

## Effects of Aqueous and Ethanolic leaves and flowerbuds Extracts of *Croton zambezicus* on the Larval Growth of African Lady Bird *Epilachna chrysomelina* F (Coleoptera: Chrysomelidae)

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### **Abstract:**

The *Croton zambezicus*, Um-Ghalaila is a common name. "Ghussga-yene" is a vernacular name used by a local community, where the plant parts were collected. It classified as aramotic plant and may be active agent as natural product (plate:7 &8). The plant leaves and flowerbuds were collected in South Kordofan State in January. The objectives of this study are: i .To determine the quantities and % of the extractable from leaves and flowerbuds of *Croton zambezicus* , ii. To evaluate efficacy of bioactive compounds present in the plant as insecticide, against 3<sup>rd</sup>- larval (L3) stage of insect, *Epilachna chrysomelina*; using both aq.extr. and EtOH-extr .Electric shaker apparatus and Rotary vacuum evaporator for aqueous extracts and Soxhlet apparatus and rotary vacuum evaporator for ethanol (Et.OH) and hexane (hex)-extracts were used. *E.chrysomelina* 3<sup>rd</sup>instars larvae (L3), average weight (30±2mg) were used. Three replicates were adopted . Only one larva placed in each Petri-dish lined with a moist filter paper, and treated or untreated leaf discs of a cucurbit were used as food source. Concentrations of 1, 2.5 and 5% were used. Control used in the bioassay was(. Discs + extracting solvent+ 1 larva) . The most important results obtained in all treatments investigated throughout this study showed that : All plant leaf and flowerbuds-aqueous extract and Ethanolic extract at all doses used were highly effec-

tive in reducing insect parameters measured compared to those recorded in control. The larval food consumption of leaf aqueous extract within 24hrs were 5.7, 6.0 and 7.3mg for the concentrations 5%, 2.5% and 1% respectively. The control consumed 30.0mg in the same period. An average weight loss/larva fed on leaf aq- extr. were 10.1, 14.1 and 4.2 at conc. 5%, 2.5% and 1%, whereas control larvae gained 80mg body weight. The larval average food consumption fed on flowerbuds aqueous extract at first and second 24hrs of test were 2.5 and 10.0mg at conc. 5% respectively, and about 6.2 and 8.5 mg at 2.5%, while 9.0 and 11.0mg at conc. 1%. The controls, were 30.0 and 128.0mg for the same periods. In the case of larval weight loss when fed on aqueous extract of flower buds during 48hrs, larval recorded 3.2mg at conc. 5% as weight loss and at 2.5% and 1% the larval gained, 2.1 and 5.6mg respectively. control weight loss was two times less than those recorded in the conc. 5%. Regarding to the effect of ethanolic leaf extract on weight loss, the larval during the second 24hr of 48hrs loss weight of 5.3mg at conc. 5%, while at conc. 2.5% and 1% it gained 5.0 and 10.8 mg respectively.

**Key word:** African Melon Lady Bird, *Croton zambezicus*, Larval Growth, bioactivity.

اثر المستخلصات المائية والايثونولية لاوراق و براعم ازهار نبات ام غليظة على نمو يرقات

حشرة خنفساء القرعيات الافريقية

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المستخلص:

نبات ام غليظة تُصنف كنبات عطري ويمكن لمنتجاته ان تكون فعالة في العديد من المحاور كمنتجات طبيعية. تم جمع اوراق وبراعم ازهار نبات ام غليظة في ولاية جنوب كردفان خلال شهر يناير. اهداف هذه الدراسة تلخص في الاتي: تحديد كمية ونسبة المواد المستخلصة من الاوراق وبراعم الازهار وتقييم الفعالية الحيوية للمستخلص المائي والكحولي لاوراق وبراعم الازهار كميبيدات حشرية للطور اليرقي الثالث للخنفساء الافريقية. استخدم للاستخلاص المائي والكحولي الاجهزة الكهربائية التالية: جهاز الاستخلاص الهزاز، جهاز التبخير الدوار وجهاز السكسليت. أُستخدمت يرقة واحدة من يرقات الطور الثالث للخنفساء الافريقية بمتوسط وزن  $2 \pm 30$  ملجرام في

كل مكرر من المكررات الثلاثة لكل معاملة . زُودت كل طبق بتري خاص بمكررات التجربة بورقة ترشيح مبللة للرطوبة واوراق القرعيات المعاملة والغير معاملة بالمستخلص في الشاهد . أُختبرت ثلاثة تركيزات (1% ، 2.5% و 5%) وكرّر كل واحد ثلاث مرات لكل معاملة . أظهرت أهم النتائج المرصودة في كل المعاملات خلال هذه الدراسة ان كل مستخلصات الاوراق وبراعم الازهارالمائية والكحولية المختبرة عند الجرعات المستخدمة ذات فعالية عالية في خفض المقاييس الحشرية المقاسة مقارنةً بتلك المسجلة عند معاملات الشاهد . متوسط الاستهلاك الغذائي ليرقات الخنفساء الافريقية لاوراق القرعيات المعاملة بالمستخلص المائي لاوراق ام غلييلة خلال 24 ساعة كان 5.7، 6.0 و 7.0 ملجرام للتركيزات 5% ، 2.5% و 1% على التوالي، بينما استهلك يرقات الشاهد 30.0 ملجرام . متوسط فقد الوزن لليرقات المتغذية على اوراق القرعيات المعاملة بالمستخلص المائي لاوراق ام غلييلة خلال 48 ساعة بلغ 10.1 ، 14.1 و 4.2 ملجرام عند التركيزات 5% ، 2.5% و 1% على الترتيب، في حين اكتسبت يرقات الشاهد وزن 80 ملجرام. متوسط استهلاك يرقات الحشرة للغذا المعامل بالمستخلص المائي لبراعم ازهار نبات ام غلييلة في اول وثاني 24 ساعة من زمن الاختبار كان 2.5 و 10.0 ملجرام للتركيز 5% على التوالي ، وكان 6.2 و 8.5 ملجرام للتركيز 2.5% بينما كان 9.0 و 11.0 ملجرام للتركيز 1% وبالمقابل بلغت متوسطات الشاهد 30.0 و 128 ملجرام لنفس الفترات اعلاه . في حالة الوزن اليرقى عند استخدام المستخلص المائي لبراعم الازهار فانه بلغ 3.2 ملجرام عند التركيز 5% كوزن مفقود وعند التراكيز 2.5% و 1% اكتسبت اليرقات وزن 2.1 و 5.6 ملجرام على التوالي وكان الوزن المفقود في الشاهد اقل مرتين من المرصود في التركيز 5%. وفقاً لاثر المستخلص الايتانولي لاوراق نبات ام غلييلة فان الوزن اليرقى المفقود خلال 24 ساعة الثانية من ال 48 ساعة بلغ 5.3 ملجرام للتركيز 5% وفي التراكيز 2.5% و 1% اكتسبت اليرقات 5.0 و 10.8 ملجرام على الترتيب .

الكلمات المفتاحية: خنفساء القرعيات الافريقية، ام غلييلة ، نمو اليرقات ، الحيوية

## INTRODUCTION

pesticide was defined as any substance or mixture of substances, intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals, causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, or animal feed stuffs. The term is also used as a substance applied to crops either before or after harvest, to protect commodity from deterioration during storage and transport(1). Pesticides also, include herbicide, insecticide, insect growth regulator (IGRs), nematocides, termiticides, molluscicides, piscicides, avicides, rodenticides, predacides, bactericides, insect repellents, animal repellents, antimicrobials, fungicides, disinfectants

tants (antimicrobial), and sanitizers (2) . Generally, a pesticide is a chemical or a biological agent that deters, incapacitates, kills, or otherwise discourages pests (3) . The pesticides applied in grocery stores and food storage facilities to manage rodents and insects that infest food, such as grain. Pesticides' use can save farmers' products by preventing crop losses by insects or any other pests . Environmentally, considerable amounts of insecticides and herbicides, reach non-target species, air, water and soil, causing contamination. In addition, pesticides reduce biodiversity, contribute in pollinator decline, destroy habitat and threaten beneficial species, attracting, seducing or mitigating any pest . Moreover, continuous application, ignorance, negligence, misuse, abuse, and misapplication of pesticides by farmers, beside unaffordable costs, both in Khartoum and Gezira area particularly, contributed in pesticides problems. Natural products are suggested to be one of the possible solutions or alternatives for such cases. The natural products based on botanical sources. Such products are expected to be ecologically-sound, in addition to the availability of rich plant biodiversity in Sudan, especially South Kordofan region. Several plants are studied in such universities, research centers and institutes. Some of them proved to have insecticidal activities, fungicidal activities, ability to control plant insects and diseases vectors. Moreover , plants with potent bioactive compounds are often characterised as both poisonous and medicinal, and a beneficial or an adverse result may depend on the amount eaten and the context of intake (4). South Kordofan State (SKS) is very rich at its flora, for about 50 medicinal plants wildy grown, of which 20 have demand in national and international markets. The present study aimed to evaluate croton zamb.. Phytochemicals or bioactive compounds, efficacy as insecticides, against 3<sup>rd</sup>-instar larva of *Epilachna chrysomelina* F., the African Melon Lady Bird Beetle (AMLB), using aqueous and ethanolic extracts.

## MATERIALS AND METHODS

### 1. INSECT COLLECTION AND CULTURE

#### 1.2 Collection

Primarily, survey and collection of the African Melon lady-bird beetle (AMLB), *E.chrysomelina*, was conducted in four different locations of the capital city of the Sudan, Khartoum. First survey was done at Dar-esalam area "western Omdurman city". Adults and eggs were collected from water melon plants. The second batch was collected from Tayba-Elhasanab village, southern Khartoum from water melon plants. Only four batches of eggs and few adults were collected from that area. The last survey was carried out at El Gaily area, northern Khartoum north (Bahari), from cucumber field. The field was completely damaged by this pest. All stages were collected from that field at season of winter.

#### 1.3 Culture

The collected adults were placed inside glass cages, 40 x 20 x 20 cm (Plate 1). The cages were lined with sterilized fine sand and kept under  $29\pm 1^{\circ}\text{C}$  and  $55\pm 10\%$  R.H. The soil inside the cages was frequently provided with a few ml of water to keep the larval and adults' feed fresh (plate2). Both, adult and larval instars were fed on cucumber and pumpkin plant parts ( plate3), which are supplied twice / day. When oviposition (O.P.) started, leaves, roots, stems or even flowers of the pumpkin or cucumber, with newly laid eggs, were transferred to Petri-dishes (9cm in dia.), lined with a moist filter paper. For the securing food, pumpkin, cucumber or water melon were planted throughout the experimental period

(plate 3).



**Plate(1). Insect rearing cage**



**Plate (2). Wet sand inside the cage with fresh leaves**



**Plate(3).Pumpkin culture for insect rearing(U of G)**



**Plate (4).*Epilachna Chrysomelina* 3<sup>rd</sup> instar larva (L3).**

## **2. Collection of Plants, Preparation of Powders and Extracts**

### **2.1. Collection**

Leaves (L) and flowersbuds (Fb) of *Croton zambezicus* Muell. Arg were collected following the rainy-season (Kharief), where the plants are still green and at the flowering stage. These plants were collected from different areas of South Kordofan State (SKS), *viz.* Nuba -Mountains , the collected plant parts were left to dry for a month under shade conditions, and at room temperature ( $29\pm 1^{\circ}\text{C}$ ).

### **2.2. Preparation of Powders**

Dried plant parts were finely ground by household grinder-mixer. The powder obtained from each plant part was passed through mesh-sieve No. 10, and kept inside black sacks under laboratory temperature ( $30\pm 2^{\circ}\text{C}$ ) to be used for extraction.

### **2.3. Preparation of Extracts**

#### **2.3.1. Aqueous- extracts (Aq-extr.; polar extr.)**

Each extract was prepared by shaking the powder in an Electric rotary shaker using 50g of the powder from the different plant parts added to one litre of distilled water in a 2L bottle and

hand-shaken for homogeneity of concentration, then divided into several conical flasks. These were subjected to shaking using the above-mentioned shaker for 24 hr at room temperature. The contents were strained by muslin cloth and filtered through Whatman No.1 filter paper. Using rotary vacuum evaporator (RVE) device, the excess water at the filtrate was removed. The filtrate placed at flat dishes or Petri-dishes for complete dryness. After 3- 4 days, residues were obtained, weighed using sensitive balance (Plate5) and expressed as % aq- extr. of the total extracted weights .The water extracts were kept in dark vials under laboratory conditions ( $30\pm 2^{\circ}\text{C}$ ) to be used for the bioassays.



**Plate (5) Sensitive balance used in the study**

### **2.3. 2. Hexane -Extracts (hex-extr.; apolar extracts)**

Forty g of the previously mentioned of two powders, as in section (2.3.1), were dissolved in 300ml of hexane (hex) as an extractant using the Soxhlet apparatus for 6-9 hr. The extract was

collected and evaporated using the RVE apparatus as in section 2.3.1), to remove excess hexane. The filtrate was left to dry at laboratory temperature ( $30\pm 2^{\circ}\text{C}$ ), weighed and the percentage extracted was calculated (Table 1). To extract the essential oil from *C. zambesicus*, leaves (L) and flower-buds (FB), similar method was adopted.

### 2.3.3. Ethanol -extracts (polar-extracts)

The hex-extr. (Table 2) were re-dissolved into 150-200 ml ethanol (Et.OH). The extract was collected and evaporated as in (2.3.2). Finally, substrates gained as Et.OH-extracts weighed and presented in (Table1).

## 3. BIOASSAY

### 3. 1. Preparation of Stock Solutions

A stock solution is a concentrate from which dilutions will be made. For example, 5% w/v contains 5g of solute /100ml of solution. For example, 1g of the extract (e.g. *Croton* leaves ext.) was added to the conical flask (20 ml volume); 5ml DW was transferred into the flask. The mixture was gently shaken or stirred to dissolve the extract in the DW, then completed to volume (20 ml). This stock solution was stored at  $5^{\circ}\text{C}$  (refrigerator) as a 5% w/v.

### 3.2 Dilutions (Treatments)

From previous stock solution 5% w/v, conc. 2.5% and 1% were prepared as follows:

1g ----- 20ml----- 5%

0.5g ----- xml -----2.5%

$X = 0.5 \times 20 \text{ ml} \div 1 = 10 \text{ ml (from stock solution)} + 10 \text{ ml (D.}$

water) =20 ml

2.5% ----- 20ml DW

5% ----- 1g ----- 20ml

1% ----- x ml

1% = 20 ml ÷5 = 4 ml (from S. solution) +16ml (DW)  
=20ml

#### 4. Effect of Aq- and O.S- Extracts on Feeding, Larval Growth (LG) and Duration of L3

##### 4.1. *C. zambesicus* leaves for 24 hr only

L3 of the same age and average wt ( $30 \pm 2$ mg) were used. The larvae were placed in Petri-dishes (9 cm dia.) lined with a moist Whatman No.1 filter paper, containing either treated or untreated pumpkin leaf-discs ( $5 \times 100$  mg/disc) prepared by a cork borer (18 cm diameter( plate6). Each Petri-dish contained only one larva. Leaf-discs were dipped into one of the following concentrations: 1%, 2.5% and 5% of the *I. carnea* L- aq— or -Organic—Solvent-extr., for 10 sec. The leaf – discs were left to dry under laboratory conditions (solvent evaporation). Discs + extracting solvent + one larva were used in the bioassay as control check. Each treatment was replicated three times. Leaf-discs (consumed food) and larvae were weighed after 24 hr and data recorded. The amount of food consumed (in mg) / larva and a larval wt (LW; gain/ or loss) were daily recorded. Leaf discs, after 24 and 48 hr test, were replaced by untreated ones during feeding the larval instars. Pupation and adult emergence were recorded daily until last adult emergence.



### **Plate (6) Cork borer used in the study**

#### **4.2. *C. zambesicus* leaves for 48 hr**

The same method in (3.4.3.1.) was followed here, but the larvae were fed on the treated pumpkin leaf discs for 48 hr. The fresh untreated leaf discs replaced the treated ones after 48 hr up to the end of the experiment (s).

#### **4.3. Feeding with untreated leaves for the whole larval duration**

In this experiment, the control/ or check larvae were fed on the untreated leaf discs during larval duration by daily renewing. Also, pupation duration and adult emergence were determined, i.e. the normal duration of each instar and stage.

### **5. Statistical Analysis**

One-Way ANOVA is a technique used to compare means of three or more groups using the (F- distribution). DMRT at 5% was adopted in the present study to find differences between means .

### **RESULTS:**

#### **1. Aqueous extracts (aq-extr.)**

The aq- extr. of the *Croton zambesicus* plant leaves & flower buds is resulted in table 1.

## 2 . Hexane extracts (hex-extr.)

The % extracted after 9 hr using Soxhlet device resulted in (table 1).

## 3.Et.OH extract (Polar).

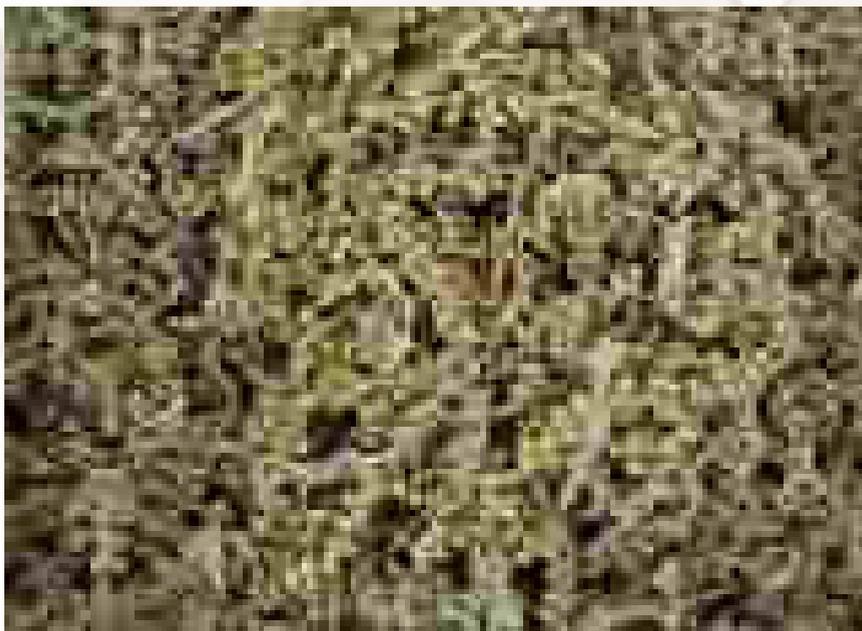
Et.OH was able to extract the material remained after hexane extraction (table1).

**Table (1) *Croton zambezicus* leaves& flower buds extract**

Type of extract	Plant part	Original wt/g	Extract wt/g	Extract%
Aqueous-ext	Leaves	50	7.3	14.6
	Flower buds	50	16.0	32.0
Hexane -ext	Leaves	40	4.46	11.16
	Flower buds	40	0.46	1.14
Ethanol-ext	Leaves	40	2.36	8.4
	Flower buds	40	1.48	3.7



## Plate (7) Summary *Croton zambezicus* in its rocky habitat



Plate(8) *C.zambezicus* flower-buds(Dalanj area).

### 4. Leaf consumption and total larval weight of *Croton zambezicus*

#### 4. 1. Leaf Aqueous -Extract (Feeding for 24 and 48 hr)

The result obtained (Fig. 1) showed the larval food consumption (FC), an average rate, in terms of mg/day larva fed on leaf-discs treated with L-aq-extr. of *C. zambezicus*, were: 5.7, 6.0 and 7.3mg for the concentrations 5%, 2.5% and 1%, respectively. The control consumed 30.0 mg in the 1<sup>st</sup> 24 hr. Larval mortality (LM) was 11.1% at conc. 2.5% only. At Larval duration (LD), the average of food consumed by larva FC within three days, were 131.1, 142.6 and 142.9 mg, following the same order of doses in first 24hr. The control consumed 158.3 mg. A statistical analysis showed insignificant reduction of FC at the 1<sup>st</sup> day of the experiment .

## Regarding

48 hr of exposure the average leaf consumption in mg/larva/day, at the 1<sup>st</sup> and 2<sup>nd</sup> 24 hr ranged between 3.0 and 6.3, 4.0 and 15.0 mg for 5%, 2.5% and 1%, respectively. Statistically, these results were insignificant, except concentration 2.5% at the second 24hr. L-discs consumption (LDC) was increasing from day to another. Control larva ceased feeding at the 3<sup>rd</sup> day. In larval duration (LD), the total amount of LDCs/larva, were: 130.2, 139.1 and 141.5mg at different treatments .

In case of effects on larval weight, gain or loss( Fig.2), an average initial larval weights (Lwt) used (the averages were 30 mg ;28.0, 30.0 and 32.0 mg) when started feeding on treated leaf, an average wt-loss /larva were. 9.3, 5.7 and 1.0 mg, were, at 5, 2.5 and 1% respectively. Starvation control, was -6.3 mg (first 24hr). The statistical analysis showed significant reduction of Lwt at different concentrations, but conc. 2.5% and the control were similar .The total larvae weight (wt-gained) (TLwt) per larva duration LD as follows 54.2, 55.5 and 69.9mg, for 5%, 2.5% and 1 % respectively. Starvation control SC showed -27.6 mg loss, while Control larva CL gained 80.6mg body weight b.wt. .

For 48 hr the larval total weight exposed to treated leaf, an average loss per larva at 5%, was 1.4 mg within first 24hr, while the larvae gained 1.2 and 1.7mg weight when fed on treated leaf discs at conc. 2.5% and 1%, respectively. In second 24 hr, larva lost 10.1, 14.1 and 4.2 mg, at conc. 5%, 2.5% and 1%, respective-

ly. The negative control larva lost 6.7 mg, whereas control larva CL gained 80.6 mg b.wt. Twt gained per larva throughout LD were 28.1, 56.3 and 64.6 mg, for 5%, 2.5% and 1% respectively .A statistical analysis showed significant reduction in Lwt in 2<sup>nd</sup> 24hrs, when compared to the CL and SC .

**When** following L3 of AMLB up till adult stage, at 24 and 48hr Croton L-aq-extr tests, 88.9 % and 100% of the treated larvae at 1% and 5% for "24hr" and different doses for "48hr", are transferred into adult stage, respectively. An average pupation (AP) and adult emergence (AE) were 4.3 and 3.7 days, for different L-aq-extr treated larvae. Starvation control larvae, survived till the 4<sup>th</sup> day, then died at 5<sup>th</sup> and 6<sup>th</sup> day of experiment .Positive control, developed into adult stage and finished emergence entirely after 11 days.

#### **4.2. Flower Aqueous Extract (Feeding for 24 and 48 hr)**

**The** average LDC /larva/day, when L3 fed on food treated by flower aq- extr. of *C. zambezicus*, were 1.5, 2.3 and 7.7mg for 5%, 2.5% and 1%, respectively. The Larva ceased feeding at 2.5% on the 3<sup>rd</sup> day, and also the control. At larval duration, the total FC/ larva, ranged from 123.7 to 148.9 mg, beginning by the higher concentration, to the lower one. Concentration 2.5% and 5% were alike, but different from conc. 1%. A statistical analysis showed significant reduction of FC by larva, when compared with the control (Fig.1) .

**In** case of 48 hr at first and second 24hr tests, larva consumed an average 2.5 and 10.0 mg from the leaf discs treated with conc.

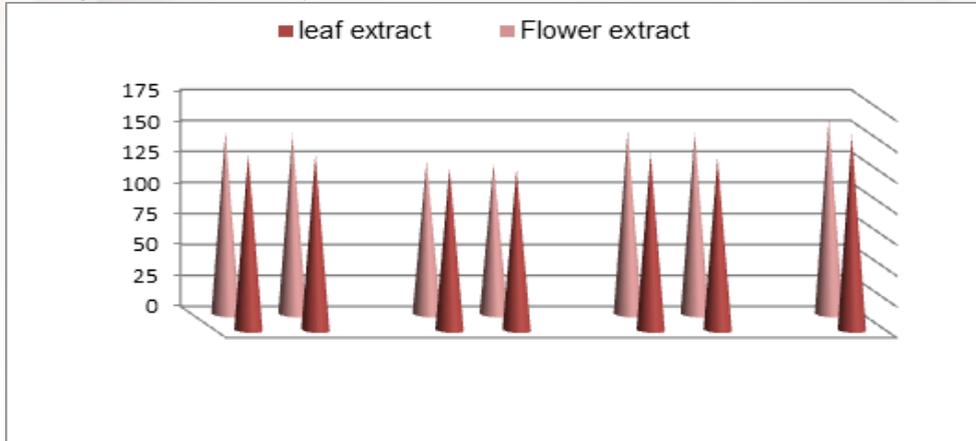
5% flower aq.- extr. of *C. zambezicus*, respectively, and about 6.2 and 8.5 mg at conc. 2.5%, while 9.0 and 11.0mg at conc. 1%. The controls, were 30.0 and 128.3mg for the same period. Total LDC, by larva, within LD, were 121.8 to 147.1 mg, at each concentration. Statistically, there were differences between CRs and the control ones.

**With** regard to the total larval weight (Fig.2), fed on leaf discs treated with flower aq.extr. for 24hr, the results recorded at conc. 5% was 0.2 mg is loss /larva. While at conc. 2.5% and 1%, larva gained an average 1.3 and 5.4 mg, respectively. The SC and CL were -6.3 and 15.2mg. Twt gained by larva within larval survival (LS): 60.9, 61.3 and 69.2mg, for 5%, 2.5% and 1% respectively. Statistically, only conc. 5% was significant in reducing Lwt.

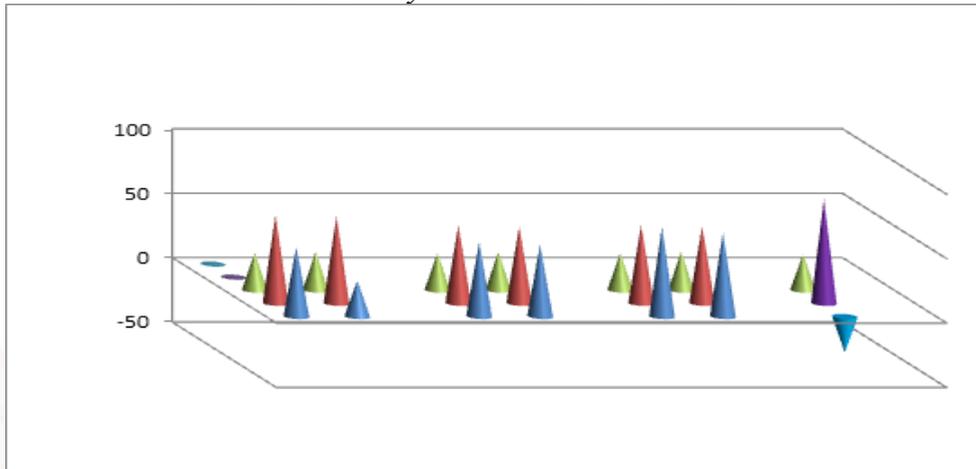
**While** the total weight of the L3 fed for 48 hr on leaf treated with flower aq-extr., the average weights gained/larva/day were: 8.7, 8.9 and 14.7mg, at conc 2.5%, 5%, and 1%, respectively, at the 1<sup>st</sup> day test. At the 2<sup>nd</sup> day, larva recorded 3.2 mg at conc. 5% as wt-loss, and at 2.5% and 1% the larva gained, 2.1 and 5.6 mg, respectively. Control wt.-loss, was two times less than conc.5%. Twts gained /larva since larval duration (LD) were 59.5, 60.0 and 68.0 mg, for 5% 2.5% and 1%, while the SC and +C were, -27.7 and 80.6 mg, respectively.

**When** L3 of *the* AMLB was followed up till adult stage, 100% of the treated larvae by F-aq-extr, successfully developed into adult insects, at all treatments and durations. An average P and

AE periods were 2.8 and 2.0 days for each . Starvation control SC larvae, survived till the 4<sup>th</sup> day, then died at 5<sup>th</sup> and 6<sup>th</sup> day of experiment. Control larvae, developed into adult stage and completely emerged after 11 days.



**Fig. 1.** Total food consumption when leaf discs were treated by *C. zambezicus* leaf and flower aqueous- extracts /larva during the third larval instar of *E.chrysomelina* for 24 and 48 hr.



**Fig. 2.** Total weight of the 3<sup>rd</sup> larval instar fed on leaf discs treated with *C. zambezicus* leaf and flower-buds aqueous- extracts during 3<sup>rd</sup> larval instar of *E. chrysomelina* for 24 and 48 hr.

### 4.3. Leaf Et. OH-extract (feeding for 24 and 48 hrs)

The results in figure(3) show an average CRs /larva within its growth, at conc. 5%, 2.5% and 1%, were: 4.3, 9.0 and 12.3mg respectively. The larva ceased feeding at 5% and 2.5% and, then continued feeding at the 2<sup>nd</sup> day. The TCRs through its LD: 148.0, 150.0 and 153.0 mg. The control consumed amounts (at 24 and 48hr) were 30.0, 128.3, with TCRs, 158.3mg. The statistical analysis, showed significant differences between treatments including the control .

In case of 48 hr in the first 24hr LG, an average CRs 7.3, 9.7 and 15.0 mg, were, recorded/larva, with total 141.7, 155.6 and 157.7 mg, at 5%, 2.5% and 1%, respectively, as LD. In the second 24hr, larval LCRs/day: 7.2, 14.2 and 20.0mg, with total leaf consumptions: 141.7, 155.6 and 157.7mg, at concentration, 5%, 2.5% and 1%, respectively. The statistical analysis showed significant differences between treatments including the control .

**Regarding** ,the larval weight when fed on *C. zambezicus* L-Et.OH-extr. for 24 hr, the result showed that an average wt / larva was, 4.1 mg at conc. 5%. while at conc. 2.5% and 1%, larva gained 4.1 mg and 10.3 mg respectively (Fig.4). The total weight (Twts) gained /larva within its duration were: 45.1 mg, 51.4 and 56.2 mg at conc. 5%, 2.5% and 1%, respectively, compared to initial weight. The statistical analysis showed significant differences in weight- loss or -gain when compared to the control .

In case of 48 hr the result showed the average wt-gain /larva

during larval stage 7.6, 8.6 and 9.4 mg, at conc. 5 %, 2.5 % and 1 %, respectively at first 24hr. In second 24 hr, the larva lost 5.3 mg at conc. 5%, while at conc. 2.5% and 1%, it gained 5.0 and 10.8 mg respectively, with total larval wt-gain: 45.1, 51.4 and 56.2 mg, at conc. 5 , 2.5%, and 1%, respectively. The statistical analysis, showed insignificant differences between treatments, except conc. 5% at 24hrs and second 24 of 48hr tests -4.1 and -5.3 respectively.

**When** followed *E.chrysomelina* L3 up to adult stage, and at first 24 or 48hr tests, 100% of the treated larvae developed into adult stage, at different treatments, and were normal. An average PD was 3.7 days. AE from pupation in average 1.2 days. In 48 hr-2, 22.2% of pupae died at 5%, the pupae that developed into adult stage, were 77.8% in average pupation period 3.7 and AE 1.2 days.

#### 4.4. Flower Et.OH extract (Feeding for 24 and 48 hr )

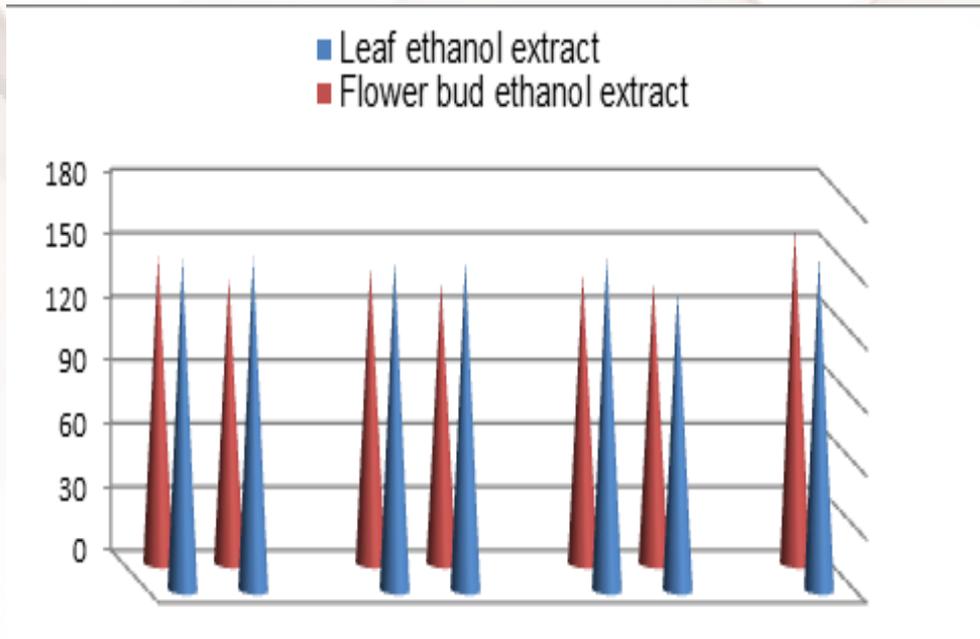
**The** results showed that an average of LDC/larva within LG were: 10.8, 14.7 and 15.2 mg, with total amounts of 137.0 , 139.5 and 145.9 mg, at conc. 5%, 2.5% and 1%, respectively, in its LD. The control consumed amounts (at 24 and 48hr) were: 30.0 and 128.3 mg. The statistical analysis showed significant difference between conc. 5%, 2.5%. and 1%. Moreover, all treatments were different from the control (Fig.3) .

**With** regard to 48 hr the results showed an average, LDC/larva/day, within first 24hr and second 24hr were 10.4, 12.6, 16.4mg and 33.8, 120.3 and 124.7mg, at conc. 5%, 2.5% and 1%, respectively. The larvae ceased feeding at conc. 5% in first 24hr, while

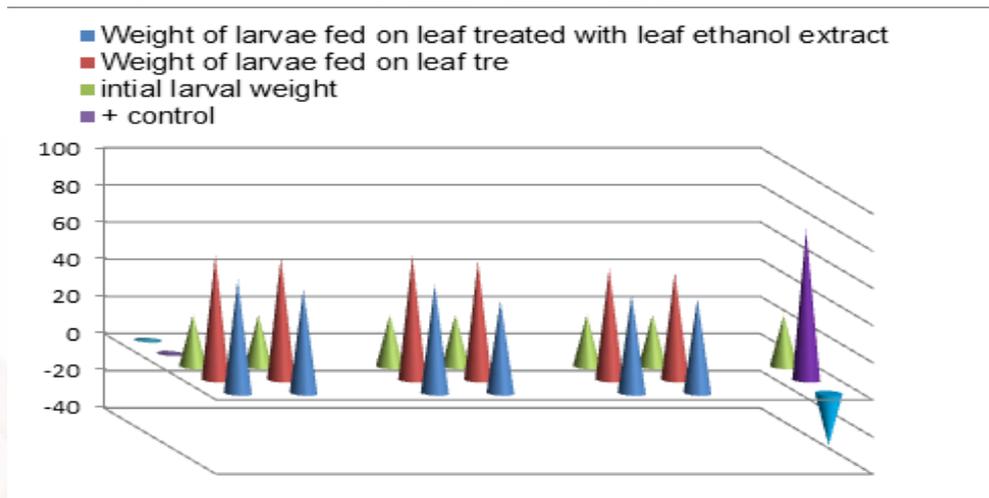
in second 24hr, the larva ceased feeding on conc. 1% and 2.5% and the total leaf CRs were 132.8, 132.9 and 135.1mg during LG. The statistical analysis showed significant differences between all treatments.

**In case of larval weight fed for 24hr on leaf discs treated with F- Et-OH-extr. (conc. 5%, 2.5% and 1%),** the results showed an average Lwt-loss/larva 15.6, 18.4 and 19.0mg, respectively, within the larval stage. The total weight gained/larva 59.6, 67.1 and 67.3mg, at conc. 5%, 2.5% and 1% respectively, during LD. Compared to the starved larvae which showed loss in weight -6.3mg, with total loss -27.7mg, and control larvae, which fed on untreated leaf discs, showed 12.2, 27.2, 10.2 and 28mg as initial weight, with total 80.6mg. The statistical analysis showed insignificant differences between treatments, but different from the control. Regarding 48 hr in the 1<sup>st</sup> 24hr of exposure the result showed that an average wt-gain/larva/day, during LG, were: 12.2, 18.6 and 19.2mg, at conc. 5%, 2.5% and 1% respectively. While in the second 24hr, the larva gained an average of 13.3, 17.5 and 18.7mg, with total 57.7, 64.1 and 65.9mg, respectively, throughout LD. Statistical analysis, showed in significant differences between all treatments (Fig4).

**Following** , L3 of *E.chrysomelina* up to adult stage, 100% of treated larvae thoroughly developed into adult stage in first 24 of 48 hrs, within an average 3.4 and AE 1.5 days. In 48 hr-2 test, 100% of the treated larvae, developed into adult in average 1.5 and pupation 3.4 days. The emerged adults were morphologically normal.



**Fig. 3.** Total food consumption /larvae fed on leaf discs treated with *C. zambeziensis* leaf- and flower- bud- ethanol extracts during 3<sup>rd</sup> larval instar of *E. chrysomelina* for 24 and 48 hr.



**Fig. 4.** Total weight of larvae fed for 24 and 48 hrs, on leaf discs treated with *C. zambeziensis* leaf and flower bud ethanol-extracts, during 3<sup>rd</sup> larval of *Epilachna chrysomelina* insect

## DISCUSSION and CONCLUSIONS

A large number of medicinal plants possess phytochemicals, such as flavonoids, anthraquinones, tannins, alkaloids ...etc, had been associated with antimicrobial, antifungal, anthelmintic, insecticidal, molluscicidal and other activities (5) . *C. zambezicus* leaves and flower buds were extracted by water, ethanol and hexane solvents. Water proved to be an efficient solvent than hexane and ethanol. This, result indicated that aq-extrs at two Croton samples, leaves and flower-buds, weighed more (in g) than Et-OH and hexane extrs (Table1) That might be used satisfactorily in different tests, or at field conditions later . Semilar findings were reported by.(6) and (7) .

In 24hr L-aq-extr tests, 11.1% was mortality of L3 at 2.5% on the 3<sup>rd</sup> day. 88.9%,was number of tested larvae that developed into adult stage . An average pupation period, at different Croton parts extracts, ranged from 2.8 to 4.3days,and an average adult emergence, from 1.2 to 3.7days.This,result was more than control, Um-Salama and less than El-Khidir reports . L Wt. and CRs at all treatments significantly decreased, when compared with the control at 24hr L-aq-extrs . At 48hrs tests, 100% of the treated larvae developed into adult stage. Conc. 5% was only significant in reducing LWt at 1<sup>st</sup> 24hr of 48hrs.At 2<sup>nd</sup> 24hr of 48hrs,all treatments reduced larval Wts . At 24hr testes of Croton L-aq-extrs all treatments reduced CRs . At 1<sup>st</sup> 24hr and 48hr ,all doses reduced leaf CRs, In 2<sup>nd</sup> 24hr trials only conc. 2.5% markedly reduced leaf CRs . At *Croton* L-Et.OH-extr,in 24hr tests,100% of the

treated larvae developed into adult stage, while at 48hrs trials, pupal mortality was 22.2% at conc. 5% . Regarding, the flower-buds aq and Et.OH extrs and at different durations and treatments, all treated larvae developed (100%) into adult stage and they were morphologically normal. The results obtained in this study shows that the mean larval consumption, mortality, weight loss or gain and development compared to the control treatment varies from one plant part extract to another, and this may explain that each part or type of extract differs in its effect, the result agreed with attribution of (8), (9) and (10) who investigated different botanical extracts, including *Catharanthus roseus*, *Balanites aegyptiaca* and *Lawsonia inermis* as antinutritional materials, repellent and inhibitory materials for some insect pests, including *E.chrysomelina* and *C.maculatus* insect and they found that the extracts vary in the degree of their effect on the activities of insects according to the amount, type of extraction and effectiveness of the chemical in each one. They also added that their general effect was proportional to the increase in the amount of dose used compared to control treatment. The latter attribution support the current results. Generally, The present findings demonstrated that only Croton L-aq and L-Et.OH extrs, were toxic. *C.zambeziacus*, is a multi-purpose plant, aromatic, medicinal and toxic one. Such plant might be beneficial at rural area levels. High cost and hazards of agrochemicals, might motivate small farmers to make use of such plants. Croton plant, could be qualified enough to solve farmers' problems when used in pest control at rural areas. In other words, farmers could

treat themselves and manage their pests at the same time. Future work, should include *Croton* volatile oil. Because, many essential oils succeeded nowadays, to manage human disease and used as pest control agents. Demonstrate different traditional uses as a medicinal, aromatic and toxic plant. Identify or detect essential-oils' constituents.

In conclusion, the current findings encourage more detailed studies in such plant parts, using more sophisticated procedures, methods and techniques to identify, quantify and isolate the active ingredients and conduct more intensive and extensive toxicological investigation.

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