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Effects of the Insecticide, Lannate, on the Land Snails, *Eopania vermiculata* and *Monacha contiana*, Under Laboratory Conditions

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Abstract: The efficacy of the insecticide lannate as a molluscicide for the control of two land snail species widely distributed all over the Nile Delta, *E. vermiculata* and *M. contiana* was investigated. The rates of mortalities of both snail species are depend on the dose and time of exposure to the insecticide, lannate. The higher mortality rates were noticed at doses higher than LD50. However, at all doses, the mortality rates increased by the time and most mortalities occurred over five days post insecticide administration, the maximum mortality rates of 95 and 75% were observed for *E. vermiculata* and *M. contiana* after 108 hours by doses of 34.8 and 39.4 mg/kg, respectively. Both species of the land snails treated with lannate have shown pathological alterations in the digestive gland and in the gonads. The vacuolated and swollen digestive cells and presence of numerous yellowish brown granules (residual bodies) in the cells are the most pathological changes observed. These indicate that lannate have toxic effects in cellular damage of the digestive glands of the snails which could be correlated with the disturbed enzyme activities. The male and female gametes on the other hand, are severely affected and probably the male gametes are inhibited to develop to the late stages of spermatogenesis. This indicates that the insecticide used did not cause a complete cessation of gametogenesis.

Key words : Insecticide , lannate, land snails, *E. vermiculata*, *M. contiana*

Introduction

The stylommatophoran species including land snails and slugs have gained an economical importance science they became among the pests attacking several crops in many parts of the world (Baker, 1988 ; Baker and Vogeizang, 1988) .

Several species of the land snails are recorded on vegetable crops, ornamental plants ,orchard trees and in field crops in different Governorates of Egypt (El-Okda, 1980, 1984; Hashem *et al.*, 1992; Mersal, 1992 ; Azzam, 1995 ; Nakhla and Tadros, 1995 ; Arafa, 1997; Shahawy, 1998) . Most of these studies concluded that the population densities of the land snails depend mainly on the relative humidity and temperature . The control of these pests either chemically or biologically requires an understanding of their biological and physiological activities with their ecological conditions . The photoperiod , temperature and starvation are the environmental factors that much affect the gonadal activity and reproductive rate (Gomot and Gomot, 1985; Medina *et al.*, 1988; El-Saadany *et al.*, 1993 a,b).

Pesticides are widely and successfully applicated in all agricultural protection purposes . Synthetic molluscicides or insecticides are still the main mean for controlling the land snails (El- Okda *et al.*,1989; Kelly and Martin, 1989; Okka *et al.*, 1996). Issa *et al.* (1977) used mesurol and copper sulphate against *Theba pisana* and *Cochlicella acuta* and found that mesurol is more toxic for both land snails. Kady *et al.* (1983) evaluated the molluscicidal activity of some insecticides as profenoles malathion, triazophos, pirimiphos methyl, endosulfan and monocrotophos against the land snails in the field . They found that insecticidal application led to a high decrease in snails numbers. El-Okda *et al.* (1989) evaluated the efficacy of the formulated local 0.5% aldicarb oxamyl, methiocarb, lannate and metaldehyde in controlling land snails *Helix aspersa*, *E. vermiculata* and *Theba* sp. They reported that aldicarb and lannate have a toxic effects against the tested snails and are more toxic than other compounds .

The present study was conducted to evaluate efficacy of lannate in the control of two land snails widely distributed all over the Nile Delta, *E. vermiculata* and *M. contiana*, through determination of the concentration-mortality curves . The histopathological alterations induced by lannate in the digestive glands and in the gonads (ovotestis) of both species were studied.

Materials and Methods

Experimental animals : Mature specimens of the land snails *E. vermiculata* weighting 1.5-2 gm and *M. contiana* weighting 0.3-0.5 gm were collected from the gardens of ornamental plants at Station of Agricultural Research , Kafr El-Sheikh Governorate and from Egyptian clover (berseem) and lettuce fields around Al Mehalla Al-Koubra city, Gharbia Governorate, respectively, during March through May . Both snail species were kept in glass jars (20 snails / jar) under laboratory conditions (photoperiod 14h. light/ 10h dark and temperature 21 + 1°C) . They were fed on lettuce leaves that were provided three times weekly. The snails were acclimatized tow weeks to these conditions before experimentation.

Insecticide used : Lannate methomyl insecticide was used through out this study. Methomyl is the common name for s-methyl N-{(methylcarbamoyl) oxy} thioacetimidate. It is a carbamate insecticide having anticholinestrase activity and having a melting point of 78-79 °C and a solubility in water of 5.8 gm / 100 ml. A formulated product containing 90 % methomyl was used in all tests . All dosages levels , unless otherwise stated , were based on the active ingredient, methomyl (Kaplan and Sherman, 1977).

Experimental design and animal groups: To evaluate the efficacy of lannate in the control of land snails as well as its acute toxicity, the following experiments were done:

1) This experiment was done to determine the dose mortality curve and the LD50 of the pesticide for both snail species. For each snail species, seven animal groups each of 60 snails/3 jars were given increasing doses of lannate (in the body cavity) and the mortalities were recorded over 24 hours after pesticide administration. A curve relating the percentage mortality with the dose was constructed and the LD50 was deduced from the curve according to the method described by Weisbrot (1985). 2) Snails in groups of 60 animals were given lannate at various increasing dose levels . Seven groups were used for each species and each was given a specific pesticide dose . Seven dose levels were tested, three over the LD50 and four lower than the LD50. The number of mortalities for each group were recorded and survival curves relating the percentage mortalities with the time were constructed.

The digestive gland and ovotestis (hermaphrodite gland) of both species of the land snails *E. vermiculata* and *M. contiana* of control as well as of experimental snails, were dissected out and fixed in Bouin's fluid or in 10% neutral buffered formalin for the histological investigation . The fixed samples were dehydrated in a series of ethanol, cleared and embedded in paraffin wax. Sections of 5 μ thickness were cut, stained with haematoxylin and eosin and studied with a binuclear microscope.

Results

The pesticide LD50 for *E. vermiculata* was found to be 30.27 mg/kg body weight, while its LD50 for *M. contiana* was found to be 37.18 mg/kg body weight (Fig. 1a, b , 2a, b). The respective LD100`s for the two species were 161.4 and 131.46 mg/kg body weight.

Observation of the survival rate over a period of 5 days of *E. vermiculata* given various doses of lannate showed that the percentage mortality of the snail depended on the dose of lannate. The higher dose given , the higher percentage death resulted . At dose range less than the LD50 (Fig. 3) and after 12 hours of the pesticide administration , mortality rates of 3, 8, 15 and 30% were observed in snails given doses of 5.8, 11.6, 23.2 and 29 mg/kg body weight, respectively . Higher mortality rates were observed at dose level higher than the LD50 (Fig. 4). A 60 % mortality was observed within 12 hours for snails given 58 mg/kg body weight. However, at all doses, the mortality rates increased by the time and most mortality occurred over 5 days post pesticide administration . Five days post treatment (Fig. 3), the maximum rates of mortalities observed were 20% at a dose of 5.8 mg/kg, 55 % at a dose of 11.6 mg/kg, 75% at a dose of 23.2 mg/kg and 83% at a dose of 29 mg/kg. No further mortalities were observed after 5 days of treatment. For snails given doses higher than the LD50 (Fig. 4), most mortalities occurred after 72 hours. Total death occurred after 84 hours by a dose of 58 mg/kg and after 108 hours by a dose of 46.4 mg/kg. A maximum mortality rate of 95 % was observed after 108 hours by a dose of 34.8 mg/kg.

As for *M. contiana*, the results (Figs. 5 & 6) showed that the percentage mortality of the snail depended on the dose of lannate. The higher dose given, the higher percentage death resulted. At dose range less than the LD50 (Fig. 5) and after 12 hours of the pesticide administration, mortality rates of 12.5, 21.7% were observed in snails given doses of 13.1 and 26.3 mg/kg body weight, respectively. Higher mortality rates were observed at dose level higher than the LD50, 36.7, 50, 65 and 92.5% were observed within 12 hours in snails given doses of 39.4, 52.6, 78.9 and 92.1 mg/kg body weight, respectively (Fig.6). However, at all doses, the mortality rates increased by the time and most mortality occurred over five days post pesticide administration. At five days post treatment, the maximum rates of mortalities observed were 32.5% at a dose of 13.1 mg/kg, 61.7% at a dose of 26.3 mg/kg. No further mortalities were observed after 5 days of treatment. Total death occurred after 120 hours by a dose of 52.6 mg/kg and after 60 hours by a dose of 78.9 mg/kg and after 24 hours by a dose of 92.1 mg/kg. A maximum mortality rate of 75% was observed after 108 hours by a dose of 39.4 mg/kg.

Histological and histopathological observations

Digestive gland

Normal snails: The digestive gland of the land snails *E. vermiculata* and *M. contiana* is a bilobed part of the alimentary canal as previously described. Each lobe of this gland consists of numerous tubules. Each digestive gland tubule is surrounded by a thin layer of loose connective tissue which fills the intertubular spaces and contains few muscle fibers. The lumina or ductules of the tubules open into a collecting duct which opens into the stomach from one side and into the intestine from another side. The epithelia of the ductules of both snail species consist of columnar cells resting on a thin basement membrane (Figs. 7a,b). These cells can be differentiated into; digestive cells, excretory cells and secretory cells which are unequal in height.

The digestive cells are numerous in the tubules and contain vacuoles. They are with round apics and flat base. Most of the digestive cells contain dark bodies or granular material which has a yellowish brown colour inside vacuoles . The rounded nuclei are basally located in the cells. The excretory cells are present in smaller numbers and less in the height than digestive cells. They are pyramidal or conical in shape and they have pointed apics and a broad bases on the basement membrane. The secretory cells are taller and smaller in numbers than the digestive cells. These cells are filled by secretion granules.

Treated snails: The tubules of the digestive gland with different cells in both snails exposed to dose range less than LC50 of lannate showed some pathological alterations. The lumina of the tubules appeared narrower than those of the normal and had irregular or branched shape. On the other hand, the tubules were seen widely separated by connective tissue. The digestive cells became swollen and more vaculated (Figs. 8a,b). The excretory and secretory cells were still differentiated . Most of these cells were abnormal by virtue of their hypertrophy and degeneration of the cytoplasm for presence of numerous large vacuoles with the yellowish brown material.

Ovotestis (gonad)

Normal snails: The reproductive system of the land snails *E. vermiculata* and *M. contiana* is composed of some elements which are common to both male and female (hermaphrodite gland, ovotestis or gonad and hermaphrodite duct), thus constituting, the hermaphrodite system as in most gastropods. This system is also composed of other organs or elements which belong either to male or to female ducts with their accessory sex glands.

The gonad of both species is located in the posterior part of visceral mass and is partly embedded in the digestive gland. It is composed of a large number of oval or rounded acini in which male and female gametes and their supporting cells (Sertoli cells and follicle cells, respectively) develop (Figs. 9a,b). The acini are separated by connective tissue.

Five different stages of female germ cells were recognized during the development. The youngest female cells are located in the proximal part of an acinus whereas the largest ones are in complete differentiation at the bottom. The oogonium is the first stage of oogenesis and is usually found in groups. This oogonium continues in development to oocytes; the first

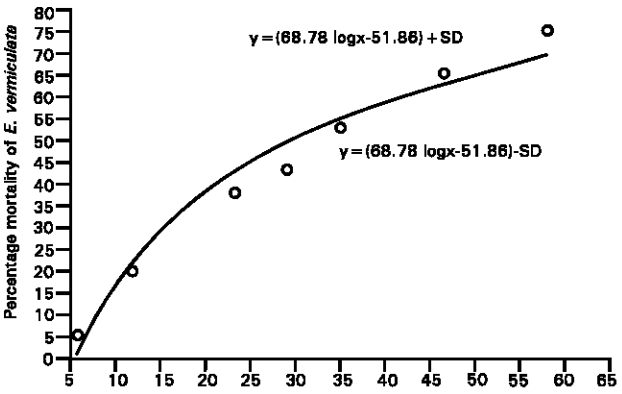

Fig. 1a: Does mortality curves of lannate against the land snail *E. vermiculata*

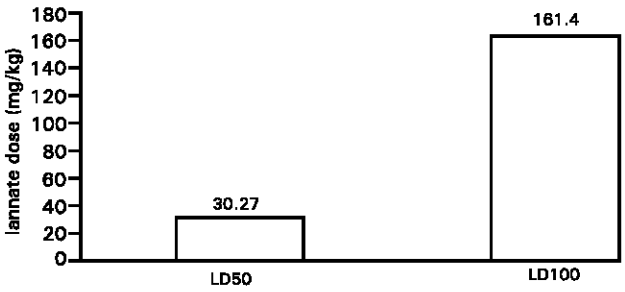

Fig. 1b: LD50 and LD100 of lannate for land snail *E. vermiculata*

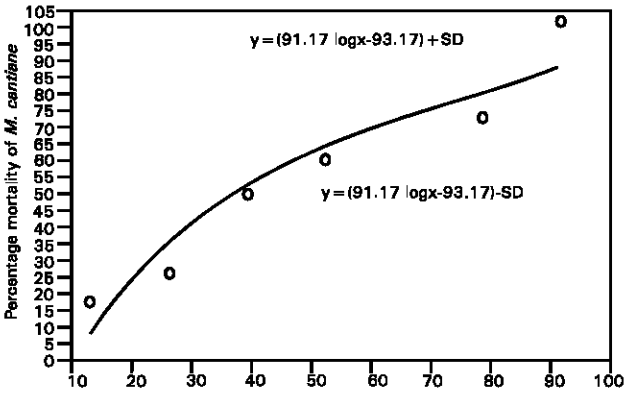

Fig.2a: Does -mortality curves of lannate against the land snail *M. cantiana*

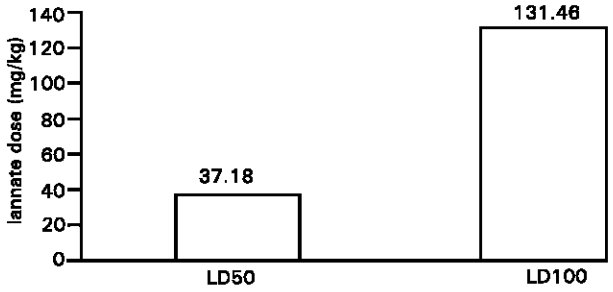

Fig.2b: LD50 and LD100 of lannate for the land snail *M. cantiana*

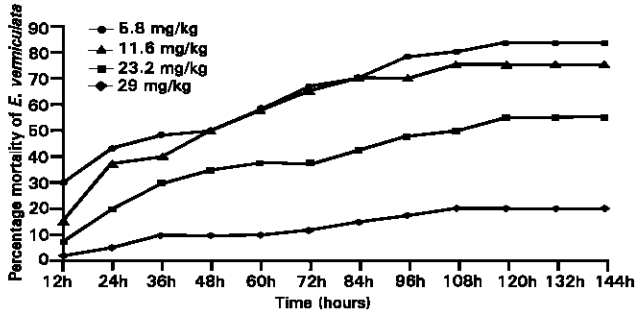

Fig.3: Mortality curves of the land snail *E. vermiculata* gives lannate at does levels less than the LD50

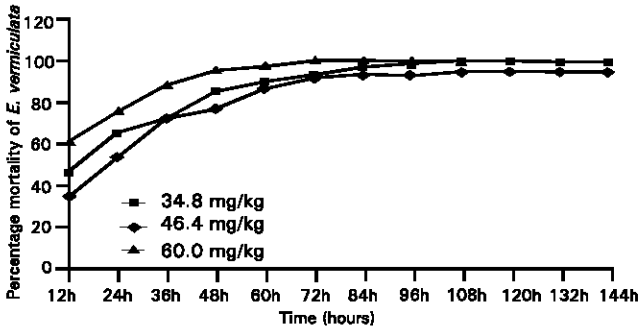

Fig.4: Mortality curves of the land snail *E. vermiculata* gives lannate at does levels more than the LD50

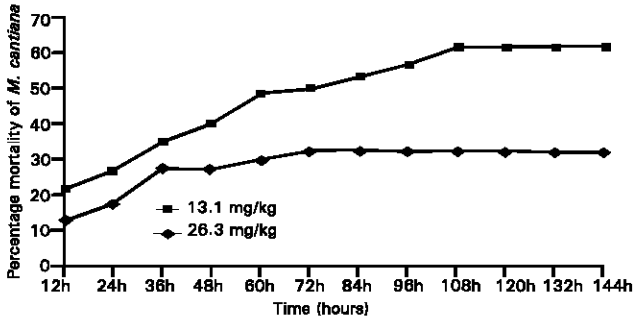

Fig.5: Mortality curves of the land snail *M. cantiana* gives lannate at does less than the LD50

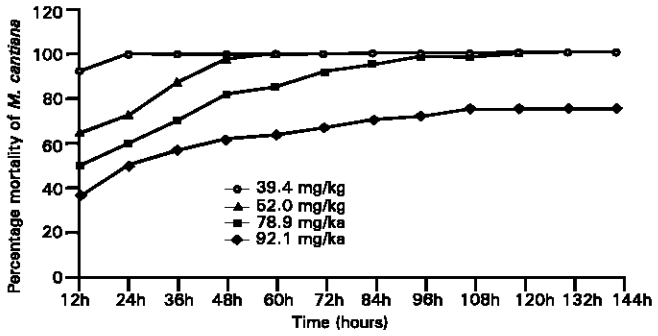

Fig.6: Mortality curves of the land snail *M. cantiana* gives lannate at does more than the LD50

Fig.7: Light micrographs of a sections of the digestive glands of the land snails, a) *E. vermiculata* and b) *M. contiana* showing the digestive tubules with the digestive cells(Dc) with the yellowish brown material (arrows),secretory cells (Sc) and excretory cells (Ec). L, lumen; N, nucleus; Sg, secretory granules; V, vacules. X 400

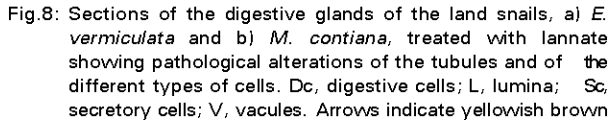

Fig.8: Sections of the digestive glands of the land snails, a) *E. vermiculata* and b) *M. contiana*, treated with lannate showing pathological alterations of the tubules and of the different types of cells. Dc, digestive cells; L, lumina; Sc, secretory cells; V, vacules. Arrows indicate yellowish brown material. X 400

stage, the second stage, the third stage and the late stage of oocyte. Little number of degenerative oocytes was observed in the lumen of the acinus. Apparently, oogenic cells were more numerous in the ovotestis of *E. vermiculata* than those in the ovotestis of *M. contiana*. Spermatogonia , on the other hand , are differentiated from the germinal epithelium and usually arranged in clusters. They are often found along the wall in the proximal part of the acinus. These spermatogonia are small rounded cells, increase in number and develop into primary and then secondary spermatocytes. These rounded cells developed into elongate cell, spermatids,which developed into spermatozoa. The spermatogenic cells were more numerous in the acini of the ovotestis of *M.contiana* than those in the ovotestis of *E. vermiculata*.

Treated snails : The acini of the ovotestis of *E. vermiculata* and *M. contiana* exposed to lannate showed pathological changes (Figs. 10a,b). Some acini of *E. vermiculata* were found filled by different stages of male and female gametes while numerous acini appeared occupied by few stages of both gametes. The late stages of oocytes had degenerated while the spermatogenic cells in most of the acini had affected or probably inhibited to develop to the late stages of spermatogenesis. This indicates that the insecticide used did not cause a complete cessation of gametogenesis in *E. vermiculata*.

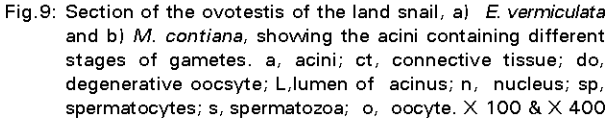

Fig.9: Section of the ovotestis of the land snail, a) *E. vermiculata* and b) *M. contiana*, showing the acini containing different stages of gametes. a, acini; ct, connective tissue; do, degenerative oocsyte; L,lumen of acinus; n, nucleus; sp, spermatocytes; s, spermatozoa; o, oocyte. X 100 & X 400

On the other hand, the acini of the ovotestis of *M. contiana* exposed to lannate appeared filled, with spermatogenic cells only except spermatozoa were not found. Most oogenic cells were not observed in the acini. Absence of the female gametes had lead to a decrease of the size of the acini and then ovotestis. Moreover, the spermatogenic cells had inhibited to develop to mature sperms.

Discussion

The land snails are considered as the most injurious of the stylommatophoran species in Egypt. These snails have been steadily increasing in recent years as an abundant agricultural pests in several areas. For this reason, the land snails gained a considerable attention due to their economic importance. In the present study, lannate was assessed for its efficacy in the control of two species of a widespread land snails *E. vermiculata* and *M. contiana* . The data obtained showed that the rates of mortalities of the snails *E. vermiculata* and *M. contiana* depended on the dose and time of exposure to the insecticide, lannate. Higher doses give higher percentage of the mortality. Generally, higher mortality rates were observed at doses higher than LD50. This is in parallel with that found by El-Okda (1978, 1979, 1984) during his studies on the molluscicidal toxicity of methomyl and aldicarb against the land snails *Helicella vestalis*, *T. pisana*, *E. vermiculata* and *Monacha* sp. In some studies, he used wetted baits of methomyl and in others he used methomyl adsorbed on filter paper. He concluded that water spraying treatments raised the molluscicidal activity of methomyl against the land snails. The selective toxicity (selectivity

) of lannate was tested on different species, land snails and mammals (mice) , i.e. target and non-target species, in close contact with each other (Al-Batal, 2001). Highly selective substances have a selectivity coefficient considerably higher than unity. Generally, selectivity based on differences in accumulation signifies that the relevant substance is toxic to both beneficial and harmful organisms, but only the latter can accumulate it in a toxic dose. At the level of light microscope, the digestive gland of *E. vermiculata* and *M. contiana* is similar to some extent to that described in other pulmonates , freshwater snails, e.g. *B. pfeifferi* (Meuleman, 1972)and *B. alexandrina* (El-Saadany, 1990) and similar to that previously described in *E. vermiculata* by Mersal (1992). The lobules of the digestive gland of *E. vermiculata* and *M. contiana* have been differentiated into digestive cells, excretory cells and secretory cells. Mersal (1992) described three types of cells in the digestive tubules of *E. vermiculata*, digestive , excretory and calcium cells as mentioned by Babua and Skowronsk-Wendland (1988) for the digestive gland of the slug *Deroceras reticulatus*. The dark bodies or the yellowish brown granules containing vacuoles which observed in the digestive cells of the present snails as a result of absorption are probably represent the indigestible residual material as mentioned in the slug *Agriolimax reticulatus* (Walker, 1972) and according to lysosome theory (Duve and Wattiaux, 1966). On the other hand, the main function of the secretory cells is synthesis and storage of the digestive enzymes.

Fig.10: Section of the ovotestis of the snails, a) *E. vermiculata* and b) *M. contiana* treated with lannate showing the acini containing different stages of male and female gametes. ct, connective tissue; do, degenerative oocyte; L, lumen; sp, spermatocytes; s, spermatozoa; o, oocyte. X200 & X 400, respectively

The present results revealed that both species of the land snails exposed to lannate have shown pathological alterations in the digestive gland. The narrows lumina to become irregular in shape, the dilation between the tubules, the vaculated and swollen digestive cells and presence of numerous yellowish brown granules in the vacuoles are the most pathological changes observed. These changes are probably due to accumulation of the insecticide in the cells of the digestive gland. There is evidence that some insecticides reduced the feeding capacity of the freshwater snails and this due to the direct effect for the insecticide on the tissues of the digestive tract of the snails (Mohamed *et al.*, 1988. They decided that the insecticides reduce the glycogen and protein contents of the snails tissue according to the reduction in the feeding capacity. The results indicate that the insecticide, lannate, have a toxic effects in cellular damage of the digestive gland of both species. This cellular damage could be correlated with the disturbed enzyme activities in different species. The gonads of the land snails *E. vermiculata* and *M. contiana* are similar in structure and in the development of the different stages of spermatogenesis and oogenesis to those of other pulmonate snails, e.g. the land snails *Helix aspersa* (Griffond and Medina, 1984), *H. pomatia* (Csaba and Bierbauer, 1981) and *Theba pisana* (Heiba, 1998), the slug *Ariolimax californicus* (Gottfried and Dorfman, 1970) and the freshwater snails (De Jong-Brink *et al.*,1977, El-Saadany, 1992). The gonads of these pulmonates consist of large number of oval or circular acini in which male and female gametes and their supporting cells (Sertoli cells and follicle cells, respectively) develop. It has been reported that the different stages of gametogenesis in the land snail *E. vermiculata* is under control of the environmental factors as photoperiod, temperature and starvation (El-Saadany *et al.*, 1993a,b, 1994).

In the present study, the ovotestis of both snails *E. vermiculata* and *M. contiana* exposed to lannate showed pathological alterations. The male and female gametes are severly affected. The female gametes were not observed in the acini of the gonad of M. contiana while the male gametes in the gonad of both snails had affected or probably inhibited to develop to the late stages of spermatogenesis. This indicates that the insecticide used did not cause a complete cessation of gametogenesis. Moreover, presence of female gametes in the acini of *E. vermiculata* and absence of these gametes in *M. contiana* give impression that the last snail is more sensitive to lannate than the first snail or that the effect of lannate is achieved by a change and release activities of the endocrine organs which play a role in reproduction. The present results are in line with the observation of Mohamed *et al.* (2000) on the freshwater snail *B. alexandrina* who found that the pesticide abamectin, inhibits the gametogenesis probably via inhibition of the gonadotropic hormones.

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