

## Effect of Aqueous and Ethanol Extracts of *Citrullus colocynthis* Seeds on the Larval Growth of the *Musca domestica vicina* Macq. (Diptera: Muscidae)

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### Abstract

The house-fly is one of the most common of all insects; it can carry and transmit diseases through contaminated food. The aim of this study is to evaluate the effects of crude seed aqueous and ethanol extracts of *Citrullus colocynthis* against house-fly (*Musca domestica vicina*) larvae. A flat plastic container containing horse feces was used to attract the females' houseflies to lay their eggs, in Wad Medani City, central Sudan and transferred to the Basic Sciences Laboratory, University of Gezira, Sudan. The study was carried over three seasons (2013, 2014 and 2015). Dry crude seed extract (aqueous or ethanol) was mixed (at the concentrations ranged from 5% to 25%; w/w) with the larval feed. Each ten 3<sup>rd</sup> instar larvae were placed in special container and fed on the prepared mixture. Larval feeding continued until pupation. The larval mortalities were recorded after 1, 2, 3, 4 and 5 days. These experiments were repeated twice. The results showed that, the ethanol extracts were more potent (produced mean of 45% mortality for three seasons) than aqueous extracts (produced mean of 17.33% mortality for three seasons) in most cases. Some morphological abnormalities in larvae, pupae and emerged adults were also observed. Based on this study we recommend using this plant in house-fly control as component of an Integrated Pest Management programs.

**Keywords:** *Citrullus colocynthis*; Seeds; Extracts; House-fly; *Musca domestica vicina*; Larval Growth; Wad Medani City

## تأثير المستخلصات المائية والإيثانولية لبذور نبات الحنظل على نمو يرقات الذبابة المنزلية (*Musca domestica vicina*)

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### مستخلص الدراسة

الذبابة المنزلية من أكثر الحشرات المعروفة التي تنتقل الأمراض عبر الأطعمة الملوثة كما أنها تحمل الكائنات المسببة للأمراض. هدف هذه الدراسة هو تقييم تأثير المستخلصات الخام المائية والإيثانولية لبذور نبات الحنظل على نمو يرقات الذبابة المنزلية. تم استخدام وعاء بلاستيكي مفلطح يحتوي على براز الخيل لجذب الإناث لتضع بيضها فيه بمدينة ودمدني وسط السودان ونقلت لمعمل العلوم الأساسية، جامعة الجزيرة، السودان. أجري البحث في ثلاثة مواسم (2015، 2014، 2013). تم خلط المستخلص الخام الجاف (المائي أو الإيثانولي) في تراكيز متدرجة من 5% إلى 25% وزن/وزن مع غذاء اليرقات. تم وضع كل 10 يرقات (العمر الثالث) في وعاء خاص وغذيت بالغذاء المخلوط. استمرت عملية تغذية اليرقات حتى مرحلة العذراء. ثم رصدت وفيات اليرقات بعد 1، 2، 3، 4 و 5 أيام. كررت هذه التجارب لمرتين. أوضحت نتائج الدراسة أن المستخلص الإيثانولي أكثر فعالية (أحدث وفيات بمتوسط 45% خلال المواسم الثلاثة) من المستخلص المائي (أحدث وفيات بمتوسط 17.33% خلال المواسم الثلاثة). تم أيضاً ملاحظة بعض المظاهر المورفولوجية غير الطبيعية في اليرقات، العذراء والطور البالغ. بناءً على نتائج هذه الدراسة نوصي باستخدام هذه المنتجات ضد اليرقات في برامج مكافحة المتكاملة ضد يرقات الذبابة المنزلية.

**كلمات مفتاحية:** نبات الحنظل؛ بذور؛ مستخلصات؛ الذبابة المنزلية؛ موسيكا

دومستيكا؛ نمو اليرقات؛ مدينة ودمدني.

## 1. Introduction

The house-fly is one of the most synanthropic insects in the world. It imposes itself on humans and eats all what is available, food and wastes. Therefore, house-fly is considered mechanical vector of pathogens (bacteria, protozoa and viruses) to human and causes economic problems to all farm animals (2001; Sangmaneedet *et al.*, 2005; Jesikha, 2012). These pathogens may cause many contagious diseases such as food poisoning, diarrhea, cholera, typhoid, paratyphoid, shigellosis, and anthrax (Banjo *et al.*, 2005; Yap *et al.*, 2008).

The most of the house-flies in Sudan are *M. domestica vicina* Macq., while *M. d. domestica* are restricted to certain humid and shade places (Kehail and Abdalla, 2012). *M. d. vicina* Macq., is found in subtropical and tropical zones. The common subspecies found in Sudan is *M. d. vicina* Macq. (Omer, 2011).

*C. colocynthis* (L.) Schrad, commonly called colocynth, bitter apple or bitter cucumber and locally known as “Hanzal”, is one of many species of the family Cucurbitaceae. It is a wild plant; it is an ancestral type of watermelon, widely distributed in Northern and Central Sudan (AbdElgadir, 1995). *C. colocynthis* tendrils are simple, slender and hairy leaves are very variable in size (Borhade *et al.*, 2013).

Fight against adult house-fly is temporary, unsatisfactory, inadequate and environmentally polluting. Larval treatment is much more effective way for managing this notorious insect, because in this stage larvae are localized and restricted to a small space due to the low mobility (Howard *et al.*, 2007). The application of easily degradable plant compounds is considered one of the safest methods of control of insect pests and vectors (Sun *et al.*, 2006). Several studies have shown the possibility of using plant extracts in the control of house-fly eggs, larvae, pupae and adults (Issakul *et al.*, 2004; Malik *et al.*, 2007).

The aim of this study was to evaluate the effect of crude seed aqueous and ethanol extracts of *Citrullus colocynthis* against house-fly (*M. d. vicina*) larvae.

## 2. Materials and methods

### 2.1. Preparation of plant extract:

The seeds of colocynth (*C. colocynthis*) were brought from the local market of Wad Medani City, Gezira State, Sudan. Samples were immediately cleaned, dried in shade under room temperature away. After being dried, the seed samples were peeled to fine powder by using an electrical grinder and kept in plastic containers for the further tests. An amount of fifty grams of the dried powder of colocynth seeds were soaked into 500 ml of distilled water or ethanol in 500 ml conical flask for 48 hours. Each solution was filtrated by using a filter paper and then evaporated to dryness under vacuum using a rotary evaporator with a water bath adjusted to 80°C. The resulted dry extracts (DE) were then weighed for estimating their yield percentages and kept until used later.

### 2.2. Collection and colony rearing of *M. d. vicina*

A flat plastic container containing horse feces was used to attract the female house-flies to lay their eggs on, from El Andalus area, west of Wad Medani City, Gezira State, central Sudan; then was transferred to the Laboratory of Basic Science, Faculty of Agricultural Sciences, University of Gezira. The emerging adults (It has four black longitudinal stripes on the dorsal surface of the thorax, antennae are not easily seen, and concealed in depressions on the front of the face (Service, 2000) of *M. d. vicina* house-fly were separated from the other species by using aspirator, in a special cage (35X35X35 cm). The newly emerged larvae (first generation) were collected and reared under room temperature in special containers and were later used in the study experiment.

### 2.3. Bioassay experiment:

The larvae used for the test were at early 3<sup>rd</sup> instar. The dry extracts of colocynth seeds were mixed separately (at the concentrations of 5, 10, 15, 20 and 25% w/w) each with larval feed (composed of 6 g of wheat bran, 4 g of milk powder, and 25 ml of water) to produce the treated larval feed. Ten larvae were placed in special container (7X7X10 cm), for each test plant and fed on the prepared mixture (treated feed), while others were taken as control (fed on DE's-free feed) until pupation stage. The mortality was recorded after 1, 2, 3, 4 and 5 days, both in treated and control groups. Each experiment was repeated twice. The survived larvae which became pupae, were transferred to the pupal container, provided with small dish containing cotton pads soaked in milk powder dissolved in water for the emerged adults to feed on; at the room temperature during rainy season. These feeding methods were suggested by Hanai (2010) with some modifications.

### 2.4. Statistical analysis:

Microsoft Office Excel (2007) Program was used to analyze the data obtained. Simple descriptive statistics (count, sum, average (mean) and variance) and ANOVA analysis that used to describe the observed variations in the toxicities exerted by any plant extract were used. The difference was considered significant whenever the f-stat value is bigger than the f-crit value at certain P value.

## 3. Results and Discussion

3.1. Effect of Colocynth (*C. colocynthis*) Seed Extracts on Percentage Mortality of *M. d. vicina* Larvae at season 2013.

### 3.1.1. Aqueous extract

Table (1) shows that at all concentrations (5%, 10%, 15%, 20% and 25%) of the aqueous extracts of colocynth seeds, the resulted mortalities at the 5<sup>th</sup> day, did not exceed 10% (about 2 larvae only).

### 3.1.2. Ethanol extract

Table (1) also shows that, at the lower concentrations (5% and 10%) the resulted mortalities at the 5<sup>th</sup> day did not exceed 10% (about 2 larvae), while at the medium concentrations (15% and 20%) the mortalities were 25% and 15%, respectively, at the 5<sup>th</sup> day. The mortality in the higher concentration (25%), reached 35% at the 5<sup>th</sup> day, which was the highest mortality recorded for all extracts of colocynth seeds against house-fly larvae.

**Table (1): Mortality percentage of *Musca domestica vicina* larvae treated with different concentrations of *Citrullus colocynthis* seed extracts at season 2013**

Day	Control	Aqueous extract					Ethanol extract				
		Concentrations %									
		5	10	15	20	25	5	10	15	20	25
1	0	0	0	0	0	0	5	5	10	0	20
2	0	5	0	0	5	0	5	10	25	15	20
3	0	10	0	0	5	0	5	10	25	15	25
4	0	10	0	0	5	0	5	10	25	15	30
5	0	10	0	0	5	0	5	10	25	15	35

### 3.2. Effect of Colocynth (*C. colocynthis*) seed extracts on percentage mortality of *M. d. vicina* larvae at season 2014.

#### 3.2.1. Aqueous extract:

Table (2) shows that the concentrations of aqueous extract (5%, 10%, 15%, 20% and 25%) resulted in mortalities of 25%, 80%, 5%, 10% and 25%, respectively, at the 5<sup>th</sup> day. The mortalities at season 2013 were 10%, 0%, 0%, 5% and 0%, following the same order, which were obviously lower than those of season 2014. All aqueous extract concentrations of colocynth seeds did not reach 100% mortality against housefly larvae.

### 3.2.2. Ethanol extract:

Table (2) also, shows that the concentrations (of aqueous extract) 5%, 10%, 15%, 20% and 25% resulted in mortalities of 5%, 35%, 80%, 85% and 100%, respectively, at the 5<sup>th</sup> day of the test period. The highest ethanol extract concentration (25%) of colocynth seeds resulted in 100% mortality against housefly larvae. The mortalities at season 2013 were 5%, 10%, 25%, 15% and 35%, following the same order, which were obviously lower than those of season 2014. At Season 2014, the colocynth extracts (aqueous and ethanol) were more toxic than those of the season 2013.

**Table (2): Mortality percentage of *Musca domestica vicina* larvae treated with different concentrations of *Citrullus colocynthis* seed extracts at season (2014) and the resulted mortalities at the 5<sup>th</sup>day of (2013)**

Day	Control	Aqueous extract					Ethanol extract				
		Concentrations %									
		5	10	15	20	25	5	10	15	20	25
1	0	0	5	0	0	0	0	0	5	50	100
2	0	5	30	0	0	5	0	5	30	65	100
3	0	25	45	0	0	15	0	10	45	65	100
4	0	25	65	5	10	25	0	35	80	85	100
5	0	25	80	5	10	25	5	35	80	85	100
Season 2013	0	10	0	0	5	0	5	10	25	15	35

### 3.3. Effect of Colocynth (*C. colocynthis*) seed extracts on percentage mortality of *M. d. vicina* larvae at (season 2015)

#### 3.3.1. Aqueous extract:

Table (3) illustrates that, the mean mortalities attributed to the concentrations (5%, 10%, 15%, 20% and 25%) of aqueous extract, were 40%, 5%, 35%, 5% and 5% respectively, at the 5<sup>th</sup> day. The highest mortality (40%) was caused by the concentration (5%), which also,

caused a maximum mortality of 10% at season 2013, whereas, the maximum mortality (80%) was caused by the concentrations of (10%) at season 2014. The aqueous extract of colocynth seeds at the tested concentrations did not result in 100% mortalities against house-fly larvae at all seasons.

### 3.3.2. Ethanol extract:

Table (3) also illustrates that, the concentrations (5%, 10%, 15%, 20% and 25%) resulted in 50%, 40%, 50%, 70% and 70%, mortalities, respectively, at the 5<sup>th</sup> day. The higher concentration (25%) produced the highest mortality (70%) at season 2015, while it produced 100% and 35% mortality at season 2014 and season 2013, respectively. It was also noticed that, the ethanol extracts of colocynth seeds at the tested concentrations, were more potent against house-fly larvae than their aqueous counterparts. Ramziet *al.* (2013) reported the pesticidal activity of the lectin of *C. colocynthis* against *Ectomyelois ceratoniae* larvae, showing the toxicity effect of *C. colocynthis* lectin on the survivorship of *E. ceratoniae* larvae. Khalid, (2015) noted that several studies on the activity of insecticides of *C. colocynthis* against other insect pests have been conducted, supporting the toxicity of *C. colocynthis* against *Hoplosiphum padi*. So the various qualitative chemical tests of *C. colocynthis* showed the presence of diterpenoids, saponin, sterols, flavonoids, carbohydrate and alkaloids (Uma and Sekar, 2014).

**Table (3): Mortality percentage of *Musca domestica vicina* larvae treated with different concentrations of *Citrullus colocynthis* seed extracts at season (2015) and the resulted mortalities at the 5<sup>th</sup> day of (2014) and (2013)**

Day	Control	Aqueous extract					Ethanol extract				
		Concentrations %									
		5	10	15	20	25	5	10	15	20	25
1	0	35	0	0	0	0	5	0	5	0	0
2	0	40	10	10	0	0	20	30	20	30	45
3	0	40	15	20	5	5	40	30	40	60	45
4	0	40	15	35	5	5	45	30	50	70	65
5	0	40	15	35	5	5	50	40	50	70	70
Season 2014	0	25	80	5	10	25	5	35	80	85	100
Season 2013	0	10	0	0	5	0	5	10	25	15	35

### 3.4. Effect of *C. colocynthis* seed aqueous extracts on percentage mortality of *M. d. vicina* larvae at seasons (2013, 2014, and 2015).

Table (4) indicates the effect of aqueous extracts of the *Colocynthis* seeds on the mortality (%) of *M. d. vicina* larvae at three seasons after 5 days. At (season 2013), the concentration of 10%, 15% and 25% did not produce any mortality, while the 10% and the 20% concentrations caused 10% and 5% mortality respectively. At the (season 2014), the 5% and 25% concentrations produced 25% mortality, the 15% concentration produced 5% mortality and the 20% concentration produced 10%, while the 10% concentration produced 80% mortality. At the season 2015, the concentration of 25% and 20% produced 5% mortality, whereas the 10% concentration caused 15% mortality. While the 15% and 5% concentration produced 35% and 40% mortality, respectively, in house-fly larvae.

The statistical analysis revealed that, the average mortality during season 2013 was 3%, during season 2014 was 29%, while it was 20%

during season 2015. Although, there were observable differences among mortalities resulted during the different seasons, the ANOVA (two factors) showed a non-significant difference in this level ( $f=2.047$ ,  $f\text{-crit}=4.46$ ), i.e. the obvious differences in the average mortality during season 2013, season 2014 and season 2015 in respect to the concentrations of all aqueous extracts of Colocynth seeds were statistically not significant.

**Table (4): Mortality percentage of *Musca domestica vicina* larvae treated with different concentrations of aqueous extracts of Colocynth seeds in three seasons (2013, 2014 & 2015)**

Season	Concentrations				
	% 5	% 10	% 15	% 20	% 25
season 2013	10	0	0	5	0
season 2014	25	80	5	10	25
season 2015	40	15	35	5	5

**Table (5): Summary of table (4) mortality percentage of *Musca domestica vicina* larvae treated with different concentrations of aqueous extracts of Colocynth seeds in three seasons (2013, 2014 & 2015)**

Summary	Count	Sum	Average	Variance
Season 2013	5	15	3	20
Season 2014	5	145	29	892.5
Season 2015	5	100	20	275
5%	3	75	25	225
10%	3	95	31.67	1808.33
15%	3	40	13.33	358.33
20%	3	20	6.67	8.33
25%	3	30	10	175

### 3.5. Effect of Colocynth seed ethanol extracts on percentage mortality of *M. d. vicina* larvae at seasons (2013, 2014, and 2015).

Table (6) indicates the effect of aqueous extracts of the Colocynth seeds on the mortality per cent of *M. d. vicina* larvae at three seasons (2013, 2014, and 2015) after 5 days. At season 2013, the concentration of 5%, 10%, 15% produced 5%, 10%, 25% mortality, respectively. But the concentration of 20% caused 15% mortality and the concentration (25%) produced 35% mortality. At the (season 2014, the mortality increased from lower to higher concentration (5, 10, 15, 20 and 25%) as 5, 35, 80, 85 and 100% mortality. At the (season 2015, the concentration of 5% and 15% produced 50% mortality, whereas the 10% caused 40% mortality. While the 20% and 25% concentration produced 70% mortality, respectively, in house-fly larvae. So the highest mortality in this study at the concentration (25% ethanol) at season 2014.

The statistical analysis revealed that, the average mortality during season 2013 was 18%, during season 2014 was 61%, while it was 56% during season 2015. The ANOVA (two factors) showed a significant difference in this level ( $f= 8.23$ ,  $f\text{-crit} = 4.46$ ), i.e. the obvious differences in the average mortality during season 2013, season 2014 and season 2015 in respect to the concentrations of 25% of all aqueous extracts of the *C. colocynthis* seeds were statistically significant.

**Table (6): Mortality percentage of *Musca domestica vicina* larvae treated with different concentrations of ethanol extracts of Colocynth seeds in three seasons (2013, 2014 & 2015)**

Season	Concentrations				
	%5	%10	%15	%20	%25
Season 2013	5	10	25	15	35
Season 2014	5	35	80	85	100
Season 2015	50	40	50	70	70

**Table (7) Summary of table (6): Mortality percentage of *Musca domestica vicina* larvae treated with different concentrations of ethanol extracts of Colocynth seeds in three seasons (2013, 2014 & 2015)**

Summary	Count	Sum	Average	Variance
Season 2013	5	90	18	145
Season 2014	5	305	61	1567.5
Season 2015	5	280	56	180
5%	3	60	20	675
10%	3	85	28.33	258.33
15%	3	155	51.67	758.33
20%	3	170	56.67	1358.33
25%	3	205	68.33	1058.33

The mean percentage mortalities (Table, 8) at all concentrations of Colocynth seed aqueous (aq.) and ethanol (eth) extracts on *M. d. vicina* larvae at all Seasons (season 2013, season 2014 and season 2015) after 5 days, revealed the highest mortalities of house-fly larvae produced by 25%eth. (mean of 68.33% for the three seasons), followed by 20% eth. (56.67%), and 15% eth. (51.67%), whereas the 10%aq. caused (31.67%), while the 25%aq., 20%aq., 15%aq., 10%eth., 5%eth. and 5%aq. produced less than 30% mortality. So ethanol extract concentrations produced mortality (mean of 45% for the three seasons) more than aqueous extract concentrations (mean of 17.33% for the three seasons). There were some cases of inconsistency of mortalities of house-fly larvae by using the aqueous extract concentrations, indicating some proves of anti feedant characteristics.

The reason of the larval death may be due to the toxicity of the Colocynth seed extracts or of hunger as a result. According to Sattiet al, (2003); and Nadeem et al, (2012), they reported, that the *C. colocynthis* showed insecticidal effects against some agricultural pests, as well as larvicidal activities against some mosquito species (Satti et al, 2010; Kehail and Bashir, 2004).

**Table (8): The percentage mortalities of *Citrullus colocynthis* seed aqueous and ethanol extracts on *Musca domestica vicina* larvae at all seasons after 5 days.**

Concentration	Aqueous extracts	Ethanol extracts	Average
5%	25	20	22.5
10 %	31.67	28.33	30
15 %	13.33	51.67	32.5
20 %	6.67	56.67	31.67
25%	10	68.33	39.17
Average	17.33	45	

### The Morphological abnormalities formed on the House-fly stages fed on the Colocynth seed extracts:

The present study on *M. d. vicina* 3<sup>rd</sup> larval instar feeding on the diets containing different concentrations of *C. colocynthis* showed 35%, 100% and 70% mortality at the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> seasons of experiments and also the mortality increased with increase in the concentrations through each season. Hamshou *et al.* (2010) reported that, the feeding on the diets containing different concentrations of *C. colocynthis* Agglutinin decreased digestive enzymatic activities in the treated *Ectomyeloides ceratoniae* larvae.

Plates (1, 2 and 3) include swelling of body, brown pigmentation, weakness in cuticle and irregular body shapes. They also showed the distinct malformations of larvae, pupae and adults of the house fly induced after treatment of the 3<sup>rd</sup> instar with *C. colocynthis* seed extracts. This result was similarly to Sexena *et al.* (1981).

The morphological abnormalities induced by *C. colocynthis* seed crude extracts were concentration dependent, in almost all cases, the higher concentration the more morphogenetic aberrations. Some deformed larvae were pigmented and larval-pupal intermediate, some individuals showed C-shaped pupae, elongated pupae and balloon shaped pupae, most of the pupae failed to reach adults, however, some

emerged adults have various degrees of morphological abnormalities. Similar abnormalities were reported by Hashem and Youssef (1991), they observed dark inter segmental pigments on the 3<sup>rd</sup> larvae of *M. domestica* and fully formed pupa but with a constricted puparium after treatment of the 1<sup>st</sup> instar larvae with methanolic extraction of leaves and flowers of *Meliaaze darach*. El-Domiatty *et al.* (2003) found shrinkage of the pupae and folding of the wing of adults as a result of treatment of 3<sup>rd</sup> instar larvae of *M. domestica* with *Pipper nigrum* volatile oil.



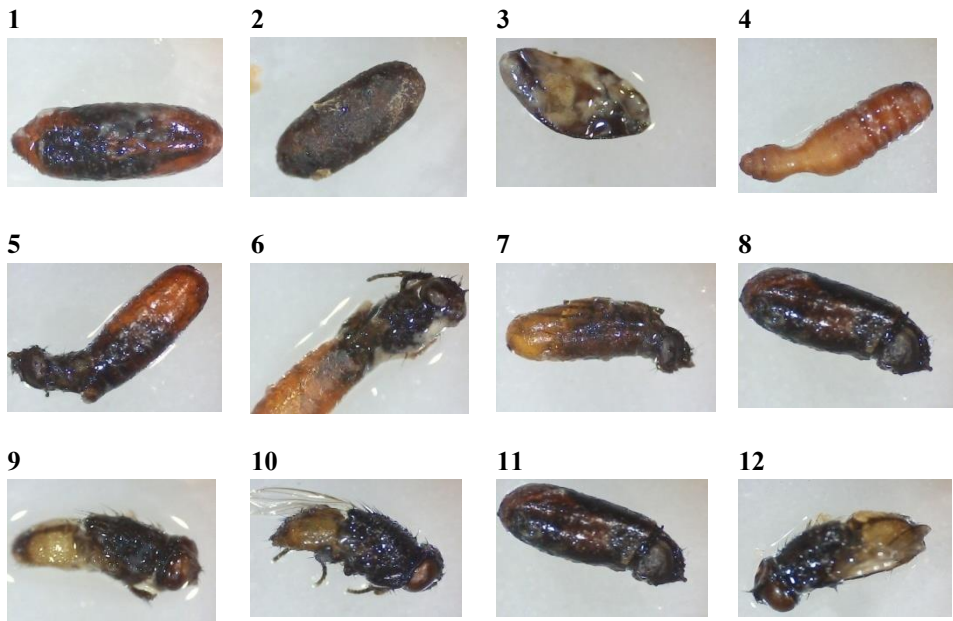
**Plate1: The Normal shape of the housefly (*Musca domestica vicina*) stages**

1- Larva    2- Pupa    3-Adult



**Plate (4.1): Deformed larvae and larval-pupae of *Musca domestica vicina* treated with Colocynth:**

1- Curved and burned larva (aqueous extract). 2- Twisted larva (aqueous extract). 3- Elongated burned larva (ethanol extract). 4- Curved larva with batches of black pigment (ethanol extract). 5- larval-pupa with segmented larval cuticle (ethanol extract). 6- Weak larval-pupa (ethanol extract) 7- Twisted larval-pupa (ethanol-extract) 8- larval-pupa with one segmented larval cuticle (ethanol extract).



**Plate.2: Deformed pupae, pupal-adults and adults of *Musca domestica vicina* treated with Colocynth;**

1- Swelled fossil pupa (aqueous extract). 2- Dry and swelled fissile pupa (aqueous extract). 3- Twisted pupa (ethanol extract). 4- Elongated pupa with slightly constricted puparia (ethanol extract). 5- Elongated pupal-adult (ethanol extract). 6- Elongated weak pupal-adult (aqueous extract) 7- Pupal-adult (aqueous extract). 8- Black

pupal-adult (aqueous extract). 9- Abdomen shrinkage, wings and legs lost adult (aqueous extract) 10- Short abdomen and without right wing adult (ethanol extract). 11- Shirked legs adult (ethanol extract). 12- Stretched wings and legs adult (aqueous extract).

## 5. Conclusions and recommendations:

The ethanol extracts produced mortality (mean of 45% for the three seasons) more than aqueous extracts (mean of 17.33% for the three seasons). Ethanol extracts were more potent than the aqueous extracts (in most cases). The used of aqueous and ethanol extracts tended to produce some morphological abnormalities in larvae, pupae and on the emerged adults. The normal adults did not produce new generation at all seasons. We recommend to use the tested aqueous and ethanol extracts as alternative larvicidal compounds for the house-fly after field experimentation. The active ingredients of Colocynth products should be determined so as to be used in formulation natural compounds in future, and to test the other parts of Colocynth against house-fly larvae and using this plant in house-fly control for Integrated Pest Management (IPM) programs.

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