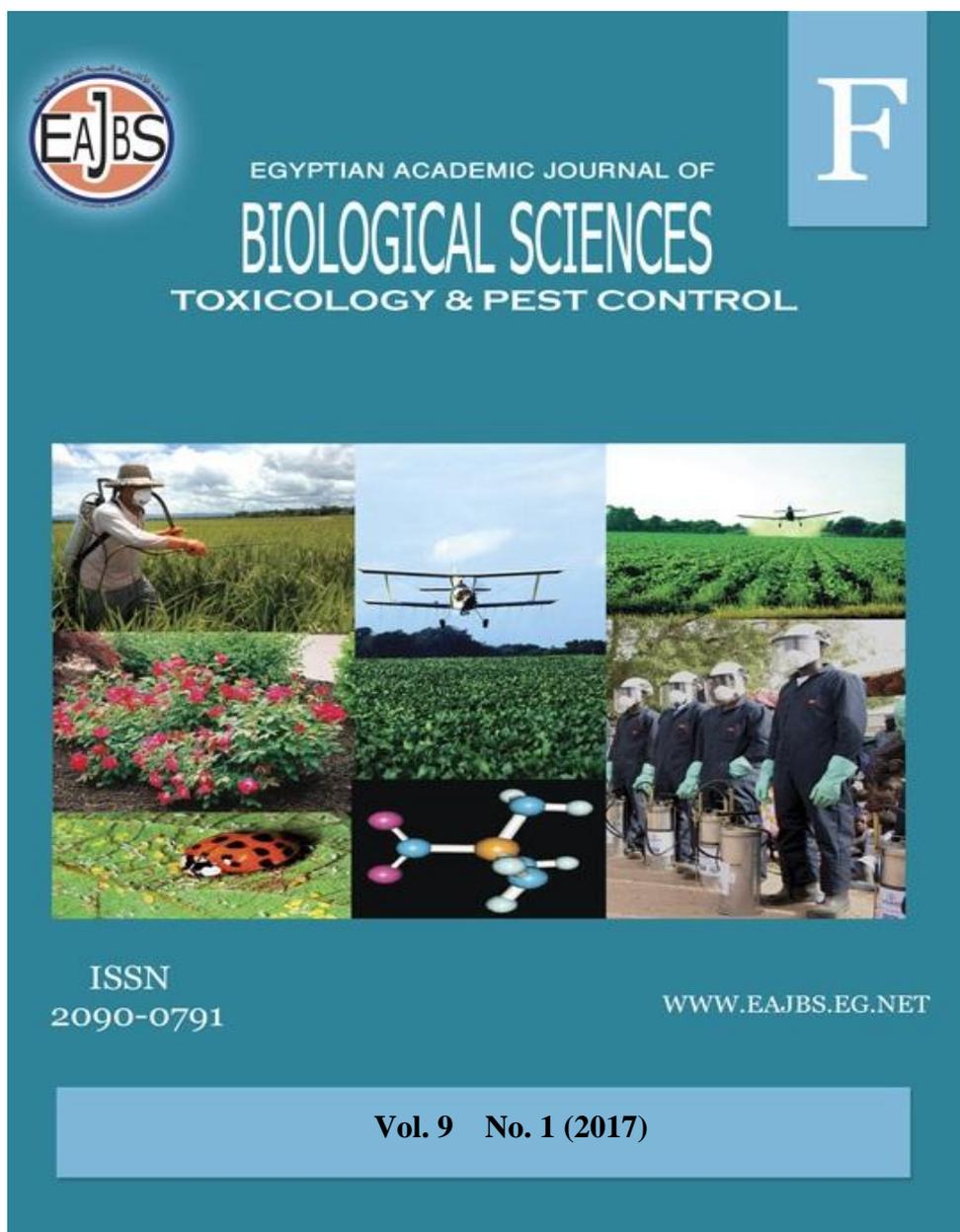


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Chemical Constituents of *Eobania vermiculata* (Müller) Mucus Before and After Treatment with Acetylsalicylic Acid and Chlorfluazuron.

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ABSTRACT

Effect of acetylsalicylic acid and chlorfluazuron (IGR) compounds on *Eobania vermiculata* ((Müller) land snails were studied as contact. LC₅₀ of the two compounds was estimated after one week. The results indicated that LC₅₀ of acetylsalicylic acid and chlorfluazuron was 210.6 and 177.7 ppm, respectively. The mucus, of untreated and treated (with half the LC₅₀ value of the two compounds) animals, was analyzed by GC/Mas/ Mas. Acetylsalicylic acid caused increasing in the volume of gland and necrosis and conglomerate in mucus. While chlorfluazuron decreased the volume of gland and atrophy cells and caused mucus disappearing in the cells. Chemical analyses of mucus indicated that 20 compounds were recorded. These compounds fluctuated between highs and lows in its concentrations compared with untreated individuals. Results revealed that chlorfluazuron was more toxic than acetylsalicylic acid. They both affect snails' mucus gland and kill them. Therefore, both compounds could be recommended for use in land snail control programs in agricultural field and orchards in Egypt.

INTRODUCTION

Land snail *Eobania vermiculata* is the most prevalent snail species in agricultural field and orchards in Egypt (El-Sayed, 2013). This species causes damage to all plant parts of vegetable crops and orchard trees (El-Okda, 1979; Mahrous *et. al* 2002). The degree of damage inflicted upon any particular agricultural or horticultural plantation depends not only on the activity of each individual slug or snail, but also, on the density of the gastropod population (Godan, 1983). Mucus is the first line of defense for snails, whereas it secretes large amounts of mucus when exposed to foreign substance. So this can cause difficulty in control. The major organ in slugs' responsible mucus production and release is the skin in which several of mucus cells can be found (Simkiss and Wibur, 1977). The mucus is secreted into the passageway. While it is possible that the sole function of the mucus is to lubricate the passage, the fluted morphology of the dart of *C. asperses* together with structural trends in the darts inferred evaluation suggest that it is adapted to carry a maximal load of mucus (Koine and Schulenburg 2005). Mucus is essential for locomotion and it is supplied by a specialized gland. The suprapedal is located in the body cavity and opening to the ventral side of the animal at the anterior margin of the sole (Barker, 2002).

Acetylsalicylic acid (Asprin) caused a dose dependent reduction in mucus synthesis (Mitobe *et al.*, 2000). Asprin enhance the recurrence of gastric ulcer. Acetylsalicylic acid inhibits cyclooxygenase, mucus secretion and mucosal blood flow may be involved (Wang *et al.*, 2007). Acetylsalicylic acid inhibits cyclooxygenase which then inhibits production of prostaglandins, including protective prostaglandins of the E- series. High levels are known to directly stimulate the respiratory center (early) to cause an initial respiratory alkalosis. High doses are also known to uncouple oxidative phosphorylation and may cause hyperglycemia and glycosuria. LC₅₀ of acetylsalicylic acid caused desiccation and adhesive for snail body and complete disappearance of necrosis and atrophy of mucus glandular tissue of *Eobania vermiculata* (Kandil *et al.*, 2014). The second compound chlorfluazuron is insect growth inhibitors (IGR). The mode of action of chlorfluazuron, acts as an anti-molting agent, inhibits biosynthesis of chitin of an important constituent in insect cuticle, loses cuticle elasticity and firmness, and results in abortive molting (Fang *et al.*, 2007). Chlorfluazuron has been globally used under the trademark of atabron for mainly controlling Lipdoptera on cotton, bean, vegetables, and fruit trees, etc. Chlorfluazuron caused fertility inhibition amount to 32% (Perveen, 2012). (IGRs) are physiological selective, non – neurotoxic compounds that disrupt the hormonal systems or the physiological development of insects. Juvenile hormone analogs such as fenoxycarb mimic juvenile hormones in many insects and reproductive processes (Robertson and Kimball, 1970; Robb and Parrella, 1984; Retnakaran *et al.*, 1985; Chen and Borden, 1989). Chlorfluazuron induced malformation on the organs of reproductive system of *E. vermiculata* land snail (Soha Mobarak, 2016).

In this study, two compounds acetylsalicylic acid and chlorfluazuron, are used to control *E. vermiculata* (Müller) land snail with the following points.

- 1- The effect of two compounds against land snail *E. vermiculata*, using contact poison technique.
- 2- Pathological changes in the mucus gland of snails after treatment with two compounds.
- 3- Chemical analysis of mucus of land snail before and after treatment with two compounds.

MATERIALS AND METHODS

Tested Compounds.

Acetylsalicylic acid: Asprin (91% Powder). It was supplied by Oxford Co.

Chlorfluazuron: Caprice (5% EC). It is an insect growth regulator. LD₅₀ for rat is 8500 mg/kg and it's effective at the rate of 10.50g a. i./ ha. It was obtained from El-Helb for pesticides and chemicals company, Egypt.

Experimental Animals.

Specimens of mature chocolate band snail, *Eobania vermiculata*, were collected from trees of a citrus nursery belongs to the ministry of agriculture, Giza Governorate, Egypt, and transported to the laboratory. Specimens were put in small glass cage containing moist soil (8-10 cm high). Each cage was placed with fresh lettuce leaves and covered with muslin secured with rubber band, to prevent snails from escaping and placed under $20 \pm 2^{\circ}\text{C}$ in laboratory.

Laboratory Experimental.

Contact (Thin film) Technique:

Contact technique was used according to Ascher and Mirian, 1981. The tested concentrations for both compounds were (100, 150, 200, 250, 300 ppm). The compounds were applied in petri-dishes using water. Ten individuals of *E. vermiculata* were exposed for one week, to 2 ml of each concentration of each tested compound. The control test was carried out using

water only. Mortality was recorded and LC₅₀ was calculated according to Finney, (1971) for each compound.

Anatomical Study.

E. vermiculata snails were anaesthetized in 1% of chloral hydrate for 12 hours after the snail was exposed for one week to ½ LC₅₀ of the two compounds separately. The mucus gland of the treated and untreated animals was removed and photographed using binocular stereoscope. Untreated animal glands were compared with that of treated animals (Shoieb, 1997).

5 ml of mucus was collected from 10 individuals of treated and untreated animals by stimulating the snails using small plastic syringe containing 0.5 ml of saline to preserve the mucus without limestone image. The samples were stored at -20 ° c in a deep freezer until analysis.

Chemical Analysis.

Mucus from *E. Vermiculata* was analyzed by GC-MS- MS. The analysis was carried out using a GC (Agilent Technologies 7890 A) interfaced with a mass selective detector (MSD, Agilent 7000) equipped with a polar Agilent HP-5ms (5%- phenyl methyl poly siloxane) capillary column (30m × 25mm i.d. and 0.25 µm film thickness). The carrier gas was helium with the liner velocity of 1ml/ min.

Operating conditions were as follow:

Injector port temperature, 250 ° c. Helium was used as a carrier gas at a flow rate of 1.0 ml/ min pulsed split less mode programmed at 10 ° c/min to 260 ° c, and held for 3 min. The total analysis time was 41 min. A 1 µl volume was injected split less. The mass

spectrometric detector (MSD) was operated in electron impact ionization mode with an ionization mode with an ionizing energy of 70 eV, scanning from m/z 50-600. The ion source temperature was 440 ° c and the quadruple temperature was 150 ° c. The electron multiplier voltage (EM voltage) was maintained at 1100V above auto tune, and a solvent delay of 3 min., was employed. The instrument was manually tuned using per fluoro tri butyl amine (PFTBA). The identification of components was based on a comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature.

RESULTS AND DISCUSSION

Data in Table (1) and Figures (1, 2, 3) show the efficacy of the acetylsalicylic acid and chlorfluazuron compounds against *Eobania vermiculata* after one week of treatment.

Results revealed that mortality increased gradually with increasing of compound concentration (Figs. 1& 2). Acetylsalicylic acid gave 10, 20, 40, 70, and 100% mortality with 100, 150, 200, 250, and 300 ppm concentrations, respectively.

The same concentrations of chlorfluazuron achieved 10, 40, 60, 70, and 90% mortality, consecutively. The calculated LC₅₀ of acetylsalicylic acid was 210.6 ppm after 7 days of treatment while it was 177.7 ppm for chlorfluazuron.

Table 1: Effect of acetylsalicylic acid and chlorfluazuron compounds against *Eobania vermiculata* land snail, after one week of treatment.

Compounds	Concentrations ppm	Mortality%	LC ₅₀ ppm	Slope
Acetylsalicylic acid	100	10	210.7	4.6 ± 1.7
	150	20		
	200	40		
	250	70		
	300	100		
chlorfluazuron	100	10	177.7	4.8 ± 1.3
	150	40		
	200	60		
	250	70		
	300	90		

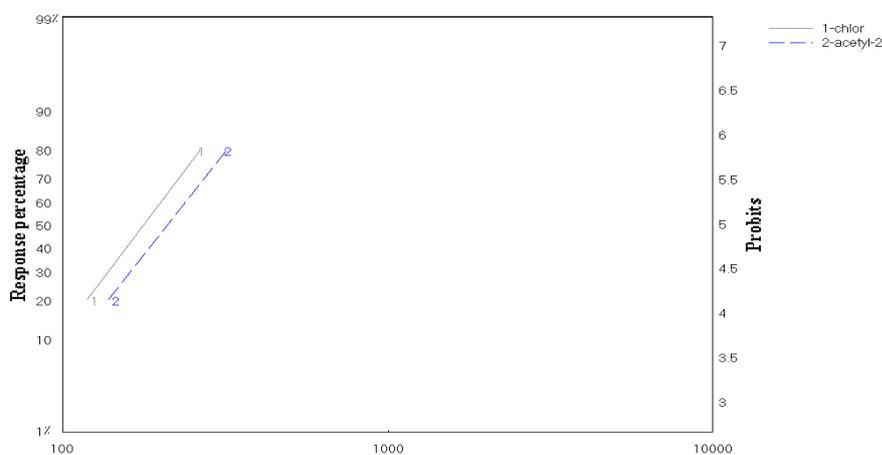
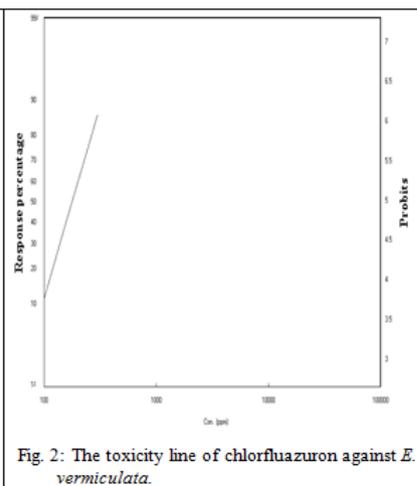
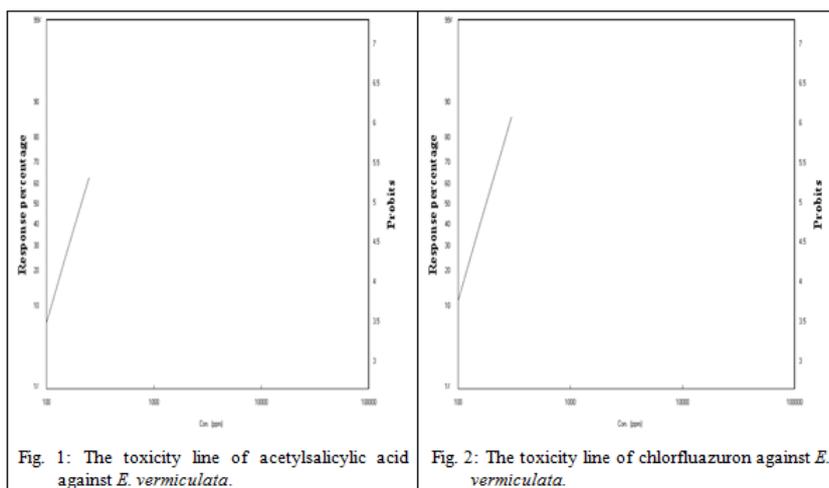


Figure (3) shows the toxicity line of both compounds. It is clear that the strain was homogeneous and susceptible to both compounds while it was more susceptible to chlorfluazuron than acetylsalicylic acid. So, IGR compound is more toxic than acetylsalicylic acid. Radwan *et al.*, (1993) concluded that chlorfluazuron was more toxic to *Helix*

aspersa than flufenoxuron when used as feeding technique on lettuce discs treated with 5% whereas the chlorfluazuron gave 100% mortality while flufenoxuron gave 86% mortality percentage after 7 days of treatment. Also, the LC₅₀ of acetylsalicylic acid was 0.67 mg/cm² against juvenile age of *E. vermiculata* using contact method (Kandil *et al.*,

2014). The same result was obtained by Soha Mobarak (2016), she stated that LC_{50} value of chlorfluazuron compound was 152.8 ppm when used as a thin film technique. Gabr *et al.*, (2007) indicated that the LC_{50} of pyproxifen achieved 0.49% against *Monacha obstructa*.

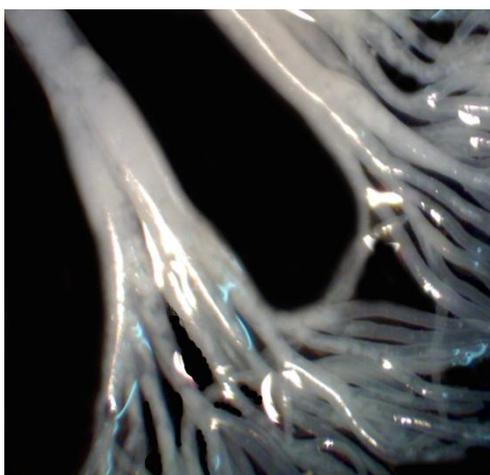
Pathological Symptoms.

The effects of acetylsalicylic acid and chlorfluazuron on mucus gland of land snail *E. vermiculata* after 7 days of treatment compared with untreated snails were explained in Figures 4, 5, and 6. Figure (4) shows that the untreated gland is a pair of tubes with regular shaped tall branches consisted of arranged secretory

cells full of mucus. Figure (5) shows the effect of the acetylsalicylic acid on the mucus gland. It is cleared that there was increase in the volume of cells of gland cells, swelling cells and necrosis in the mucus. Also, the mucus was conglomerate in the branches of the mucus gland. While, Figure (6) revealed the effect of chlorfluazuron on the mucus gland. It was observed that there is a decrease in the volume of the cells of gland cells. It shows that the pair of tubules in the treated gland was irregular with atrophy cells, with no mucus in the cells.



Fig. 4: Mucus Gland of untreated *Eobania vermiculata*, much branched, arranged tubules.



Fig, 5: Mucus gland of treated *E. vermiculata* with Acetylsalicylic acid, swelling cells, necrosis, and conglomerate the mucus in the branches.



Fig. 6: Mucus gland of treated *E. vermiculata* with chlorfluazuron, atrophy cells, irregular tubules, and disappeared the mucus in the branches.

From the previous results, it is illustrated that both compounds caused sever effect on snail mucus glandby causing disappearance of mucus. This effect may be due to sever damage to mucus producing cells by both compounds. The results agree with (Kandil *et al.*, 2014), who mentioned that the acetylsalicylic acid caused inhibition of mucus synthesis which is very important for snail life. On the other hand, the results were in agreement with Samah Abd-El- Kader (2001), she indicated that the death of snails is primarily correlated with the complete destruction of the foot tissues and the consequent greet loss of the total body water content. Also, Maha Fouad (2005) stated that malathion and methyl caused increase in the mucus cell volume of *E. vermiculata* land snail with some change in the color and reducing the quantity of mucus secretion. Also, Rita *et al.*, (1998) cleared that after metaldehyde application, the quantity and quality of mucus produced by slugs are modified. Mucus structure, enzymes, histochemical, and immunocy to tested chemical revealed that metaldehyde induced an effect in mucocytes to be revealed to influences of the molluscicide on serotonin and on energy metabolism. Gland mucus cells showed degenerative change with boric acid and necrosis was noticed in the cells with alum compound (Maha Fouad, 2012). On the other hand, Mitobe *et al.*, (2000) mentioned that aspirin caused a dose- dependent reduction in mucus synthesis. On the other side, Bieri (2003) cleared that the main effect of metaldehyde on mollusca is irreversibly damage of mucus cells. Also, Al- Akraa (2010) studied the effect of dimethoat and benomyl compounds on mucus gland of *Helicella vestalis* at 0.25% after 24h post-treatment. The results revealed that the mucus gland branches were increased in volume with decrease in the amount of mucus

exsiccation. Also, diflubenzuron (IGR) compound caused teratogenic effect on jaws of three ages of *E. vermiculata* and *Theba pisana* land snails which fed on lettuce leaves sprayed with the compound (Maha Fouad, 2002). On the other side, Soha Mobarak (2016) investigated how chlorfluazuron caused malformation effect on reproductive system of *E. vermiculata* land snail whereas it caused severe swelling in the organs of the reproductive system and preventing the egg product.

Effect of Tested Compounds on Mucus Constituents.

The effect of acetylsalicylic acid and chlorfluazuron on mucus components are shown in Table (2). Data showed that 20 compounds were found in mucus analysis. These compounds were differed in their area percentages in the treated mucus compared with untreated. The area % of (IS 2R)- 1,2 Diphenyl -2 [(1,1- Dimethyl ethyl) Dimethyl Siloxy]- Ethyl amine was 47.1% in control while it was 71.9 and 70.9% in mucus of animals treated with acetylsalicylic acid and chlorfluazuron, respectively. The area % of Z- pyridineald oxime decreased from 23.1 in control to 3.2 and 7.2% in mucus of treated snails with acetylsalicylic acid and chlorfluazuron compounds, consecutively. The same trend was observed with 5- Amino -2-trimethylsiloxyacetophenone as it recorded 4.26% concentration in control while decreased to 0.9 and 0.9% in the treatment with acetylsalicylic acid and chlorfluazuron, respectively. In contrast P- Toluidine, N (p- methyl benzylidene, the concentration increased after treatment with both compounds from 1.67 in control to 1.85 and 3.24%. The rest of the components varied between the increase and decrease after treatment compared to the control. This diversity between the increase and decrease in ingredients needs several studies to interpret. Smith and Morin (2002)

recorded that mucus consists primarily of large carbohydrate- rich molecules with some relatively small proteins. Skingsly *et al.*, (2000) investigated that mucus components are proteins, glycosaminoglycans and proteoglycan, carboxylate, and lactins.

Table 2: Chemical components of mucus of *Eobania vermiculata* treated with acetylsalicylic acid and chlorfluazuron.

NO	Components	RT (min.)	Area % of untreated	Area % of treated by A.	Area % of treated by Ch.
1	(1S,2R)-1,2- Diphenyl-2-[1,1-Dimethylethyl) Dimethylsiloxy]- Ethylamine	3.34	47.1	71.8	70.9
2	2-Pyridinealdoxime	3.9	23.1	3.2	7.2
3	5-Amino-2-trimethylsilyloxy-acetophenone	4.8	4.2	0.8	0.8
4	p- Touluidine, N-(p-methyl-benzylidene)	5.4	1.6	1.8	3.2
5	Isoelmicin	5.5	2.2	2.4	2.3
6	Benzoic acid,4-amino-,trimethy-lsilylester	5.9	4.7	3.1	0.8
7	Heptamethyl-,cyclotetrasiloxane	6.5	0.4	0.8	0.7
8	Hexylresorcinol	6.7	0.6	1.0	0.9
9	1,1-Binaphthalene,2,2-dimethyl	7.8	2.5	1.6	3.0
10	3,4-Dihydroxybenzylalcohol,tris(trimethylsilyl)	8.2	0.7	0.9	0.7
11	Cyclopentasiloxane, decamethyl	8.3	2.19	1.1	0.7
12	Vanillylalcohol,bis(trimethylsilyl)-deriv.	8.6	3.0	1.5	0.8
13	Butanoic acid, 4-(dimethylamino)-3-hydroxy	9.3	0.7	1.0	0.7
14	stilbene,4,4-dimethoxy- α - α -dimethyl	9.5	0.7	1.2	2.5
15	3-Hydroxydihydro-2 (3H)-furanone	9.9	0.5	0.9	0.8
16	1,3,5,7,9-Pentaethyl-1-methoxycyclopentasiloxane	10.2	0.7	0.9	1.2
17	Butanoic acid, 2-amino-4-(methylsulfinyl)	10.4	1.1	1.0	1.1
18	Propanedioic acid, Dihydroxy	11.1	0.7	1.1	0.8
19	Benzoic acid, 3,5-dimethoxy-4-[(trimethylsilyl)oxy]-trimethylsilyl ester	11.2	1.1	1.4	0.7
20	Butanedioic acid.	12.7	1.5	1.7	3.0

The same results were obtained by Davies and Hawkins (1998) who pointed that molluscan mucus is produced continuously from a series of glands that exit through the epidermis. The most obvious being that of the pedal glands used to aid locomotion of the animal over a variety of terrestrial and aquatic terrain. This mucus is made up of a range of lactins, uronic acid sialic acid, and hexosamine.

From previous results, it can be concluded that acetylsalicylic acid and chlorfluzuron achieved good results as contact poisons, against land snail by their impact on the mucus gland which is essential for snails production against foreign substance in the environment.

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ARABIC SUMMERY

المكونات الكيميائية للمادة المخاطية لقوقع الخطوط الشيكولاتية قبل وبعد المعاملة بحمض الاستيل ساليسليك ومركب الكلورفلوزورون

سها عبدالله مبارك- راندا عبدالسميع قنديل- نعمة محمد العبد
معهد بحوث وقاية النباتات - مركز البحوث الزراعية- الدقى -جيزة - مصر.
وزارة الزراعة واستصلاح الاراضى- مصر

تم دراسة تأثير المركبين حمض الاستيل ساليسليك والكلورفلوزورون (منظم نمو حشرى) على قوقع الخطوط الشيكولاتية. اختبر المركبين على القوقع بطريقة الملامسة ، وتم تقدير التركيز النصف مميت بعد اسبوع من المعاملة. ايضا تم فحص التغيرات المرضية على الغدة المخاطية بعد المعاملة بنصف قيمة التركيز النصف مميت للمركبين بعد اسبوع من المعاملة. كما تم تحليل المادة المخاطية للقوقع باستخدام جهاز GC-MS-MS قبل وبعد المعاملة بكل المركبين والمقارنة بينهم.

أظهرت النتائج ان مبيد الكلورفلوزورون اكثر سمية من حمض الاستيل ساليسليك ضد القوقع. حيث كان التركيز النصف مميت لحمض الاستيل ساليسليك ٢١٠,٦ جزء فى المليون بينما كان ١٧٧,٧ جزء فى المليون لمركب الكلورفلوزورون. وبالنسبة للتغيرات المرضية فقد احدثت المعاملة بحمض الاستيل ساليسليك تضخم فى الغدة المخاطية ونخر فى خلايا الغدة وتكتل للمادة المخاطية داخل انابيب الغدة المخاطية. ايضا ادت المعاملة بمركب الكلورفلوزورون الى صغر حجم الغدة وضمور خلاياها كما ادت الى اختفاء المادة الخاطية داخل الانابيب كما اشارت نتائج تحليل المادة المخاطية الى وجود ٢٠ مركب اختلف تركيزها ما بين الارتفاع و الانخفاض بعد المعاملة بكل المركبين عنها فى حالة الافراد الغير معاملة . لذلك يمكن ان يستخدم كلا المركبين ضمن برنامج مكافحة القواقع