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DEPARTMENT OF BIOLOGICAL
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EFFECT OF CERTAIN PLANT PRODUCTS ON SOME PHYSIOLOGICAL AND HISTOLOGICAL ASPECTS OF THE TERRESTRIAL SNAIL *EOBANIA VERMICULATA* IN EGYPT.

THESIS SUBMITTED FOR THE FULFILLMENT OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN SCIENCE TEACHER'S PREPARATION (Zoology)

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تأثير بعض المنتجات النباتية على بعض النواحى الفسيولوجية والهستولوجية فى القوقع الأرضى *ايوبانيا فيرميكيولاتا* فى مصر.

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ABSTRACT

Effect of certain plant products on some physiological and histological aspects of the terrestrial snail, *Eobania vermiculata*, in Egypt

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In the present study; laboratory bioassays were carried out for evaluating the efficacy of certain plant materials including Nicotine, Thymol, Menthol, Caffeine and Camphor as molluscicides against the brown garden snail, *Eobania vermiculata* using the topical application method. The obtained results proved that Nicotine and Thymol were the most promising from the molluscicidal point of view with $LD_{٥٠}$ of ٢٠٤,٠٢ and ٥٥١,٢٠µg/snail for the two materials, respectively.

The effects of sublethal doses ($LD_{١٥}$ and $LD_{٥٠}$) of the most potent materials, Thymol and Nicotine, on certain physiological and histological aspects of *E. vermiculata* snails were evaluated. Most of the estimated physiological indices were estimated along three post treatment time intervals (١, ٧ and ١٥ days) as recovery periods to evaluate the ability of the treated snails to eliminate the adverse and/or toxic effects of the applied materials.

Snails treated with $LD_{٥٠}$ Thymol exhibited significant decrease in their heart rate and oxygen uptake; while the rest treatments caused marked elevations in these parameters along the three experimental periods. A general significant increase in levels of some biochemical parameters (total proteins, total lipids, total cholesterol and glucose level) of the haemolymph were detected in most cases. Both examined materials caused a marked enhancement in the activity of acid phosphatase (ACP), aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) enzymes in the haemolymph; while the activity of alkaline phosphatase (ALP) exhibited a significant suppression. In addition, the examined biochemical parameters (total soluble proteins, total lipids and glycogen content) in the digestive gland exhibited marked reduction in response to both examined materials at the three post exposure periods. Also, qualitative changes in the digestive gland proteins were detected in response to both applied materials. Furthermore, the examined materials caused severe histopathological changes in the digestive gland and ovotestis of treated snails.

It was concluded that application of both tested materials interfered with the snails' physiology and caused great damage in architecture of the digestive gland and gonads; while the post treatment period did not enable the treated snails to eliminate the adverse effects of the applied materials; so Thymol and Nicotine may be of great value in controlling the terrestrial snails; but further studies are needed to evaluate their efficacy as safe and economic molluscicides in the field.

Keywords: Molluscicides; Thymol; Nicotine; Snails; *Eobania vermiculata;* Oxygen Consumption, digestive gland, ovotestis, electrophoresis, haemolymph.

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INTRODUCTION

Land snails and slugs are destructive agricultural pests causing economic damage to a wide variety of plants including vegetables, forage crops, tree fruits, shrubs, flowers, ground green cover and newly sown lawn grasses. Moreover, they play an important role in transmitting and spreading diseases to cultivated plants **(Kassab and Daoud, 1964; Barry, 1969; El-Okda, 1980 & 1984; Godan, 1983; Baker, 1989).**

In Egypt, the brown garden snail, *Eobania vermiculata* (Mollusca: Gastropoda: Stylommatophora: Helicidae) is considered one of the most predominant agricultural pests and is becoming a real threat to several vegetations including orchard trees, vegetable crops as well as ornamental plants causing considerable damage to all plant parts **(El-Okda, 1979 and Shetaia *et al.*, 2009).** It is also an eminent threat to agricultural expansion in the Egyptian desert reclamation projects.

Chemical control using metaldehyde and many of the synthetic carbamate compounds such as carbaryl, methomyl, methiocarb and mexacarbate are considered to be the most effective measures against mollusc pests and are still the main means for controlling the land snails **(Agarwal and Singh, 1988; Miller *et al.*, 1988; El-Okda *et al*, 1989; Kelly and Martin, 1989; Okka *et al*, 1996; Heiba *et al*, 2002).** However, extensive application of synthetic molluscicides had led to great threat to human health in addition to toxicity to non-target organisms, long-term persistence in the environment and they are cost-intensive **(Godan, 1983).**

In recent years, the development of botanical molluscicides as a possible substitute for chemical molluscicides is gaining wide attention. Molluscicides of plant origin are environmentally friendly, culturally more acceptable than synthetic ones, less

expensive, rapidly biodegradable and have low toxicity to non-target organisms **(Marston and Hostettmann, 1985; Singh *et al.*, 1996).**

Monotrpenoids, including Thymol, menthol and camphor, are naturally occurring compounds that are found in many higher-order plants. These compounds are secondary metabolites that seem to play no role in the metabolic functioning of the plants. They give plants their unique odoriferous properties. One role of monoterpenoids in the plants is to defend against plant-directed pathogens, herbivores, or competing plant species **(Grodnitzky and Coats, 2002).**

Monoterpenes have been shown to possess remarkable pesticidal activities, including insecticidal (**Isman, 2000; Grodnitzky and Coats, 2002; Abdelgaleil, 2010)**, herbicidal **(Duke *et al.*, 2000; Singh *et al.*, 2002)**, fungicidal **(Wuryatmo *et al.*, 2003; Cärdenas-Ortega *et al.*, 2005)**, and bactericidal **(Cristani *et al.*, 2007; Cantore *et al.*, 2009)** properties. Moreover, some monoterpenoids exhibited molluscicidal activity against the white garden snail *Theba pisana* **(Radwan and El-Zemity, 2007; Abdelgaleil, 2010)**; Schistosomiasis snail vector *Biomphalaria alexandrina* (**Sharaf, 2006; Radwan and El-Zemity, 2007; Radwan *et al.*, 2008-a);** *Bulinus trancatus* snails **(Lahlou and Berrada, 2001; Sharaf, 2006);** and the land snail *Helix aspersa* **(El-Zemity *et al.*, 2001).**

Nicotine is a naturally occurring alkaloid found in a wide variety of plants **(Doolittle *et al.*, 1995).** It is commonly used to control aphids, thrips, spider mites and other sucking insects on most vegetables, some fruits, flowering plants and ornamental shrubs and trees **(Schmelz, 1971; Duke *et al.*, 2010; Hassan and Amupitan, 2010).** Organic nicotine products are available and approved for use under several names "e.g.,Tobacco Dust" **(Dayan**

***et al.*, 2009).** However, to the best of our knowledge, practical studies on the molluscicidal efficacy of nicotine are nearly lacking.

Caffeine is one of the most widely used plant secondary metabolites **(Kim *et al.*, 2006).** It is a typical purine alkaloid, which is produced in a variety of plants, including coffee, tea, kola nuts, and cacao beans **(Ashihara and Crozier, 1999).**

Application of caffeine was shown to be effective not only as a repellent and pesticide for tobacco hornworms **(Nathanson, 1984)** but also to disturb the reproductive ability of several species of moths **(Mathavan *et al.* 1985).** A recent report also showed that caffeine repels and kills slugs and snails **(Hollingsworth *et al.*, 2003).**

REVIEW OF LITERATURE

PLANT-ORIGIN MOLLUSCICIDES

The stylommatophoran species including land snails and slugs have gained an economical importance since they became among the pests attacking several crops in many parts of the world **(Godan, 1983).**

The chemical control of snail populations through application of synthetic molluscicides is remaining the most effective method, particularly over large areas. Conventional pesticides especially carbamates are successfully used in Egypt as well as in many other countries, as sprays or baits to control these pests **(Miller *et al*., 1988; Radwan *et al*., 1992 and Heiba *et al*., 2002).** However, their use in higher concentrations has a deleterious effect on non-target species like vertebrate animals and human beings and lead to pollution of environment. Therefore, much effort has been focused on plant materials for potential use as commercial pesticides in the hope that they might provide cheap, locally produced, biodegradable, environmentally safe and effective control agents.

Large numbers of phytochemicals have already been isolated and shown to have molluscicidal activity against aquatic snails **(Lemma, 1970; Baalawy, 1972; Medina and Ritchie, 1980; Shoeb *et al*., 1982; Twaij *et al*., 1988; Singh and Agarwal, 1988&1990; Thiilborg *et al*., 1993; Lemmich *et al*., 1995; Ekabo *et al*., 1996; Schall *et al*., 1998; Singh and Singh, 1998, 2000, 2001, 2005 & 2009; Hamed, 1999; Al-Zanbagi *et al*., 2000; Bilia *et al*., 2000; Dos Santos *et al*., 2000; Sparg *et al*., 2000; Tripathi and Singh, 2000; Dos Santos and Sant' Ana, 2001; Tiwari *et al*., 2003; Meepagala *et al*., 2004; Abebe *et al*., 2005; Zahran, 2005; El-Din, 2006; Hamed *et al*., 2006; Kumar and Singh, 2006;**

Ahmed, 2007; Osman *et al*., 2007; Adenusi and Odaibo, 2008; Jaiswal and Singh, 2008; Yadav and jagannadham, 2008; Upadhyay and Singh, 2011).

On the other hand, few work has been carried out to detect natural molluscicides or antifeedants against land mollusks. The following trials can be reported:

Hagin and Bobnick (1991) isolated 6-hydroxy-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid from the quackgrass *Agropyron repens* (Linnaeus) Beauvois (Gramineae). It was both dermal and gastrointestinal toxic to the slug species *Deroceras reticulatum* (Müller), *D. leave* (Müller) and *Arion subfuscus* (Draparnaud).

Ghamry (1994) tested the toxic effects of five cruciferous seeds powder from cabbage, cauliflower, garden rocket, radish and turnip against the land snails *E. vermiculata, Monacha contiana* and *Cepaea nemoralis* under laboratory conditions. Seeds powder of cabbage and cauliflower and their extracts were the most effective for killing snails.

Hussein *et al.* (1994) isolated a cardenolide, Usharin, from the Egyptian wild shrub *Calotropis procera* and found that it was highly toxic by contact to the land snail *Thepa pisana* since it was 128 times more toxic than methomyl against the same snails.

El-Hwashy *et al.* (1996) examined the molluscicidal activity of six plant leaf extracts from Cauliflower, Pergularia, Khilla, Radish, Oshar and Datura against *E. vermiculata* land snails. Results revealed that crude ethanolic leave extracts of Cauliflower, Oshar and Pergularia were the most effective when tested as residue film with LC_{50} values 72.9, 39.97 and 36.47 ppm, respectively.

Zidan *et al.* (1997) studied the molluscicidal activity of certain weed extracts against the brown garden snail, *Helix aspersa*. They found that Damsisa extract was the most toxic preparation followed by Hanzal and Halfa-bar; while Gobberia was the least effective extract. A gradual increase in mortality occurred by the prolongation of time until the end of the experiment.

Hussein *et al.* (1999) evaluated the molluscicidal activity of a cardiac glycoside extract from *Pergularia tomentosa* (Asclepiadaceae), methomyl and methiocarb against the land snail *Monacha obstructa* (Ferussac) at 25^{o}C. They reported that, the LD_{50} value of the plant extract after 24h of treatment was 60.9 µg/snail whereas the LD_{50} values of the two tested carbamates pesticides after 72h of treatment were 11.9 and 27.4 µg/snail respectively.

Abd-El-Karim (2000) studied the molluscicidal activity of some wild herbs existing in the Egyptian flora against three mollusk species, *Monacha obstructa*, *E. vermiculata* and *Theba pisana*. Damsisa extract was found to be the most toxic followed by cabbage extract, Negrum extract and then Datura extract; while pepper seeds extract showed no toxicity against the snails tested.

El-Zemity *et al.* (2001) examined the molluscicidal activity and repellent properties of thirteen monoterpenoidal compounds against the land snail *Helix aspersa*. Camphor, Thymol, (R)-carvone and carvacrol proved to be potent molluscicides. Of the compounds tested, only citronellol, geraniol, (±) menthol and thymol were highly effective as repellents.

Youssef (2001) studied the response of two land snail species *Monacha contiana* and *E. vermiculata* for 2, 4 and 8% of vertemic (abamectin) as poison baits under the laboratory conditions. Results showed that percentages of mortality were (90, 100), (100, 100) and

(100, 100) for the three tested concentrations respectively against the two tested snail species after 9 days of exposure.

Zidan *et al.* (2001) studied the molluscicidal and antifeedant effects of 10 plant extracts (Neem, Spotted gum, Oshar, Cauliflower, Cabbage, Santonica, Radish, Peppermint, Khella, and Alocasia) against three land snail species (*E. vermiculata, Monacha obstructa and Theba pisana*). Among the tested plants, Neem, Spotted gum, Oshar, Cauliflower and Cabbage proved high potency against the three tested snails when extracted with ethanol. The antifeedant potential of the tested extracts differed according to plant species, solvent used and snail species. All tested ethanol extracts exhibited high antifeedant effects; while hexane failed to gain satisfactory antifeedant effects against the three studied snails.

Rao and Singh (2002) evaluated the toxic effect of single and binary treatments of synthetic and plant-derived molluscicides against the harmful terrestrial snail *Achatina fulica*. *Cedrus deodara* oil was the more toxic among molluscicides of plant origin against the snails tested.

Hollingsworth *et al.* (2003) proved that caffeine acts as both a repellent and toxicant against slugs and snails since drench treatment using 1% or 2% solutions of caffeine caused 100% of slugs, (*Veronicella cubensis*), to exit off treated soil, and the majority of these slugs subsequently died from caffeine poisoning. In addition, a 2% solution of caffeine applied to the growing medium of orchids killed 95% of orchid snails (*Zonitoides arboreus*) and gave better control than a liquid metaldehyde product representing the standard commercial control for this pest.

The efficacy of Vertemic against two land snail species, *Monacha obstructa* and *Helicella vestalis* was evaluated by **Khidr**