

EFFECT OF NEEM EXTRACT, GARLIC ACID AND DIFLUBENZURON ON JAWS MORPHOLOGY OF SOME TERRESTRIAL MOLLUSCS

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Abstract

The present study aimed to elucidate the differences in the morphological characteristics of jaw between two land snails, *i.e. Eobania vermiculata* (Muller), *Theba pisana* (Muller) and one land slug, *i.e. Limax flavus* Linnaeus at different ages (juveniles of 2 months old, adults of 9-10 and 20-24 months old) under laboratory conditions. Also, the teratogenic effects of two plant extracts (neem and garlic) and one insect growth regulator (igr), diflubenzuron on jaw of these terrestrial molluscs were investigated.

Results indicate that, the morphological features of the jaw were differed considerably according to the terrestrial molluscs species their ages and used either of the two plant extracts or insects growth regulator.

INTRODUCTION

The problems of land snails and land slugs increased during the last two decades. Moreover, these gastropods had little attention and few studies were done about their external teratisms on jaw which considered the most important external organs of both animals.

The activity rhythms of snails and slugs depend on several environmental factors, such as temperature, humidity and photoperiod (Hommay, 1994). Also, activity rhythms depends on an endogenous factor, called the internal clock which affected by several external factors. The endogenous factors allowing individuals placed under constant conditions to express the endogenous component if their rhythm (Wareing and Bailey, 1985). This biological clock is synchronized by external factors, which are generally light and/or temperature cycles for terrestrial gastropods. It was found that other internal factors, such as the stage of maturity are likely to influence the amplitude of activity (Lorvelec, 1988).

The aim of the present work is to describe and draw the external morphological characteristics of the jaw of two land snails [*Eobania vermiculata* (Muller) and *Theba pisana* (Muller)] and land slug (*Limax flavus* Linnaeus) at three different ages for both snails and slug (*i.e.* juveniles of 2 months old, adults of 9-10 and 20-24 months old) species under laboratory conditions as well as after the application of two plant extracts (neem extract and garlic acid 96 %) and one insect growth regulator (Dimilin 48 % SC).

MATERIAL AND METHODS

The present study was carried out during the season activity of land snails and slugs (from March to July) under laboratory conditions.

1. Tested animals : Two snail species; *E. vermiculata* (Muller) and *T. pisana* (Muller) family : Helicidae and one slug species (*L. flavus* Linnaeus) family : Limacidae were used in the present study.

Healthy snails and slugs were collected from the nursery of ornamental plants at Giza Governorate during period extended from March to July. The collected animals were transported periodically in plastic bags to the laboratory. After identification and separation, animals of each species were individually caged in plastic boxes (35 x 12 x 18 cm) containing 10 cm moistened optimal soil (clay : peat : sand, 1 : 1 : 1) and covered with muslin with rubber band to prevent snails and slugs from escaping. Each cage was supplied with fresh lettuce leaves for feeding when needed. The terrarium was examined weekly for egg - laying . Two weeks after hatching , the juveniles were transferred to a small plastic boxes (20 x 10 x 8 cm) under the same conditions to supply these investigation juveniles of 2 months ,adults of 9-10 and 20-24 months, respectively .

2. Chemicals tested

2.1. Plant extracts

- Neem extract (Azadirachtin 1.0 %) , at the concentration of 1 %.
- Garlic extract (Garlic acid 96%) at the concentration of 0.5 %.

2.2. Insect growth regulator

- Dimilin 48 % SC (diflubenzuron), a chitin synthesis inhibitor, produced by Duphar, B.V., Netherland, at the concentration of 1.0 %. Chemical name : 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea.

3. Chemical treatments : The three tested chemicals were prepared just before application. Appropriate amount of each compound was diluted with water to obtain the required concentration as mentioned above . Fresh lettuce leaves were sprayed with the candidate compound and offered to the maintained land molluscs species after 24 h starvation . Three replicates in addition to another one for comparison were used for each treatment . All groups of treated and untreated animals were examined for their jaw structures.

4. Anatomical studies : Animals of each land molluscs species, under investigation, were placed in a wide beaker with suitable amount of water and heated in a water bath up to 60°C. The animals are thus killed in a well expanded condition. In each animal, the gross anatomy, the sodium hydroxide (7.5%) dissolved all tissues surrounding the jaw of the three species. Jaws were then washed in water, dehydrated in 70 % alcohol, cleared in xylol and mounted in canada balsam and photographed the jaws under light microscopy (X40) and compared with those of untreated (Shoieb, 1997).

RESULTS AND DISCUSSION

Data indicate that the jaws of tested specimens were varied according to species and with advanced in age. Usually, the jaw is singly, placed behind the upper lip, it has a dark brown colour, large and arcuate. The range of difference in jaw types was described in Figs. 1-3 for the three studied ages of each land snails and slug.

1. Morphological characteristics of jaws of different ages

a. The juveniles (2 months) : The jaws were relatively smooth and contains thin prominent ridges for *E. vermiculata* and *T. pisana*, but the jaw of *L. flavus* was thin and usually with a median anterior projection, Fig. 1.

b. The adults (9-10 months) : The jaws were found contained four prominent ridges and one thin for *E. vermiculata*, while it consists three prominent ridges and one

unapparent partially hide ridge for *T. pisana*.

On the other hand, a considerable increase in jaw size of adult slug *L. flavus* was recorded, Fig.2.

c. The older animals (20-24 months) : The prominent ridges were probably reabsorbed. Jaw was decidedly discriminated by its number of ridges. It contains four thin prominent ridges of *E. vermiculata* and two thick prominent ridges and one thin for *T. pisana*, Fig.3. In the same time, jaw of old slug was not changed as compared with those of adult, Fig.3.

From the previous results, it is clearly evident that jaws of land snails and slugs varied considerably according to species and ages. These findings are in accordance with those obtained by Runham and Hunter (1970) who found that jaw form varies with different families and it is lacking in the family Testacellidae. Runham (1975) reported that jaw of most slugs may be divided according to their range into two groups, relatively smooth and usually with a median anterior projection. Also, Abd-Allah (1990) suggested that a real differences was occurred between the jaws of 5 species of the Egyptian desert snail *Eremina desertorum*, *E. aschersoni*, *E. hasselquisti*, *E. rhodia* and *Xerophila icmalea*. Mersal (1990) showed that the alimentary tract of *E. vermiculata* is divisible into six morphologically distinct regions [buccal mass (the jaw, the odontophoral cartilage, the radula and radular sac), oesophagus, crop, stomach, intestine, and rectum]. Lastly, Shoieb (1997) showed that the morphological features of the jaw and radula differed in number of ribs and number of teeth in the different species *E. vermiculata*, *T. pisana*, *Monacha obstructa* and *Cochelicella acuta*.

2. Teratogenic effect of some compounds : External teratisms on jaws of adult snails (*E. vermiculata* and *T. pisana*) and slug (*L. flavus*), resulted from animals fed on lettuce leaves sprayed with either of plant extracts (neem and garlic) or IGR (Dimilin), during their feeding activity, compared with those from untreated ones were examined and illustrated in Figs. 4-6.

2.1. Effect on jaws of adult land snails

a. *E. vermiculata* : Morphological observations of jaws of adult land snails show that, the jaw of *E. vermiculata* was small or thin and contains only three thin prominent



(a)



(b)



(c)

Fig. 1. Jaw in juvenile (2 months) of (a); *E. vermiculata* (b); *T. pisana* (c); *L. flavus*

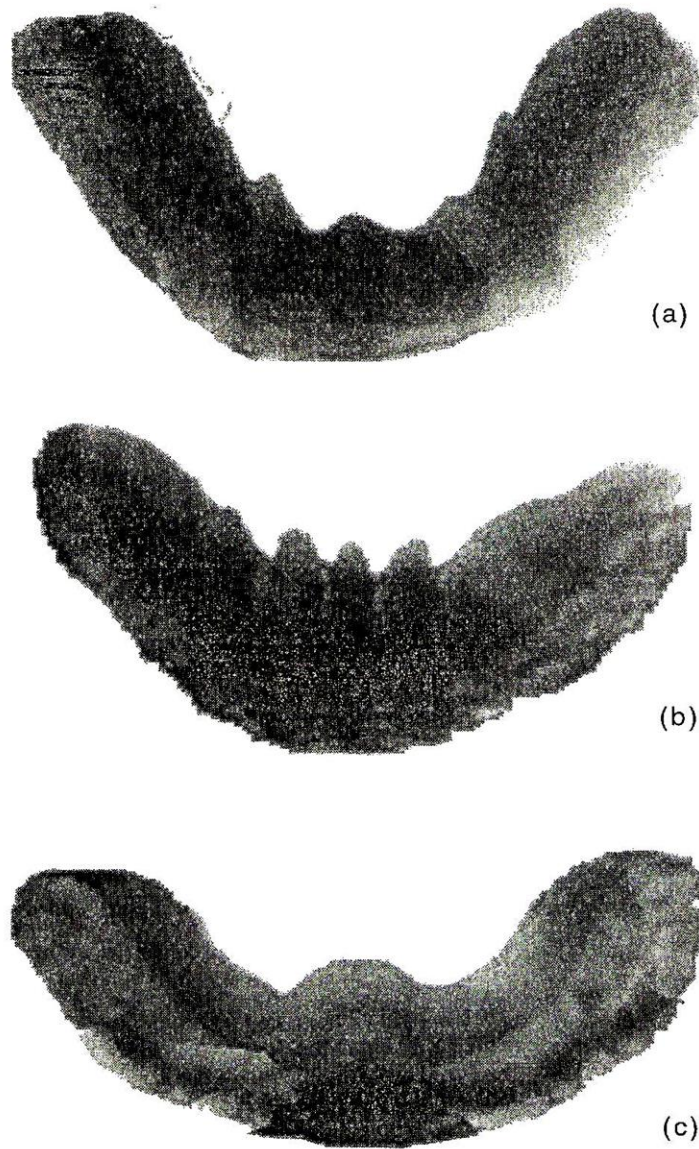


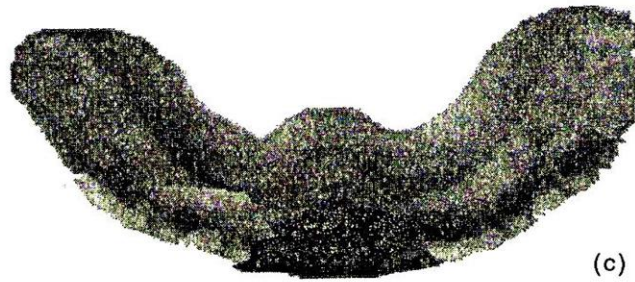
Fig. 2. Jaw in adult (9-10 months) of (a); *E. vermiculata* (b); *T. pisana* (c); *L. flavus*



(a)



(b)



(c)

Fig. 3. Jaw in adult (20-24 months) of (a); *E. vermiculata* (b); *T. pisana* (c); *L. flavus*

ridges, Fig.4a, affected with neem extract treatment, while the jaw was thick and consists of three thick prominent ridges and one unapparent partially hide ridge with garlic acid treatment, Fig.4b. As shown in Fig. 4c, *E. vermiculata* jaw is decidedly discriminate by four thin prominent ridges and changed the colour of this ridges (lighten) for animals treated with dimilin compared with for untreated animals. [the jaw of the adults contains four prominent ridges and one thin for *E. vermiculata*.]

b. *T. pisana* : Fig. 5 illustrated the morphological changes in jaws of *T. pisana* adults. The jaw of *T. pisana* was thick and possess three thick prominent ridges with neem extract treatment, Fig. 5a. On the other hand, Fig. 5b shows that *T. pisana* jaw was thin and contains three long prominent ridges for animals treated with garlic acid treatment, while effect of dimilin on jaw for *T. pisana*, Fig. 5c, is a considerable one long and thin and contains three apparent prominent ridges compared with the results for untreated animals.

[the jaw consists three prominent ridges and one unapparent partially hide ridge for *T. pisana*.]

2.2. Effect on jaws of adult land slug *L. flavus* : Fig. 6 showed that few differences were observed in jaws among slugs treated with neem extract, garlic acid and dimilin compound. Moreover, the jaws size of *L. flavus* were relatively smaller than that of untreated one, but it was thick. The beak of jaws for the slugs of three treatments was longer than that of untreated slug. [The jaw of *L. flavus* was thin and usually with a median anterior projection.]

The morphological teratisms of jaws of the three studied species fed on lettuce leaves treated with different compounds indicated considerable differences according to the type of chemical. Moreover, South (1992) described how the number of ridges and strength of the jaw could be modified by the type of food, with the jaw becoming stronger in the food was tough.

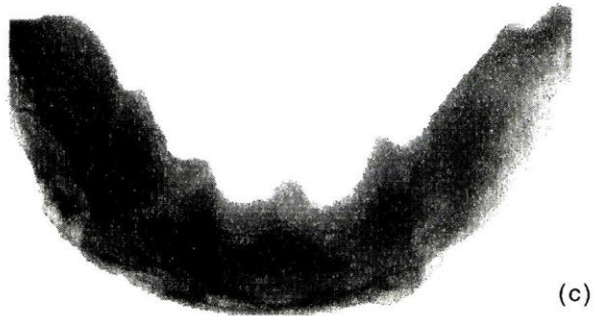
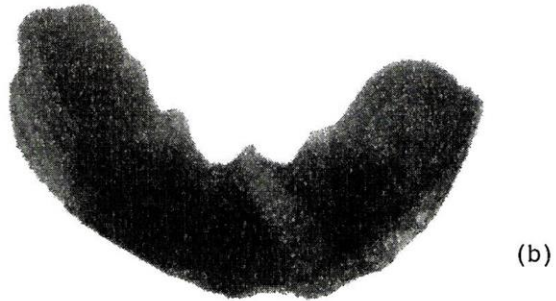


Fig. 4. Effect of (a) neem extract (b) garlic acid and (c) Dimilin on jaw of adult *E. vermiculata*

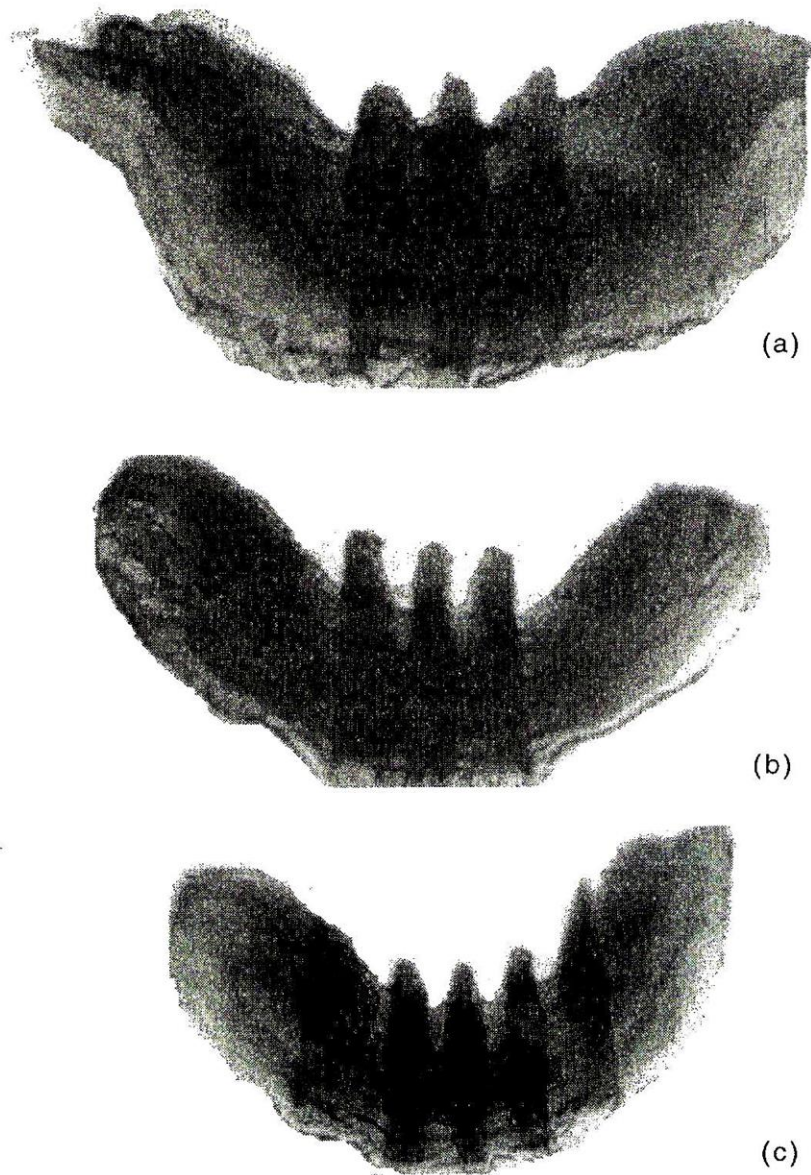


Fig. 5. Effect of (a) neem extract (b) garlic acid and (c) Dimilin on jaw of adult *T. pisana*.



Fig. 6. Effect of neem extract, garlic acid and Dimilin on jaw of adult slug *L. flavus*.

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تأثير مستخلص النيم وحامض الجارليك والدايفلوبنزايرون على مورفولوجيا الفك في بعض الرخويات الأرضية

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تتميز القواقع والبزاقات بوجود فك واحد فقط يقع في مقدمة أجزاء الفم. تم دراسة الأشكال المختلفة للفك في ثلاث أعمار مختلفة (عمر شهرين، عمر ٩-١٠ شهور، عمر ٢٠-٢٤ شهر) لتويعين من القواقع الأرضية وهي *E. vermiculata*، *T. pisana* ونوع واحد من البزاقات وهو *L. flavus*. وأظهرت الدراسة الآتي:-

- أن الفك في الأعمار الصغيرة من القواقع يكون ناعما وأملس ولا توجد به أية ارتفاعات واضحة في كلا النوعين، أما الفك في الأفراد البالغة (٩ - ١٠ أشهر) يتكون من ٤ ارتفاعات واضحة وارتفاع واحد غير واضح في حالة *E. vermiculata* أما في حالة *T. pisana* لوحظ أن الفك يتكون من ٣ ارتفاعات واضحة وواحد غير ظاهر جزئيا. وفي الأفراد المسنة من القواقع (٢٠ - ٢٤ شهر) حدث تاكل أو امتصاص لبعض هذه الارتفاعات مرة أخرى، ففي النوع الأول تكوّن الفك من ٤ ارتفاعات ضعيفة فقط، بينما وجد ارتفاعين سميكين وآخر ضعيف في النوع الثاني.

- في البزاقة *L. flavus* يتميز الفك بوجود بروز وسطى فيما يشبه المنقار ولا يختلف شكل الفك في الأفراد الصغيرة عن البالغة عن المسنة إلا في الحجم، حيث يكون الفك صغيرا وناعما في الأفراد الصغيرة، ويزداد تدريجيا في الحجم مع زيادة عمر الحيوان دون أى تغير في الفك.

أظهرت ملاحظة وتصوير التغيرات أو التشوهات المورفولوجية التي تحدث للفك بعد تغذية الحيوانات على المركبات المختبرة أن فك جميع الحيوانات تعرضت لتشوهات مورفولوجية كبيرة اختلفت تبعا لاختلاف المركب المستخدم وذلك عند مقارنتها بالأفراد غير المعاملة وذلك في حالة القواقع. أما في حالة البزاقات لم تحدث تغيرات كثيرة في الفك، ولا يتجاوز الاختلاف صغر حجم الفك وزيادة طول البروز الوسطى أو المنقار الموجود في وسط الفك. كذلك لوحظ أن الأفراد المعاملة سواء بالمستخلصات النباتية أو بمنظم النمو الحشرى مع القواقع أو البزاقات قد ماتت نتيجة إمتناعها عن التغذية بسبب التشوهات التي تحدث في الفك.