for derivatives of Dimilin have been synthesized along several years ago.

Lufenuron (Fluphenacur or Match or CGA-184699) and Diofenolan (Aware or CGA-59205) are chitin synthesis inhibitors or IGRs, in general, manufactured by Ciba Gaigi, Basel, Switzerland. The first was assessed against several insect pests, such as summer fruit tortix, *Adoxophyes orana* (Charmillot *et al.*, 1991; Ioriatti *et al.*, 1993); cat flea, *Ctenocephalides felis* (Hink *et al.*, 1991). The second compound was assessed against some species, such as scale insects: *Hemiberlesia rapax*

(Tomkins *et al.*, 1994), *Quadraspidiotus pyri* and *Q. ostreaeformis* (Hippe *et al.*, 1995); lepidopterous pests (Sechser *et al.*, 1994; Streibert *et al.*, 1994) and some citrus pests (Grout *et al.*, 1997).

However, different results had been obtained about the effects of these acylureas on survival, growth and development of those insects. The promising results, of the aforementioned works and others, encouraged to carry out the present study for investigating the possible effects of these two IGRs on the weevil *Rh. ferrugineus* through the following criteria.

### **1) Survival responses of *Rh. ferrugineus*:**

Lethality of Lufenuron and Diofenolan was studied, both in laboratory and in the field, against some insect pests and parasites. Shortly reviewing the available information may be useful. At 50 ppm Lufenuron had little effect on newly hatched larvae of the lepidopteran *A. orana* but was effective as larvicide against 8- to 20-days old larvae (Charmillot *et al.*, 1991). Feeding of cat fleas on orally administered cats with Lufenuron resulted in prevented development of the progeny. Most deaths of progeny occurred in the egg stage and the hatched eggs provided larvae which failed to develop into adults because they died. In addition, Diofenolan has demonstrated excellent selectivity at rates of between 50 and 200 ppm against the predators of scale insects: *Orius majusculus* and *Aphytis melinus* under laboratory conditions (Sechser *et al.*, 1994). On citrus trees in Egypt, Diofenolan caused only a low reduction of larvae of *Aphytis* spp. attacking the citrus purple scale (*Lepidosaphes backii*) and showed no significant effect on the predatory mite *Typhlodromus pyri* in an Italian apple orchard in a control Programme against the codling moth, *Cydia pomella* (Sechser, 1994).

In the present study, the action of Lufenuron and Diofenolan (at dose levels: 500.0, 100.0, 50.0, 10.0, 1.0, 0.1 and 0.01 µg/prepupa) was investigated on the survival potential of prepupae which had not been pronouncedly affected except at the higher two dose levels of Lufenuron. The mortal potency of the latter IGR was obviously observed as run parallel to the dose-level. Also, survival potential of prepupae had not been affected, except at the highest dose-level of Diofenolan. Otherwise, pupal survival was remarkably affected and such effect increased by the increasing dose-level of these IGRs.

A lot of research works showed various degrees of mortal potency or lethal action of different chitin inhibitors within which our Lufenuron and Diofenolan are assorted. Dimilin had been exhibited high activity as a

larvicide, pupicide and adulticide against *Spodoptera littoralis* (Radwan *et al.*, 1978; Sobeiha *et al.*, 1981; Ishaaya *et al.*, 1984; Osman, 1984; Watson *et al.*, 1984; Radwan *et al.*, 1986). Several dimiloids were, also, reported to have high toxicity against several insects such as mosquito species by Chlorfluazuron (IKI-7899), Teflubenzuron (CME-134) and Hexaflumuron (XRD-473) (Mulla and Darwazch, 1988; Bakr *et al.*, 1989; Mulla *et al.*, 1988; Vasuki, 1992a,b; Montada *et al.*, 1994; Mohapatra *et al.*, 1996); lepidopterous species by IKI-7899, XRD-473, CME-143, DPX and Triflumuron (Bay SIR-8514) Granett and Hejazi, 1983; Moustafa and El-Attal, 1984; Osman, 1984; Watson *et al.*, 1984; Atkins and Wright, 1985; Radwan *et al.*, 1986; Horowitz *et al.*, 1992; Furlong and Wright, 1994; Naguib *et al.*, 1994); muscoid flies by IKI-7899 or Bay SIR-8514 (Ghoneim *et al.*, 1992; Ghoneim and Ismail, 1995; Nassar, 1995); subterranean termites by XRD-473 (Su, 1994; Forschler and Ryder, 1996; Su and Scheffrahn, 1996 a,b; Su *et al.*, 1997). Also, larval feeding of *Muscina stabulans* on dietary concentrations either of Dimilin, CME-134 or by larval topical application with IKI-7899 or XRD-473, caused remarkable larval and adult mortalities (Basiouny, 2000). Also, Wright and Harris (1976) recorded non considerable effect for TH-6040 on the adult stage of stable fly *Stomoxys calcitrans*. Furthermore, Dimilin showed very low toxicity against *S. exigua* larvae owing to its rapid elimination from the larva and rapid metabolism of the materials remained in the body (Van Laeck and Degheele, 1993 a,b). Also, young *Spodoptera exigua* larvae owing to its rapid elimination from the larva and rapid metabolism of the materials remained in the body (Van Laeck and Degheele, 1993 a,b). Also, young *S. exigua* larvae have shown a tolerance to Dimilin and CME-134.

The toxicity differences among different species may be due to innate differences in the degradative metabolism, absorption and excretion (Wellington *et al.*, 1973 and Granett *et al.*, 1980). In addition, lethal action of several juvenoids, antijjuvenoids, ecdysteroids, antiecdysteroids, and other IGRs are available in the literature but a few references may be suffice for saving effort, area, and time (Ghoneim *et al.*, 1992, 1998; Sundaram *et al.*, 1998; Dedos and Fugo, 1999; Bakr *et al.*, 2000).

However, these different results of toxic effects and lethal action exhibited in various mortalities by several chitin inhibitors, as well as Lufenuron and Diofenolan in the present study on *Rh. ferrugineus*, may be ascribed to a direct inhibition of chitin synthesis within the integument rather than to any indirect extracuticular effects on hormone levels (Hunter and Vincent, 1974; Ishaaya and Casida, 1974; Sowa and Marks,



1975; Hajjar and Casida, 1978; Sundaramurthy and Balsubmanian, 1978). Also, the actual cause of insect death by chitin inhibitors may be attributed to either a rupture of the newly formed cuticle (Beenackers and Brock, 1974; Salama *et al.*, 1976; Sundaramurthy, 1977; Abid *et al.*, 1978; Fytizus and Mourikis, 1979).

There is an appreciated suggestion for explicating the death or mortality of different insect stages by the action of IGRs, general. According to this suggestion, mortalities are not directly related to the hormonal activity of the IGR, but to other factors or causes, such as: suffocation, bleeding and desiccation due to imperfect exuvation, starvation due to morphological defects, failure of vital homeostatic mechanisms, etc.. (Sehna, 1983; Smagghe and Degheele, 1994). The latter suggestion is, at least, partially conceived in the present study upon *Rh. ferrugineus* since water loss of pupae increased parallel to the increasing mortality % with ascending dose-level of Lufenuron or Diofenolan which indicated an adverse condition of the water body content.

## **2) Influence on growth and development of *Rh. ferrugineus*.**

Diflubenzuran the pioneer of benzoylphenyl urea, affects the development, among other vital criteria, of several insect species (El-Sayed *et al.*, 1984; Osman, 1984, Soltani-Mazouni and Soltani, 1994; Soltani *et al.*, 1996; Basiouny, 2000; Chebira *et al.*, 2000).

In the present study on *Rh. ferrugineus*, prepupal treatment with Lufenuron or Diofenolan resulted in significant depletion of body weights, especially at the two higher dose-levels. Also, the produced pupae had reduced body weights especially at the beginning and end of the stage (in the case of Lufenuron application) or along the age (in the case of Diofenolan application). Reduction of body weights, or the weight gain, by some other chitin inhibitors, and IGRs in general, had been reported for several insect species, such as *M. domestica*, *Spodoptera exempta*, *S. exigua*, *Mamestra brassicae* and *Galleria mellonella* (Smagghe and Degheele, 1994), as well as *S. littoralis* (Ghoneim *et al.*, 1998). Likewise, no effect on the body weights was recorded by some IGRs against *Leptinotarsa decemlineata*, *Diabrotica virgifera*, *Podisus sagitta* and *Locusta migratoria* (Smagghe and Degheele, 1994); *M. domestica* (Ghoneim *et al.*, 1991); *Periplaneta americana* and *Oncopeltus fasciatus* (Darvas *et al.*, 1992).

However, the suppressing action of Lufenuron and Diofenolan, in the present study, may be due to an ecdysonergic activity as suggested by

Smagghe and Degheele (1994) after using an ecdysone agonist, tebufenozide (RH-5992). With regard to the effect on the developmental durations and rates, results of the present study clearly showed remarkably hastened development of pupae which lasted a short duration, irrespective of the dose-level of both IGRs. On the contrary, many authors measured a suppressing action of several chitin inhibitors on the development during prolonged durations of immature stages (see, as for examples, Osman, 1984; Bakr *et al.*, 1989; Soltani *et al.*, 1989; Ghoneim *et al.*, 1992; Vasuki and Rajavel, 1992; Van Laeck and Degheele, 1993 a,b; Ghoneim and Ismail, 1995; Mohapatra *et al.*, 1996, etc.). However, the presence of variation in developmental effects of chitin inhibitors may be largely due to the large species - variation in respect to relative potency of these various compounds. This variation may, also, be resulted from the different mechanisms of ecdysteroid metabolism existing in different insects (Whisenton *et al.*, 1989).

The shortening effect of Lufenuron and Diofenolan in the present study on the weevil *Rh. ferrugineus*, or the lengthening effect of other chitin inhibitors on various insect species, may be explicated by causing an imbalance in the hormone titers at critical times of moulting because the proper balance in the hormone titers is necessary for normal growth, transformation into the pupal stage (Richards, 1981; Retnakaran *et al.*, 1985; Sehnal and Bryant, 1993). That relationships between chitin inhibitors, especially Dimilin and ecdysteroids were investigated in several species (Soltani *et al.*, 1993, 1996; Rehimy and Soltani, 1998; Chebira *et al.*, 2000). Moreover, shortening or elongating the developmental periods by IGRs, other than the chitin inhibitors, may be attributed to their effect on the release of ecdysteroids indirectly, by interfering with the neuroendocrine sites responsible for the release of tropic hormones (especially the prothoracicotropic hormone) (1985; Schmutterer, 1989; Subrahmanyam *et al.*, 1989).

### **3) Morphogenic effects on *Rh. ferrugineus*:**

Topical application of Lufenuron onto the prepupae of *Rh. ferrugineus*, in the present study, at dose-levels: 500.0 and 100.0 µg/insect resulted in pronouncedly prohibition of the pupation. In respect to Diofenolan, only the highest dose caused only a slight prohibition of this process. Similar results had been obtained by using Dimilin and its analogues (or chitin inhibitors) against *S. littoralis* (Gamal *et al.*, 1994), *Tribolium confusum* (El-Sayed *et al.*, 1984), *M. domestica* (El-Kordy *et al.*, 1989), *C. tarsalis* (Mulla *et al.*, 1989), some mosquito species (Montada *et al.*, 1994), some muscoids (Ghoneim *et al.*, 1992; Ghoneim and Ismail, 1995; Basiouny, 2000). Also, Diofenolan inhibited the

pupation in *Coccinella septempunctata* and *Chrysoperla carnea* (Sechser *et al.*, 1994) and *Hemiberlesia rapax* (Tomkins *et al.*, 1994). It is noteworthy to remember here that Lufenuron and Diofenolan are classified in the category of chitin inhibitors, so various works concerning only with it have been referred for saving time and effort because there are a big lot of data and results about the effects of IGRs - other than chitin inhibitors - on the pupation rate of a great variety of insect species, as well as on the adult emergence beside the affected pupal and adult morphogenesis.

Dealing with the action of Lufenuron and Diofenolan on the adult emergence of *Rh. ferrugineus*, in the present study, prepupal treatments reduced it in an effect reversibly correlated with the dose-level. The two higher dose-levels of Diofenolan completely prevented the adult eclosion but some adult weevils enclosed at other dose-levels. Similar effect was reported for various insects by Dimilin and Dimiloids (Salama *et al.*, 1976; Abo Elgar *et al.*, 1978; Bakr *et al.*, 1989; El-Kordy *et al.*, 1989; Ghoneim *et al.*, 1992; Ghoneim and Ismail, 1995; Basiouny, 2000). On the contrary, no effect of some dimiloids on adult emergence was reported by some authors, such as: Schmidt *et al.*, (1993).

However, inhibition of pupation and blockage of adult eclosion, as distinctly found in the present study by the action of Lufenuron and Diofenolan, may be considered as a result either to the haemolymph ecdysteroids or to a delay in the appearance of the last ecdysteroid peak, with or without a reduction in peak height and a slow abnormal decline in the peak (Handler, 1982; Redfern *et al.*, 1982; Sieber and Rembold, 1983). In other words, inhibition of pupation and blockage of adult emergence may be explained by the reduction of eclosion hormone production release, since this hormone is responsible for some prerequisite processes of the completion of moulting (Ghoneim *et al.*, 1998).

To clarify the possible morphogenic action of Lufenuron and Diofenolan on the pupation and adult eclosion programs, available data in the present study unambiguously prevailed increased pupal deformity, approximately, by the increasing dose-level of Lufenuron; while at the highest dose-level, the compound defused this pupal program in 25% only. The pupal malformation varied between charred body color, collapsed appendages, dorso-ventrally compressed body and presence of some prepupal skin remains, irrespective of the used IGR. No effect of Lufenuron on the adult morphogenesis was observed while Diofenolan, at its two lower doses, only, of Diofenolan impaired this phenomenon in 12%. Whether the used IGR, Lufenuron or Diofenolan, deformities of

adult weevils comprised pupal-adult intermediates, remains of the pupal exuvia and abnormal wings.

Various pupal and adult deformities were observed by several authors for different insect species, belonging to several orders by many chitin inhibitors such as: Dimilin against *Glossina morsitans* (Jordan *et al.*, 1979), *Simulium vittatum* (Lacy and Mulla, 1979), *Culex pipiens* (Bakr *et al.*, 1989), and *M. stabulans* (Basiouny, 2000); Bay SIR-8514 against *T. confusum* (El-Sayed *et al.*, 1984), *M. domestica* (Miller and Schmidtman, 1985), *C. pipiens* (Bakr *et al.*, 1989), *M. stabulans* (Ghoneim *et al.*, 1992); IKI-7899 against *C. pipiens* (Bakr *et al.*, 1989), *P. argyrostoma* (Ghoneim and Ismail, 1995), *M. stabulans* (Basiouny, 2000); ... etc. As well as, Diufenolan disrupted the insect transformation of the lepidopterans *Cydia pomonella* and *C. molesta* (Streibert *et al.*, 1994).

Several hypotheses have been made to explain the mode of action of the IGRs including direct inhibition and/or interference with chitin synthesis (Grosscut *et al.*), effect on the chitinase levels comprising that chitin is being digested faster than deposited (Soltani *et al.*, 1993), interference with juvenile hormone and ecdysteroid metabolism causing a disruption in the chitin metabolic system (Yu and Terriere, 1975), inhibition of chitin synthase by metabolites of chitin synthesis inhibitors (Cohen and Casida, 1980), inhibition of protease (s) that activate the chitin synthase zymogen (Leighton *et al.*, 1981), inhibition of DNA synthesis (Mitlin *et al.*, 1977), inhibition of glycosyl transferases that are involved with synthesis of lipid linked oligosaccharids in cell membranes which possibly provide primer molecules for chitin synthase (Marks and Sowa, 1979; Mayer *et al.*, 1980 a,b), and/or inhibition of facilitated diffusion and active transport across cell membranes of nucleosides and amino acids (Deloach *et al.*, 1981; Mayer *et al.*, 1988). However, and whatever the degree of pupal or adult deformation, it is suggested that chitin inhibitor (including Lufenuron and Diufenolan, in the present study) suppressed the chitin synthesis and prevented the normal deposition of new cuticle during apolysis, hence moulting abnormalities during larval-pupal or pupal-adult transformation may occur (*cf.* Yu and Terriere, 1975; Retnakaran *et al.*, 1985; Degheele, 1990). Finally, the exact mode of action of most IGRs almost remains poorly understood (Doannio *et al.*, 1993).

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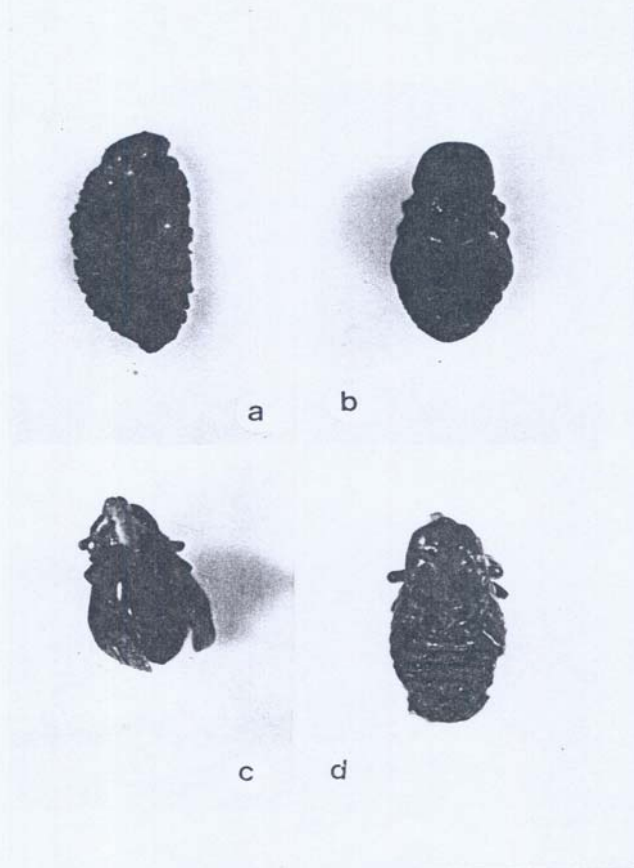


Fig. (1): Topical application of 500.0, 100.0, 50.0, 0.5, 1.0, 0.10 and 0.01  $\mu\text{g}/\text{insect}$  of Lufenuron (CGA-184699) onto prepupae of *Rh. ferrugineus* resulted in the following categories of deformed pupae: a) Dead prepupae with charred body. b) Dead pupae with charred body. c) Ventral side of pupae to show collapsed antennae, mouth parts and legs, d) Atrophied elytral pads and arched legs.

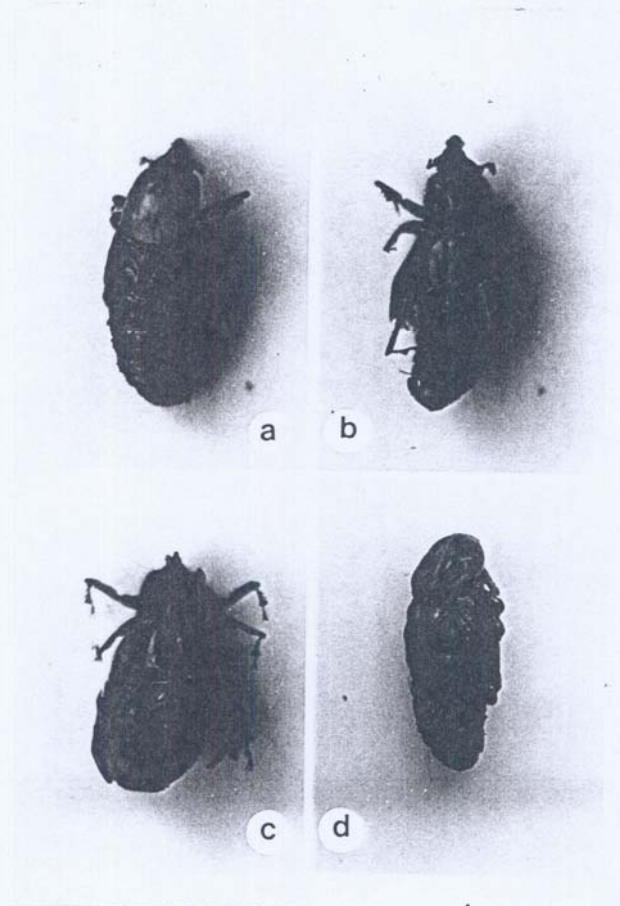


Fig. (2): Topical application of 50.0, 5.0, 1.0, and 0.01  $\mu\text{g}/\text{insect}$  of Lufenuron (CGA-184699) onto prepupae of *Rh. ferrugineus* resulted in the following categories of deformed adult weevils: a) Pupal-adult intermediate with posterior pupal portion and anterior adult portion. b) and c) Ventral side of adults which could not to emerge from the pupal exuvia. d) Lateral side of deformed adult weevil with atrophied wings and legs.

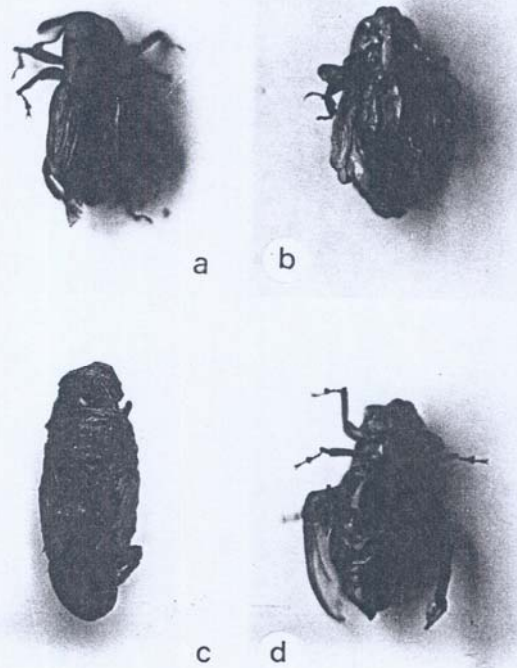


Fig. (3): Topical application of 500.0, 100.0, 50.0, 5.0 and 1.0  $\mu\text{g}/\text{insect}$  of Diofenolan (CGA-59052) onto prepupae of *Rh. ferrugineus* resulted in the following categories of deformed pupae: a) Dorso-ventrally compressed pupa. b) Assymmetrically formed pupa with zigzagged wing pad. c) Dorsal side of a pupa partially enveloped in the prepupal skin. d) Dorsal side of deformed pupa with a tubercled thorax and a dwarf wing pad.

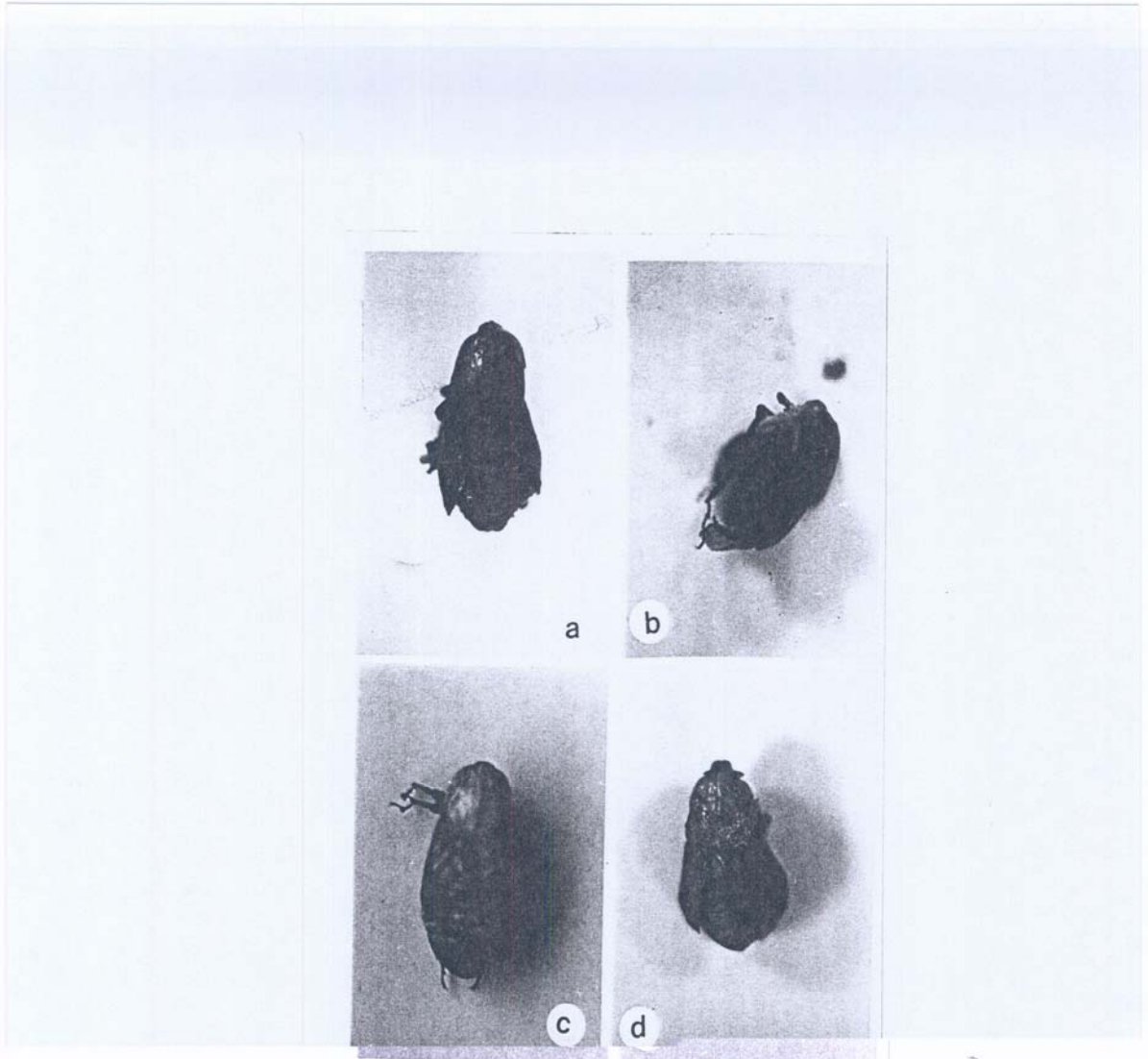


Fig. (4): Topical application of 1.00 and 0.10  $\mu\text{g}/\text{insect}$  of Diofenolan (CGA-59052) onto prepupae of *Rh. ferrugineus* resulted in the following categories of deformed adult weevils: a) Adult with permanently expanded membranous wings and evaginated elytra. b) Ventral side of a deformed adult with collapsed antennae and mouth parts, as well as with remains of pupal exuvium. c) Adult with splitted abdomen. d) Adult with permanently expanded membranous wings.

**Table (1): Prepupal pupal and adult mortalities (%) caused by Lufenuron (CGA –184699) applied topically onto prepupae of the red palm weevil *Rhynchophorus ferrugineus*.**

Dose (µg / insect)	Prepupal mortality	Pupal mortalities					Adult mortality
		Early–age	Mid-age	Late–age	General mortality	Water loss (%)	
500.00	12.5	25.0	25.0	25.0	87.5	38.17	12.5
100.00	12.5	12.5	12.5	25.0	62.5	21.73	37.5
50.00	00.0	12.5	12.5	12.5	37.5	16.88	37.5
5.00	00.0	00.0	12.5	12.5	25.0	15.77	25.0
1.00	00.0	00.0	12.5	12.5	25.0	16.82	12.5
0.10	00.0	00.0	12.5	00.0	12.5	16.32	12.5
0.01	00.0	00.0	00.0	00.0	12.5	15.67	00.0
Control	00.0	00.0	00.0	8.3	8.3	13.52	00.0

Early–aged: 1-day old pupae, Mid-aged: 4-day old pupae, Late–aged: 7-day old pupae. Eight prepupae were used as replicates

for treatment, but 12 corresponding prepupae were used as controls.

**Table (2): Prepupal pupal and adult mortalities (%) caused by the Diofenolan (CGA-59205) applied topically onto the prepupae of red palm weevil *Rhynchophorus ferrugineus***

Dose ( $\mu\text{g}$ / insect)	Prepupal mortality	Pupal mortalities					Adult mortality
		Early-age	Mid-age	Late-age	General mortality	Water loss (%)	
500.00	12.5	50.0	12.5	25.0	87.5	40.3	12.5
100.00	00.0	37.5	25.0	12.5	75.0	38.7	12.5
50.00	00.0	25.0	25.0	12.5	62.5	33.6	12.5
5.00	00.0	12.5	12.5	12.5	25.0	34.5	12.5
1.00	00.0	00.0	00.0	12.5	12.5	27.2	12.5
0.10	00.0	00.0	00.0	12.5	12.5	28.7	00.0
0.01	00.0	00.0	00.0	12.5	12.5	26.8	00.0
Control	00.0	00.0	00.0	00.0	8.3	24.28	00.0

Early-aged, Mid-aged and Late-aged: see the footnote of table (1). Eight prepupae were used as replicates for treatment,

but 12 corresponding prepupae were used as controls.

**Table (3): Effects of Lufenuron (CGA-184699) applied topically onto the prepupae on growth and development of red palm weevil *Rhynchophorus ferrugineus***

Dose ( $\mu\text{g}$ / insect)	Prepupae				Pupae			
	Duration (days $\pm$ S.D)	Develop. rate	Weights (mg $\pm$ S.D )	Growth index	Duration (days $\pm$ S.D )	Develop. rate	Weights (mg $\pm$ S.D )	
							Newly- formed	Late- aged
500.00	5.55 $\pm$ 0.45d	29.59	3.38 $\pm$ 0.37c	5.00	4.31 $\pm$ 0.46c	23.20	2.63 $\pm$ 0.32d	1.83 $\pm$ 0.29d
100.00	5.88 $\pm$ 0.87c	28.99	3.45 $\pm$ 0.50c	1.56	4.57 $\pm$ 0.81b	21.88	3.26 $\pm$ 0.29c	2.18 $\pm$ 0.35c
50.00	6.00 $\pm$ 0.67c	25.91	3.86 $\pm$ 0.56a	5.24	5.68 $\pm$ 0.35a	17.61	3.32 $\pm$ 0.25c	2.38 $\pm$ 0.36c
5.00	6.15 $\pm$ 1.28b	26.04	3.84 $\pm$ 0.75a	5.08	6.00 $\pm$ 1.26a	16.67	3.38 $\pm$ 0.39b	2.75 $\pm$ 0.27b
1.00	6.38 $\pm$ 1.48a	25.19	3.96 $\pm$ 0.66a	4.98	6.08 $\pm$ 0.99a	16.45	3.57 $\pm$ 0.47a	2.77 $\pm$ 0.37b
0.10	6.75 $\pm$ 0.70a	25.19	3.97 $\pm$ 0.77a	4.93	6.10 $\pm$ 1.77a	16.39	3.68 $\pm$ 0.77a	2.88 $\pm$ 0.68a
0.01	7.33 $\pm$ 1.03a	24.94	4.01 $\pm$ 0.40a	8.64	6.12 $\pm$ 1.28a	16.34	3.99 $\pm$ 0.63a	3.10 $\pm$ 0.56a
Control	7.45 $\pm$ 0.93a	22.03	4.54 $\pm$ 0.90a	8.57	6.16 $\pm$ 1.58a	16.23	4.10 $\pm$ 0.85a	3.46 $\pm$ 0.77a

Means  $\pm$  SD followed with the same letter (a) are not significantly different ( $P > 0.05$ ), (b): significantly different ( $P < 0.05$ ), (c): highly significantly different ( $P < 0.01$ ) (d): very highly significantly different ( $P < 0.001$ ). Develop. rate : Developmental rate. Eight prepupae were used as replicates for treatment, but 12 corresponding prepupae were used as controls.

**Table (4): Effects of Diofenolan (CGA-259205) applied topically onto the prepupae on growth and development of the red palm weevil *Rhynchophorus ferrugineus***

Dose ( $\mu\text{g}$ / insect)	Prepupae				Pupae			
	Duration (days $\pm$ S.D)	Develop. rate	Weights (mg $\pm$ S.D )	Growth index	Duration (days $\pm$ S.D )	Develop. rate	Weights (mg $\pm$ S.D )	
							Newly- formed	Late-aged
500.00	5.25 $\pm$ 0.55d	19.05	3.51 $\pm$ 0.33c	00.0	4.20 $\pm$ 0.13d	23.81	2.58 $\pm$ 0.37c	2.32 $\pm$ 0.28d
100.00	5.88 $\pm$ 0.88d	17.01	3.77 $\pm$ 0.50b	00.0	4.88 $\pm$ 0.27b	20.49	2.65 $\pm$ 0.36c	2.62 $\pm$ 0.56d
50.00	6.16 $\pm$ 0.64d	16.23	3.95 $\pm$ 0.65a	2.23	5.01 $\pm$ 0.23b	19.96	2.73 $\pm$ 0.30b	3.25 $\pm$ 0.39c
5.00	6.25 $\pm$ 0.70c	19.05	3.93 $\pm$ 0.56a	3.26	5.25 $\pm$ 0.88a	19.05	3.11 $\pm$ 0.88a	3.41 $\pm$ 0.65b
1.00	6.55 $\pm$ 0.98a	15.27	3.30 $\pm$ 0.52a	3.98	6.00 $\pm$ 1.41a	16.67	3.23 $\pm$ 0.58a	3.59 $\pm$ 0.72a
0.10	6.43 $\pm$ 1.32a	15.55	4.20 $\pm$ 0.45a	6.63	6.75 $\pm$ 1.03a	14.81	3.28 $\pm$ 1.11a	3.90 $\pm$ 0.83a
0.01	6.80 $\pm$ 1.41a	14.71	4.50 $\pm$ 0.99a	6.83	6.00 $\pm$ 1.51a	16.67	3.32 $\pm$ 0.25a	4.25 $\pm$ 1.58a
Control	7.45 $\pm$ 0.93a	13.42	4.54 $\pm$ 0.90a	6.74	6.16 $\pm$ 1.58a	16.23	3.46 $\pm$ 0.76a	4.35 $\pm$ 0.86a

a, b, c and d, Develop. rate : See the footnote of Table (3). Eight prepupae were used as replicates for treatment, but 12 corresponding prepupae were used as controls.



**Table (5): Morphogenic and Metamorphic effects (%) of Lufenuron (CGA–184699) applied topically onto the prepupae of the red palm weevil *Rhynchophorus ferrugineus*.**

Dose ( $\mu\text{g}$ / insect)	Pupal stage		Adult stage	
	Pupation	Deformities	Emergence	Deformities
500.00	87.5	50.0	37.0	--
100.00	87.5	37.5	12.5	--
50.00	100.0	37.5	50.0	37.50
5.00	100.0	25.0	50.0	25.0
1.00	100.0	12.5	50.0	12.5
0.10	100.0	25.0	50.0	00.0
0.01	100.0	12.5	87.5	12.5
Control	100.0	8.3	91.7	--

Eight prepupae were used as replicates for treatment, but 12 corresponding prepupae were used as controls.

**Table (6): Morphogenic and Metamorphic effects (%) of Diofenolan (CGA-59205) applied topically onto the prepupae of red palm weevil *Rhynchophorus ferrugineus*.**

Dose ( $\mu\text{g}$ / insect)	Pupal stage		Adult stage	
	Pupation	Deformities	Emergence	Deformities
500.00	87.5	25.0	00.0	--
100.00	100.0	37.5	00.0	--
50.00	100.0	25.0	25.0	--
5.00	100.0	25.0	37.5	--
1.00	100.0	25.0	50.0	--
0.10	100.0	--	87.5	12.5
0.01	100.0	--	87.5	12.5
Control	100.0	--	91.7	--

Eight prepupae were used as replicates for treatment, but 12 corresponding prepupae were used as controls.