Morphogenetic abnormalities of *Musca domestica* vicina induced by glycosidic groups from *Calotropis procera* plant

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Abstract: Latex samples were collected under cold ethanol (95%) from *Calotropis procera* plant which was obtained from border desert districts of Jeddah City, Saudi Arabia. The extraction of latex for its glycosidic groups was carried out by soaking or soxhelt extraction by using several solvents with different polarity. The contents were then separated by alumina – charcoal column chromatography with several solvent systems (chloroform, chloroform: ethylacetate (3:1, 1:1, 1:3) and ethylacetate. The pure components were tested against *Musca domestica* larvae to demonstrate its toxic effects on the morphogentic characters of the developmental stages. The present investigation revealed that the morphogentic aberrations has been induced by all the used plant extracts when applied topically on early 3rd larval instar of *M. domestica*.
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Key words: Structure – house fly – developmental stages – botanicals – toxicity – extraction – chemical components – chromatography.

1. Introduction

There is ample evidence to show that the plant kingdom is a vast store-house of chemical substances manufactured and used by plants in their own defense from attack by insects, bacteria, fungi and viruses. Many authors isolated and identified more other substances affected the growth of insect pests.

Much of the efforts to develop these nontoxic, safe and biodegradable natural products, have been concerned by their use as antifeedants that influence chemosensory behavior of insects growth regulators and growth inhibitors (**Deshmukh and Renaparkar, 1987**) that act upon the physiological processes of insects and as agents of strong fecundity reducing effects (**El-Zoghby *et al*., 1985**).

These substances may act as brain hormone action (**Kobayashi *et al*., 1962**), ovicide and larval growth inhibitor (**Nakajima and Kawazu, 1982**), growth regulators (**Reynold *et al*., 1984; Deshmukh and Renapurkar, 1987; and Coffelt and Schultz, 1988**), substances with juvenile hormone activity (**Bowers, 1968; Bowers and Nishida, 1980**), alkaloids (**El-Gayar *et al*., 1979 and Saxena *et al*., 1986**) and substance with moulting hormone activity (**El-Zoghby *et al*., 1985**).

Successful fly control is achieved when various methods are integrated in an overall program. Modern control of *Musca domestica* involve three interrelated approaches, namely source reduction, use of biological control methods, and control with insecticides. Conventional insecticides play an important role in the overall fly suppression programs. However, the intensive use of insecticides been challenged, especially by the house fly, and by the development of resistance of these chemicals, (**Abudulai *et al*., 2001**). Botanical insecticides, microbial pesticides and anti parasitic are highly effective, safe and ecologically acceptable (**Weinzbrt and Henn, 1991; Nathan *et al*. ,2005; Nathan and Kalaivani, 2005; Massoud *et al*., 2008; Khatter and Abuldahb, 2010; Gamal and Abuldahb, 2012**). So, the literature has directed our attention to the poisonous plant, *Calotropis procera*. A new steroidal hydroxyl ketone and calopenyl acetate were isolated from an ethanolic extract of *Calotropis procera* fresh flowers with evaporation and partitioning into chloroform and water. The aim of the present study is to isolate the toxic groups of the latex against the house fly *Musca domestica* developmental stages.

2. Material and Methods:

The tested plant (*Calotropis procera*)

This plant was reported to be insecticidal (**Farnsworth *et al*., 1975**). Its latex is very poison and yielded five crystalline bodies; calactin C29 H40 O9, calotoxin C29 H40 O10, calotropin C29 H40 O11, uscharidin C29 H38 O9 and uscharin C31 H41 O8 (**Pharmacological Exp., 1970**).

Extraction and separation of pure groups by soaking method:

Latex samples (300 ml) were immediately collected from the plant and soaked in ethanol 95% (300 ml) for 48 hours at 40-60°C on a thermostatic water bath, then it was filtered off (A). The latex coagulate was resoaked in ethanol 95% (400 ml) in the same manner and filtered off (B). The two filtrates (A+B) were mixed together and kept in a

freezer at 0-4°C for 48 hrs as crude latex and filtered off (C), the precipitate (In) was found to be white crystals (5.98 gm, m.p. 84-88°C range). The filtrate (C) was kept under cooling for further 48 hrs and filtered off (D), giving further portion of white crystals (If1) (1.8 gm, m.p. 82-90°C range). It was found that some crystals did not melt up to 290°C The filtrate (D) was also kept under cooling (0-4°C) for three weeks and filtrated off (E) to give pale yellow flakes precipitate II (1.12 gm, m.p. 84-90°C). However, If1, If2 and II portions are considered as solid mixture of crude latex cardenolides.

The filtrate (E) was concentrated to about one third of its volume using rotary evaporator, the concentrate (F) was kept under deep cooling for 48 hrs, giving a brownish yellow precipitate (3.77 gm) which was dissolved in acetone and the insoluble part was separated and dried to give pale yellow crystals (0.8 gm, m.p. 248-249°C). Another portion of concentrate (F) was further concentrated using rotary evaporator, producing an oily brown layer which was filtered off and clear filtrate was kept on water bath at 40-60°C for one hour and then cooled (0-4°C) . Silvery crystals (0.65 gm, m.p. 221.5°C) were produced.

Test insects

a- Sources of colony

Adult susceptible strain of house fly *M. domestica vicina* used in the present study were obtained from well established colony originated from the King Abdulaziz University, Faculty of Science for Girls- Biology Department.

b- Rearing technique

Egg masses were used to maintain a colony in the laboratory under constant conditions of temperature and humidity (27 ± 2°C and 60 ± 10% R.H.). Each egg mass was placed in a clean Petridish (10 cm diameter), previously constant technique described by (**Lewallen, 1954**). Full grown larvae were allowed to pupate in clean glass Petridishes. Following emergence, the adults were provided with a piece of cotton soaked in 10% sugar, 2% milk solution as a source of food.

Treatment of newly emerged 3rd larva instars (2-days old larvae):

Studies were conducted in a rearing chamber at 27± 2°C and 60 ± 10% R.H. Extracted groups (calactin, calotoxin and calotropin) were applied topically on the dorsal surface of newly moulted early 3rd larval instar (twenty five larvae) with different doses of 80 µg/larvae by using Hamilton serange. After application, larvae were put in small plastic cups, 7 cm in diameter, and covered with larval medium . This experiment was replicated three times. The larvae of control groups were treated with 1 µL of the solvent only and replicated two times. Mortality percentage of the treated and control larvae were calculated after 24, 48 and 72 hours and corrected by Abbott's formula (**Abbott, 1925**). Treated and control larvae were also noticed periodically for moulting disturbances until pupation. Larval pupation and adult emergence were also recorded. All the pupae and adults obtained were collected and checked for abnormalities.

3. Results and Discussion

A. Toxicity of latex groups:

Results of latex crude constituents are shown in table (1). It can be reported that the solvent system of chloroform, methanol (9:1) developed different constituents in the different isolated groups. The groups were found to have similar constituents with RF values equal to 0.48, 0.61 and 0.97; whereas, three other constituents were closely developed near the baseline with RF values equal to 0.03, 0.05 and 0.15. This means that the polarity of the latex three constituents was not compatible with the solvent system used (chloroform: methanol, 9:1). The three fractions in the solvent system of ethyl acetate: ethanol, 96.4 appeared three similar constituents with RF values equal to 0.95, 0.96 and 0.98. The cold ethanolic fraction for 48 hrs and 3 weeks gave two similar constituents with 0.86 and 0.88 RF values in the same solvent system, whereas, the cold fraction for 96 hrs gave also another constituents with RF values equal to 0.75. There are tow similar constituents with RF values equal to 0.1 and 0.12, which indicated the polarity of ethyl acetate: ethanol (96:4) solvent system was not suitable for running them from latex fractions cooled for 48 hrs and 3 weeks, respectively. In case of ethylacetate : ethanol (97:3) solvent system, it was found that one compatible constituents with RF values equal to 0.93 in fraction with cooling for 48 hrs whereas, three constituents with RF values equal to 0.38, 0.75 and 0.97 were found in the fraction with cooling for 96 hrs. In the fraction with cooling for three weeks, two incompatible constituents were developed (RF values 0.1 and o.19) in addition to two suitable constituents with RF values equal to 0.87 and 0.97. So, the development of the different constituents from ethanolic crude isolated extract was based on the suitable solvent used and the ability of solvent for running with the constituents or the compatibility of polarity between the isolated constituents and solvents system used.

B. Morphogenetic abnormalities

The present investigation revealed that the morphogenetic aberrations induced by all the used

plant toxic groups when applied topically on early 3rd larval instars of *M. domestica*. *So,* exhibited various morphological abnormalities in response to all latex groups used. Most of treated larvae were able to form puparia. Yet abnormal ones and other regarding the inability of this early 3rd larval instar accomplish metamorphosis (from a pupae). These larvae become pigmented with block and brown pigment, Fig (1- A, B,C). Similar observation were mentioned by (**Saxena *et al.*, 1981; Schmutterer, 1985 and AL-Sharook, 1991**).

Many investigators described the nature of this phenomenon as follow, the formation of black bodies starts from the idea that the epidermal cells are going to secret the exuvial fluid. This fluid secreted as droplets contain inactivated lytic enzyme systems. These are activated before the cuticulin layer protecting the epidermal cells against digestion is deposited. Therefore not only parts of the endocuticle, but also the epidermis, are digested. The lytic material accumulated in the subcuticular region, where it forms the center of the black bodies. Haemocytes containing further lytic material cluster around developing black body and after flattening, form a coat. The content of these clusters undergo melanization (**Chaieb *et al.* , 2001; Roy *et al.* , 2002 and Mitchell *et al.* , 2004**).

Larval-pupal intermediate stages were also observed. The cuticle of this individual contains pupal parts which parts of still persisting last larval skin. In other cases, the individual completely covered with pupal exuvia, Fig (2- A, B, C).

Similar finding were recorded by (**Jagannadh and Nair, 1992**). Pigmented pupae, small pupae, constricted pupae, deformed pupae which do not produce adults, Fig (3 A, B, C). pupal-adult intermediate also were shown in which this type of deformed individual possess the external character of pupae and has a distinct adult head, (Fig 4).

Incomplete adult eclosion was the most observed frequently. This varied from partial to complete eclosion of adults with legs or wings glued to the puparium. In most cases, only head, or head and part of thorax managed to be release from the puparium. In other cases the head, thorax, part of the abdomen or entire abdomen, and some of the legs emerged, but the adults still attached to the puparium by 1 or more legs; by tarsi or by wing. Partially eclosed adults having crumpled wings were fairly common (Fig 5), (**Ahmed, 1981; Kandil A. M. , 1985 Fouad, 2000; Delco and Gallerani, 2002; Abel-Hady *et al.* , 2005; and Pineda *et al.*, 2007**).

In conclusion, plant extracts are similar to insect ecdysteroid (**Nakanishe, 1975**) and apparently act as an inhibitor of ecdysis (**Kubo and Klocke, 1982**) by influencing the quantity or quality of the "Pool" of moulting hormone (**Rembold *et al.* ,1982**). This mode of action was suggested by the general condition of the larvae deformed by extracts. Many were unable to shed their pupal cuticle completely, those that succeeded often and crumpled or twisted wings, misshapen abdomen and other gross morphological abnormalities.

Table (1): Rf Values and melting points of the crude constituents isolated from latex of *Calotropis procera*.

Isolated groups	Rf Values			m. p (C°)
	Chloroform : Methanol 9 : 1	Ethylacetate : Ethanol 96 : 4	Ethylacetate : Ethanol 97 : 3	
Calactin	0.3, 0.48, 0.61, 0.97	0.1, 0.2, 0.86, 0.95	0.1, 0.93	84 - 88nm.p
Calotoxin	0.05, 0.89	0.75, 0.96	0.38, 0.75, 0.97	82 – 90 m.p
Calotropine	0.15, 0.47, 0.55, 0.62, o.86, 0.97	o.12, 0.21, 0.88, 0.98	0.1, 0.19, 0.87, 0.95	84 – 95 m.p

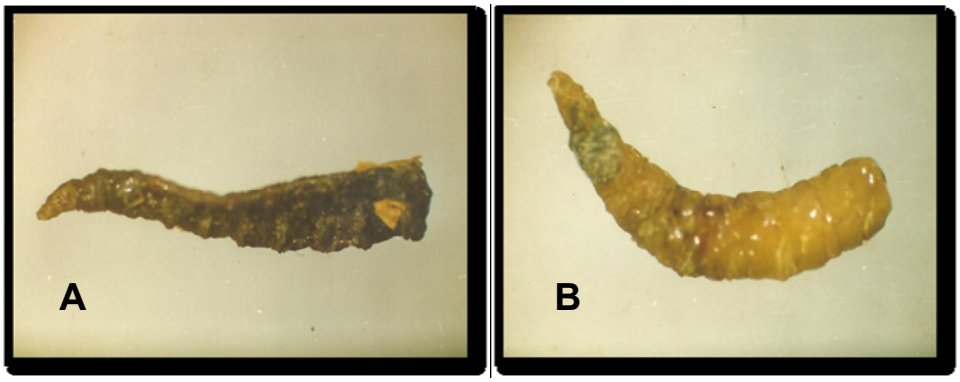

Fig (1): Deformed larvae A- Normal larva of normal size, B- larva of normal size with batches of brown pigment, C- C-shaped larvae pigmented with brown pigment, D- Burned larvae with normal size and shrankage and black pigment. (X = 25)

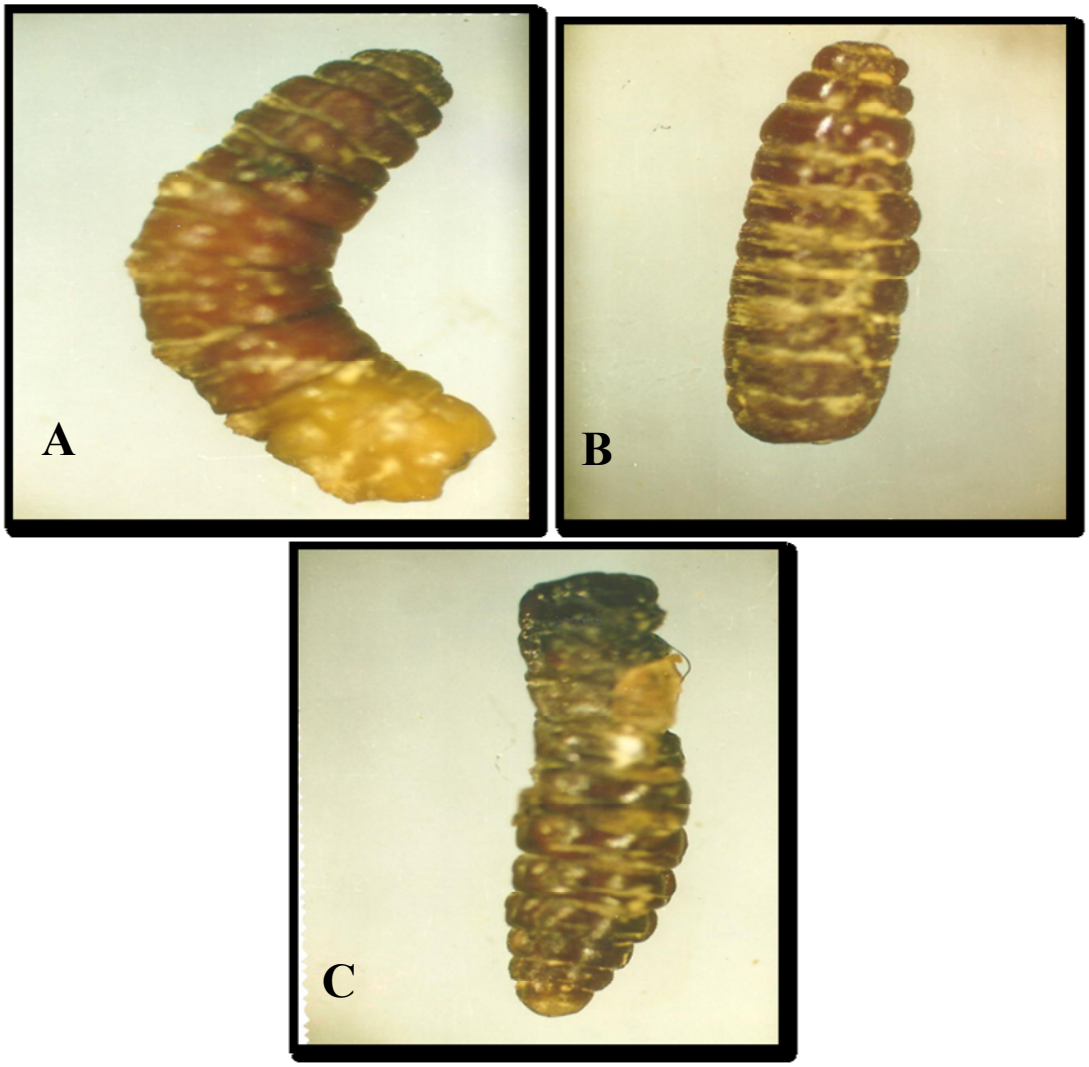

Fig (2): Larval-Pupal Intermediate.
A- C-shaped larval-pupal intermediate, the cuticle of these abnormal individuals contains part which still persisting a last larval instar skin.
B- elongated larval-pupal intermediates with normal color, it has a larviform puparia and intermediated larval cuticle.
C- Pigmented elongated larval-pupal intermediates with segmented larval cuticle (X=25).

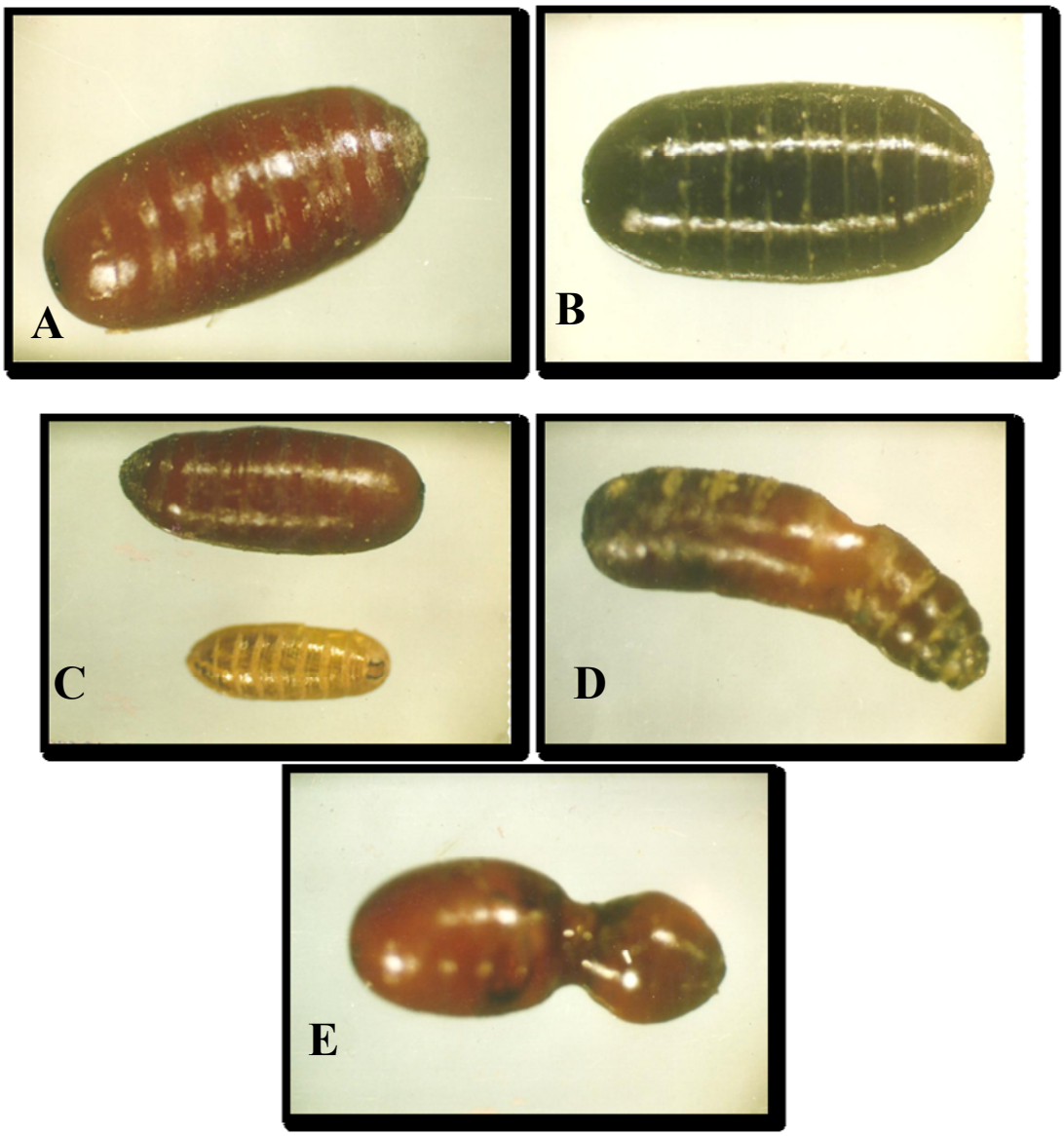

Fig (3): Deformed pupae.

A- Normal pupae.
B- B- Pupae with normal appearance, but pigmented with dark back pigment.
C- Deformed pupae. Small pupae compared with normal one, it has normal appearance but have relatively small size.
D- Fully formed pupae with conspicuous in their puparia, so that they failed to give adult.
E- Elongated melanized pupae with slightly constricted puparia. (X = 25).

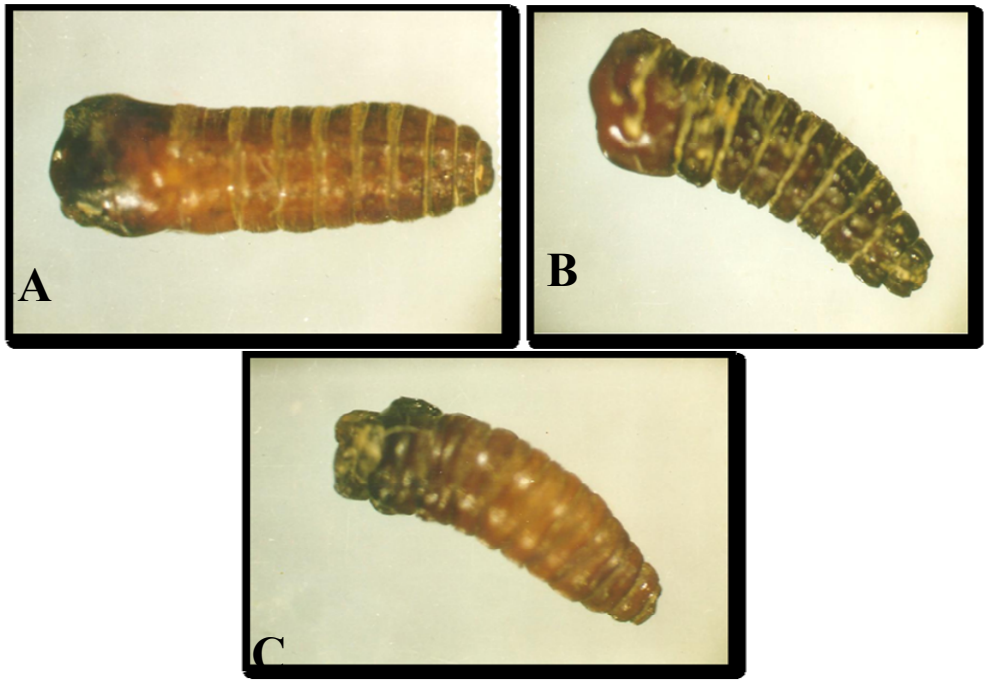

Fig (4) : Pupal – Adult Intermediates.

A- Elongated pupal – adult intermediate with distinct adult head.
B- Twisted pupal – adult intermediate with larviform puparium and distinct adult head.
C- Twisted pupal – adult intermediate with larviform puparium and distinct adult thorax. (X = 25)

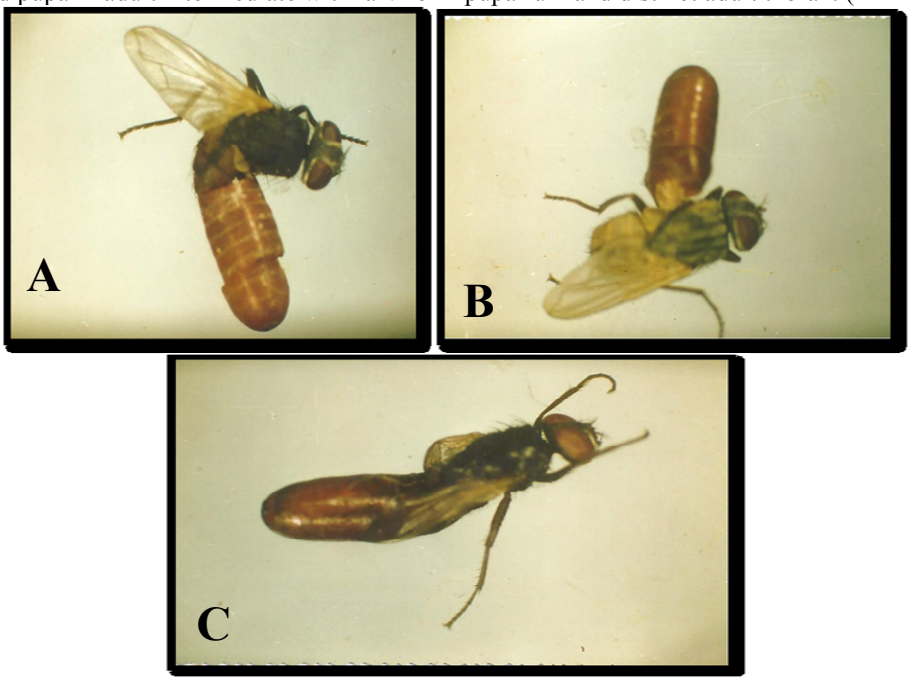

Fig (5): Deformed Adult and incompleted adult eclosion.

A- Head, thorax, one wing, legs of one side only and part of abdomen exuviated, but the remainder of the adult (abdomen, wings and legs of the another side) still retained within the pupal exuvia.
B- Pigmented thorax, crumpled wings and stiff legs are exuvated only. Abdomen and part of the wings are still retain in the pupal exuvia.
C- All the fly eclosed with undeveloped last abdominal segments. Wings and mid leg of one side failed to wirggle out the pupal exuvia. (X= 25)

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