MATERIALS AND METHODS

1. Ecological studies.

The ecological studies were conducted in two different localities; the first was Samanoud district, Gharbia Governorate to represent the Lawer Egypt, while the second in Sennores district, Fayoum Governorate to represent the Upper Egypt. In both districts, two field crops were chosen, Egyptian clover (*Trifolium alexandrinum L.*) as a Winter field crop, and Cotton (*Gossypium hirsutum L.*) as a Summer field crop.

a. Survey of the terrestrial land snail species infesting Egyptian clover and cotton crops in Fayoum and Gharbia Governorates.

Samples of land snails were collected from the fields cultivated with Egyptian clover and cotton crops. Collected samples were singly kept in tightly closed polyethelene bags. Labels including all necessary information concerning habitat, locality and date of collection were stuck on each bag, then transferred to the laboratory for identification.

b. Population dynamics of land snails on Egyptian clover and Cotton crops.

Seasonal population dynamics of land snails were conducted on two field crops; Egyptian clover and cotton during the two successive growing seasons; 2004 / 2005 and 2005 / 2006 at Sammanoud and Sennoures districts. One feddan was selected for each crop in the two districts, five replicates of quadrate plots (one meter^2) were randomly examined at biweekly intervals, during the whole growing season of each crop.Examination was undertaken during early morning before sun rise. All snail species found on plants as well as soil surface in the quadrate plot were classified and counted.

Population fluctuation for the four terrestrial snails glassy clover snail *Monacha obstructa* (Pfiffer),brown garden snail *Eobania vermiculata* (Muller),small sand snail *Theba pisana* (Muller) and small desert snail *Helicella vestalis* (Pfiffer) were monitored at 15 day intervals allover the growing small season for each field crop.

Necessary metreological data concerning air temperature and relative humidity during the period of study were obtained from metreological Station of Aga, Gharbia and Fayoum Governorates.

Results were statistically analysed to show the influence of locality, habitat, seasons, temperature and relative humidity.

2. Toxicological Studies:

A. Tested snail:-

Adults of glassy clover snail; *Monacha obstructa* (Pfiffer) were collected directly, from infested fields in Fayoum Governorate. The collected snails were transferred to the laboratory, kept under room conditions at 20 ± 3° C and 80 ±5 R.H%. Healthy and similar individuals were chosen and kept in glass terrariums , (40 cm long × 25cm wide and 40 cm. deep) which was filled with moist soil adjusted at 75% of water field capacity and provided daily with fresh green lettuce leaves for two weeks before treatment for acclimatization (Godan, 1983).

B. Tested materials:

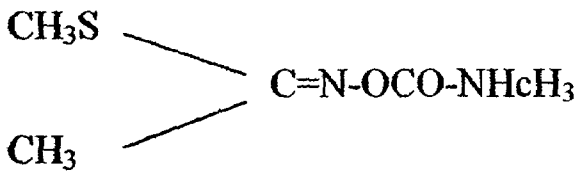
1. Chemicals:

Insecticides:

One insecticide was used in this study; Methomyl (Lannate) 90% soluble powder: it is a recommended pesticides for controlling land snails supplied from Kafr El-Zayaat Co. for Chemicals and Pesticides, Kafr El-Zayaat, Egypt.

Common name: Methomyl

Chemical name: S-methomyl N-(methomyl carbamoyloxy) thioacetimidate.



Inorganic salts:

Copper sulfate ($CuSO_4.5H_2O$) and **Sodium borate** ($NaBO_3$) whiche were supplied from El- Gomhoria Co. for Chemicals, Egypt.

Mineral oils:

CAPL- 1: it is a sulfonated solar cut of petroleum oil prepared as emulsifiable concentrate contained 96.62% (V /V) base oil, registerated at No. 501.

CAPL- 2: it is a lubrication cut of petroleum oil, prepared as emulsifiable concentrate contained 96.629% (V./V.) base oil, registerated at No. 502.

SOL.EC: it is a crude solar cut of petroleum oil, prepared as base oil. All oils are produced by Central Agricultural Pesticides Laboratory, A. R.C.

2. plant extracts:-

Three plants were chosen to be extracted by boiled water. These plants were; foliage of Santonica (*Artemisia herba . alba-Asso*), fruits of Red Pepper (*Capsicum annum L.*), fruit's Skin Peel of Pomegranate (*Punica granatum L.*). These plants in addition to seeds of Fennel (*Foeniculum vulgare l.*), Fruits of Black pepper (*Piper nigrum l.*) and Seeds of Neem (*Azadirachta indica*) were also extracted by solvent (Acetone).

Table 1. Scientific and arabic names of the plants used in the present study

Part Used	English name	Scientific name	Family	Arabic name
Seeds	Fennel	*Foeniculum vulgare l.*	Umbelliferae	الشمر
Fruits	Black pepper	*Piper nigrum l.*	Piperaceae	الفلفل الأسود
Aerial parts (Foliage)	Santonica	*Artemisia herba . alba-Asso*	Compositae	الشيح البلدى
Fruits	Red Pepper	*Capsicum annum L.*	Solanaceae	الشطة
Fruit's Skin Peel	Pomegranate	*Punica granatum L.*	Punicaceae	قشر الرمان
Seeds	Neem	*Azadirachta indica*	Meliaceae	النيم

Extraction procedure:

The parts of each plant (250 gm) were ground in an electeric grinder and extracted using water and acetone. The used plant parts of Santonica, Red Pepper, Pomegranate were put in 350 cm water, boiled for 24 minuets, then filtered and the obtained extracts were used as crude extracts. The solvent (Acetone) was used to extract the six plants at rate of 2 ml /g plant material, compared with the method described by (Su and Horvat 1981). After 24 hours, the extracts were filtered and evaporated to dryness under vacuum using a rotary evaporator in a water bath at 50 °C. The crude extracts were then weighted and adjusted to 10 ml volumes with acetone, and kept in a refrigerator until testing.

C. Bioassay Tests:

Laboratory evaluations of the tested compounds were tested against land snail *M. obstructa* using four different techniques at three concentrations of each compound.

a. Bait technique:

Bait technique was prepared according to the method described by Ebenso, (2004).

Experiments were conducted to evaluate the efficacy of insecticide, inorganic salts , mineral oils and plant extracts. The poison baits were prepared by mixing (1 ml from tested concentrations of each compounds + 5 ml molasses +80 gram bran

+ 14 ml water). Sample of five grams of the poison bait was put on plastic sheet, placed on the surface of the soil in each box. Fifteen animals of *M. obestructa* adults were divided into three replicates and exposed to the candidate concentration of the tested compound. A control test was parallel conducted using the same technique without any treatment. Mortality percentages were calculated after one, four and seven days and corrected by Abbott; s formula, (Abbott, 1925). LC_{50}, LC_{90} and slope values were calculated for each experiment.

$$\text{Corrected mortality \%} = \frac{\text{observed mortality \% - control mortality \%}}{\text{100 - control mortality \%}} \text{ X 100}$$

b. Residual film technique:

Thin layer film technique was conducted as a method of application according to Ascher and Mirian (1981). The forementioned compounds were tested, whereas the tested concentrations were applied in Petri-dishes of 5 cm diameter. One ml of each concentration of the tested compounds were spread on inner surface of a Petri-dish, by moving the dish gently in circles. Acetone was evaporated under room condition in a few minutes leaving a thin layer film of tested material on the inner surface of Petri-dish. Five adults of *M. obstructa* were exposed to the candidate concentration film for 24 hours in Petri-dishes. A parallel control test was conducted using acetone only. Mortality percentages were calculated after 24 hours.

c. Leaf Dipping technique:

Leaf discs of lettuce (3 cm diameter) were immersed in the tested material for 3 seconds. After that, the discs were air dried then each disc was placed with 5 snails which were over night starved in a box fillet with soil. Snails were allowed to feed on the treated lettuce for one week. Mortality percentages were calculated after one, four and seven days, as described by (Salama and Radwan, 1995).

d. Repellency tests:

Repellency tests were conducted according to McDonald *et al.* (1970) with some modifications. Substrates were prepared from 9 cm diameter filter papers (Whatman No. 1) which were cut in two halves. One ml of the desired material concentration was applied to a half filter paper as uniformly as possible with a pipette. The treated half discs were then air-dried to evaporate the solvent completely. Full discs were then remade by attaching treated halves to untreated (treated with solvent only) halves of same dimensions with cellotape. Precautions were taken so that an attachment did not prevent the free movement of snails from one half to another.Ten snails were put at the center of each filter – paper disc and the Petri dish was covered. Three replicates were conducted for each concentration. Counts of the snails present on each strip were done after 1 and 6 hours. The results were converted to express Repellency percentage (RP) by the formula of Talukder and Howse (1994).

$$RP\ \% = \frac{(N - C)}{C} \times 100$$

Where:

N = the number of snails present in the control half.

C = half the number of total snails present.

Positive values (+) expressed repellency and negative values (-) expressed attractancy.

Repellency percentages values were assigned repellency classes by using the following scale: classes 1, 11, 111, 1Vand Vdesignated repellency percentages values of < 0 - 20, 20.1 - 40, 40.1 - 60, 60.1 - 80 and 80.1 – 100, respectively

Toxicity lines:-

Convenient stock concentrations of each extract in acetone were prepared on the basis of the tested plant (W/V), then kept in glass stoppered bottles, and stored under refrigeration. Such stock solutions were prepared periodically. Serial dilutions were prepared. Four to five diluted concentrations per toxicant were used to draw the toxicity (LC- P) lines. There were at least four replicates for each concentration.

D. Identification of Molluscicidal components in plant extracts using thin layer chromatography:-

A layer of silica gel, about 0.75 mm. thickness, was spread on 20x20 cm glass plates. Aslurry of 7.5 grams of silica in about 15 ml. water was prepared and spread over the plate with the applicator. The plate was allowed to stand for 2 hours at room temperature, and then activated in an oven for 2 hours at 110°C. Marks were made near the edge of each plate at a distance of 1.5 cm. to define the spotting line. Spots of the plant extracts were put along the plate. The developing solvent system; consisted of Toluene: Acetic acid (9:1), was used for all tests. The developing solvent added to the chamber to a depth of 10 mm, and spotted plate was placed in the chamber so that the bottom edge was in contact with the solvent, and the lid was then replaced when the solvent was developed to a high of about 15 cm, the plate was removed and the solvent was allowed to evaporate.

The dry plates were exposed to iodine vapors as a general detection. The visualization with iodine was carried out by placing the developed, dried plates in a jar containing crystalline iodine. After closing the jar, the iodine vapors was absorbed into the dreas of the layer containing organic compounds, yielding brown spots on a white background. The spots become darker when left in the iodine, but generally fade rapidly when the layer is removed from the chamber.

Statistical analysis.

Ecological data were statistically analyzed for standard error (S.E.) and least significant difference (L. S. D.) according to Steel and Torrie (1968).

Toxicological data were statistically onalyzed to estimate LC_{50} , LC_{90}. and slope according to the method described by (Finney, 1952).