Molluscicidal Activity of Eco-friendly natural compound (Rutin) Gained from Ethanolic Flowers Extract of *Calendula officinalis* on *B. alexandrina*, *B. truncatus* and *Lymanea* snails

El-Sheikh, Y. W. A. [1]; Eltamny, H. M.[2]; Soliman, H. A.[2]; Farag A.A.[1] and El Behary M.H.H[2]

[1] Pesticide Formulations Department, Central Agricultural Pesticides Laboratory, Agricultural Research Center, Dokki, Egypt [2] Suez Canal University Faculty of Science
yasserwahied@yahoo.com

Abstract: Within 3000 intermediate host snails of *Schistosomiasis and Fasciolasis* were originated from Delta regions at Egypt mainly Beba in Beni Seuf governorate. They were selected on the basis of snails selection Schistosomiasis and Fasciolasis mainly *Schistosoma haematobium, Schistosoma mansoni* and *Fasciola* ova. Preliminary screening of *C. officinalis* flower ethanolic extract showed molluscicidal activity against *Biomphalaria alexandrina*, *Bulenus truncatus* and *Lymanea* snails under studying. Rutin as eco-friendly natural compound was separated from the ethanolic extract of *C. officinalis* flowers by TLC and column chromatography and was identified by different tools of spectral analysis. Ten snails per one liter water jars were applied. Four serial concentrations of rutin with comparing to the recommended molluscicide niclosamide were prepared as (0.05, 0.1, 0.2 and 0.4 g). Mortality percentages were observed after six, twenty four, forty-eight and seventy two hours. Our study revealed that rutin separated from ethanolic extract of *Calendula officinalis* flowers can be used in the control of both *Schistosomiasis* and *Fasciolasis* in Egypt.

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Keywords: *C. officinalis*, rutin, snails, *Biomphalaria alexandrina*, *Bulenus truncatus*, *Lymanea*, spectral analysis

1. Introduction:

Recently, a large number of synthetic pesticides have been banned in the world because of their undesired attributes such as chronic and acute toxicity, long degradation periods, accumulation in the food chain and extension of their power to destroy both useful and harmful pests **(Barnard *et al*., 1997).** Consequently, there is an urgent need for alternative agents for pest management **(Bolkan and Reiner, 1994, Rice *et al*., 1998).** Green plants represent a reservoir of effective chemotherapeutants and can provide valuable source of natural pesticides **(Balandrin *et al*., 1985; Hostettmann and Wolfender, 1997).** Reports are available on the use of active agents form higher plants to replace chemical pesticides that are not-phytotoxic, more systemic and easily biodegradable **(Fewcett and Spencer, 1970).**

Schistosomiasis is a parasitic disease which is considered a worldwide problem in various parts of the world including Egypt. As estimated by **WHO (1993)** 600 million people are at risk of infection and more than 200 million are currently infected **(Mckerrow and Salter, 2002).** The importance of the disease lies in the fact that it affects not only the overall health status and fitness of the infected people, but also the human productivity and national economy **(El-Garem *et al.*, 1994).**

Actually, *Schistosomiasis* is considered to be the second most prevalent tropical disease and a leading cause of severe morbidity in several foci in Africa, Asia and South America. There are many endemic areas where *Schistosomiasis* is not yet recognized as an important public health problem and where it receives only a low priority for control. It is estimated that around 200 million people are infected by one or another form of *Schistosomiasis* parasites throughout the world which are responsible for 800,000 deaths per year **(WHO, 1992).**

Each of *B. alexandrina*, *B.truncatus* and *lymanea* snails cause serious infectious diseases such as, liver cirrhosis, esophagus varices, renal failure and ascitis due to the infection of either *Schistosoma haematobium* ,*Schistosoma mansoni or Fasciola* ova. Successful control of both *Schistosomiasis* and *Fasciolasis should* be based on an integrated approach which includes the control of intermediate snail hosts **(WHO, 1993).**

Pesticides used to control these snails such as niclosamide accumulated in the muscles of fishes are toxic and these molluscicides are not destroyed by freezing or cooking or roasting or salting of the fish, would affect the metabolic enzymes of the human consuming such fishes **(Zinada, 2000).** As these pesticides affect both of human and environment and reduce the efficiency, potential and economics besides to, the cost of money paid in snails' control

are too much **(Joblin *et al.*, 1989).** *Calendula officinalis (Marigold)* has many pharmacological properties for skin disorders, pain and also as a bactericide, antiseptic and anti-inflammatory **(Cordova *et al.*, 2002)**

This study aimed to separate and identify the effective compound from ethanolic flower extract of *Calendula officinalis (Marigold),* then the effective compound will be used to control snails under studying and determining its LC_{50} and LC_{90} compared with niclosamide as the recommended molluscicide.

MATERIAL AND METHODS

1. Collection, Preparation of Plant Material and extraction:

Calendula officinalis flowers were collected at the flowering season from different regions in Egypt. They were kept to dry in open air and away from direct sunlight, then milled well by a mixer miller machine to get a powder sample.

Ground flowers were extracted according to **(Kato-Noguchi *et al.*, 1994)**. Weight of 750 grams flower sample was shacked in a dark vessel with 2.25 liters of hexane, chloroform and ethanol consequently and individually for 8 hours. The ethanolic extract was filtered in dark vessel purified and evaporated under vacuum with rotary evaporator at 40ºC to dryness.

2. Separation:

The chemical constituents of the ethanolic flowers extract were isolated by TLC and column chromatography techniques using eluant solvents (petroleum ether 60-80: diethyl ether: ethanol) ratio (7: 2: 1). There were five bands separated. Every band was collected, cleaned up, purified, re-purified and crystallized by a proper solvent **(Duke, 1991).**

3. Preliminary Qualitative of Phytochemical Constituents:

The preliminary phytochemical constituent such as steroids, triterpene, tannins, carbohydrates, flavonoids, alkaloids, saponins and glycosides as a (phenolic, cyanophore, anthraqunone and cardic) were carried out on fraction which exhibited *molluscicidal* activity according to **Claus (1961); Venkatroman (1962); Wall *et al.* (1964)**; **Balbaa (1981); Zedan (1992) and El Kadi (1997).**

4. Chemical identification:

The structure of the effective compound was confirmed by different tools of spectroscopic analysis, elemental analysis, IR Spectra, Mass Spectra and ^{1}H-NMR. All the analysis was carried out in Elemental Analysis Laboratory, Faculty of Science, Cairo University, Arab Republic of Egypt.

5. Bioassays:

Each of *Biomphalaria alexandrina*, *Bulenus truncatus* and *Lymanea* snails were collected from Delta regions at Egypt mainly Beba in Beni Seuf governorate. They were selected on the basis of snails *Schistosomiasis* and *Fasciolasis* mainly *Schistosoma haematobium, Schistosoma mansoni* and *Fasciola* ova. The snails were reared under laboratory conditions three days before being used in testing using lettuce plant and algae as daily food and kept in laboratory temperature (20±5°C). Ten snails per liter water jars were applied. Four serial concentrations of effective compound were prepared as (0.05, 0.1, 0.2 and 0.4 g/l). The recommended molluscicide niclosamide was used as a reference for our effective compound with the same serial concentrations which used against the snails under studying. Mortality percent was observed after 6, 24, 48 and 72 hours **(Borai *et al.*, 2005).**

6. Statistical analysis:-

Morality was scored after 6, 24, 48 and 72 hrs of treatment. Snails were considered dead if they showed no sign of movement. Data were corrected for control morality **(Abbott, 1925)**. The corrected mortality percentage was used to calculate the LC_{50} values according to **Finney (1971)**. Toxicity index was calculated according to **Sun (1950).**

$$\text{Toxicity Index} = \frac{LC_{50} \text{ of the most effective sample}}{LC_{50} \text{ of the sample}} \times 100$$

RESULTS

1. Separation:

The crude ethanolic flower extract was separated by thin layer chromatography (TLC) plates to know the number of its chemical constituent compounds. The crude was eluted by a mixture of solvents which appeared five bands under ultraviolet lamp with different R_F where the third band was the most intense band. 10 grams of crude extract was separated by column chromatography 1.6 m using silica gel for column and eluted by petroleum ether (60–80), diethyl ether and ethanol in ratio 7: 2: 1. Every fraction was followed up by TLC, collected, purified, re-purified and evaporated by a vacuum rotary evaporator till dryness. Every compound was re-crystallized by a proper solvent and tested against snails under studying.

The data of table (1) illustrated the R_F, solvent of crystallization, percentage of yield and molluscicidal effect of the collected separated fractions, which demonstrated that, the third fraction

was re-crystallized from ethanol and its % of R_F was 55% and the yield percentage was 35%. This fraction showed only molluscicidal activity against the tested snails.

Table (1): The R_F, solvent of crystallization, percentage of yield and molluscicidal effect of the collected separated fractions

Test \ No. of fraction	1	2	3	4	5
Solvent of crystallization	Benzene	Methanol	Ethanol	Ethanol	Ethyl acetate
% of R_F	80	67	55	35	23
% of Yield	5	7	35	11	8
Molluscicidal effect	-ve	-ve	+ve	-ve	-ve

-ve = No molluscicidal effect +ve = molluscicidal effect

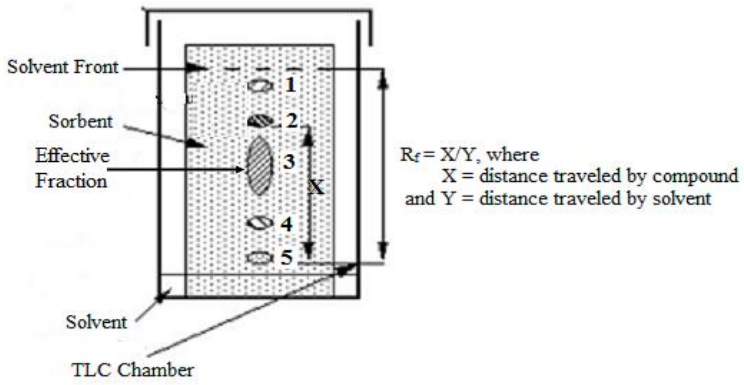


Fig. (1): Diagram showing the TLC separation processes.

2. Phytochemical identification of the effective fraction:

The results obtained from the phytochemical identification of the effective fraction separated from ethanolic flowers extract appear the functional groups of the studied compound which has molluscicidal potency (Table 2). The effective fraction gave +ve test for carbohydrates, flavonoide, phenolic glycosides and hydrolysable tannins as catechol and –ve test for sterols, triterpene, alkaloids, saponin and condensed tannins. Also has –ve test for cyanophore, anthraquinone and cardic glycosids.

Table (2): Phytochemical identification of ethanolic flowers extract of *C. officinalis*

Test	Result	Test	Result
Carbohydrates	++	Glycosides:	
Sterols/ Triterpenes	Nd	Phenolic Glycosides	++
Flavonoide	+	Cyanophore Glycosides	nd
Alkaloid	Nd	Anthraquinone Glycosides	nd
Saponins	Nd	Cardic Glycosides	nd
Hydrolysable Tannins	++ (Catechol)	Condensed Tannins	nd

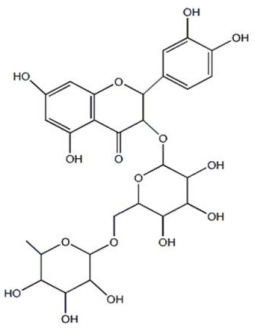
nd: not detected + : Found ++ : Excess

3. Chemical identification:

The data obtained from spectroscopic tools of analysis showed that:

- The melting point of the effective compound was 209 – 211.5 °C.
- The IR spectra absorption bands in the region of wave length (cm^{-1}): 3400 – 3300 (υ_{OH}); 3010 – 2980 ($\upsilon_{CH\ aro}$); 2980 – 2680 ($\upsilon_{CH\ aliph}$); 1680 – 1650 ($\upsilon_{C=O}$) and 1595 – 1575 ($\upsilon_{C=C}$).
- Correct elemental analysis for the separated compound give the following data ($C_{calcu./found}$ = 50.81/50.42) and ($H_{calcu./found}$ = 4.92/4.75).
- The mass spectra of the separated compound revealed molecular ion peak (M^+/at m/z = 614) with 10.1 % as relative abundance corresponding to C_{26} H_{30} O_{17}.
- The strong evidence for the suggested structure of separated compound comes from the ^{1}H-NMR spectrum where the spectrum showed signals at δ(ppm) = 7.5 – 6.8 (m, 5H, aromatic proton), 5.7 (2d, 2H, tetrahydrobenzopyrane proton), 5.4 (d, 1H, tetrahydropyran C-OH proton), 5.03 (d, 2H, H-C-O tetrahydropyrane proton), 5.0 (S, 4H, 4-OH benzene rings), 3.6 (d, 2H, methylene protons), 3.5 (t, 7H,

tetrahydropyrane protons) and 2.0 (s, 7H, 7-OH tetrahydropyrane rings).



The chemical structure of the effective compound which confirmed by different types of spectral tools and melting, mixed melting point estimated that the compound is **rutin**

4. Bioassay:

- **Molluscicidal effect of the separated compound in comparing with niclosamide against *Biomphalaria alexandrina* snails after different time intervals of treatment:**

Table (3) represents the results obtained from treatment of *Biomphalaria alexandrina* with rutin concentrations at different time intervals; 6, 24, 48 and 72 hrs in comparison with niclosamide. From which, after all time intervals we found that, the percentage of mortality increased as the concentrations increased. We can also notice that, as the time of exposure to rutin increased the LC_{50} decreased and accordingly, the effectiveness of the compound increased. The LC_{50}'s of the rutin compound after 6, 24, 48 and 72 hrs of treatment were 0.0885, 0.0555, 0.0379, 0.0272 g/l, respectively. On the other hand, the LC_{50}'s of the recommended molluscicide (niclosamide) were 0.0696, 0.0536, 0.0328 and 0.0352 g/l, respectively at the same time intervals. In comparing the effectiveness of rutin and the niclosamide molluscicide on the *Biomphalaria alexandrina* snails we found that, niclosamide was found to be more effective on the tested snail than the separated rutin. This note can be approved that the toxicity indexes of Niclosamide were 100 % at all time intervals, where the toxicity indexes of rutin were 78.64, 96.58, 86.54 and 84.62%; respectively at the tested time intervals 6, 24, 48 and 72 hrs after treatment.

The ldp-lines of the rutin and niclosamide at different time intervals against *Biomphalaria alexandrina* were plotted in Figures 2A and 2B, respectively. From the obtained lines the highest slope recorded for rutin was after 6 hrs of treatment (2.62), while the slopes after 24, 48 and 72 hrs were 2.1, 2.11 and 1.62, respectively. Finally, the highest slope recorded for niclosamide was after 6 hrs of treatment also (2.5) and the slopes after 24, 48 and 72 hrs were 2.03, 1.8 and 1.12, respectively.

Table (3): Effects of different concentrations of rutin on *Biomphalaria alexandrina*, snails in comparison with niclosamide

		Concentrations (g/l)				LC_{10}	LC_{25}	LC_{50}	LC_{90}	Toxicity Index	slope
		0.05	0.1	0.2	0.4						
6 hrs	Rutin	25.79	55.54	82.34	95.69	0.0287	0.0489	**0.0885**	0.2727	78.64	**2.62**
	Niclosamide	35.99	65.35	87.46	97.13	0.0214	0.0374	**0.0696**	0.226	**100**	**2.5**
24 hrs	Rutin	46.19	70.46	87.92	96.41	0.0137	0.0265	**0.0555**	0.2257	96.58	**2.1**
	Niclosamide	47.53	71.85	87.69	96.16	0.0125	0.025	**0.0536**	0.2296	**100**	**2.03**
48 hrs	Rutin	60.03	81.31	93.62	98.39	0.0094	0.0182	**0.0379**	0.1534	86.54	**2.11**
	Niclosamide	62.87	90.79	92.1	97.42	0.0064	0.0139	**0.0328**	0.1693	**100**	**1.8**
72 hrs	Rutin	55.58	81.99	91.94	97.04	0.0044	0.0104	**0.0272**	0.1686	84.62	**1.62**
	Niclosamide	56.75	69.36	80.01	55.06	0.0025	0.0088	**0.0352**	0.4953	**100**	**1.12**

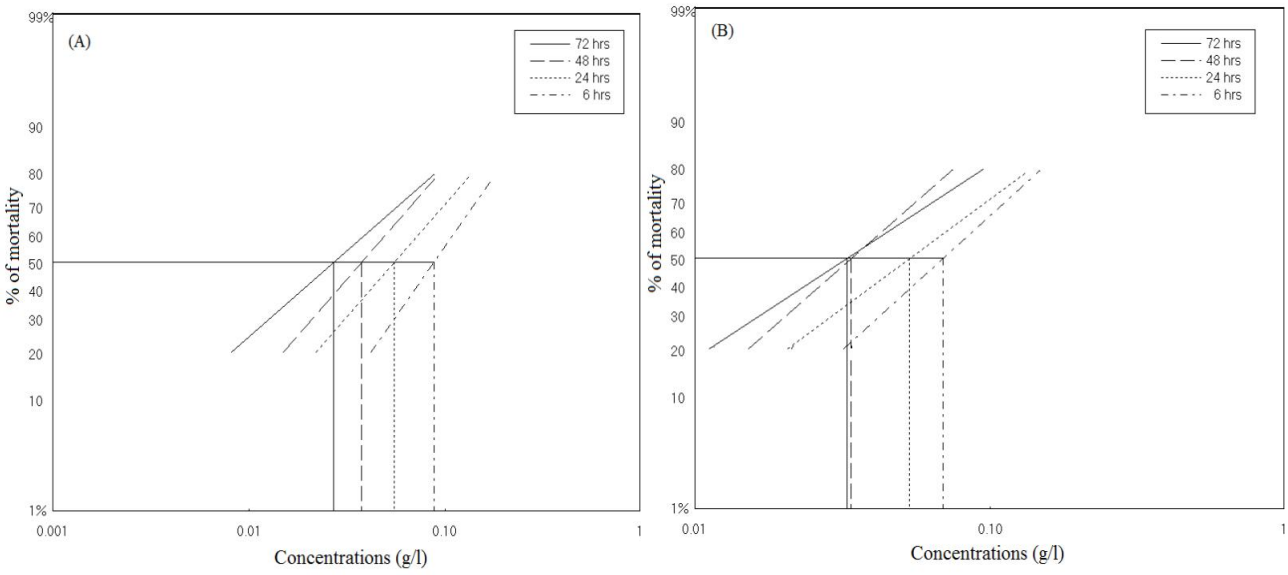


Figure (2): The ldp-lines of the rutin (A) and niclosamide (B) against *Biomphalaria alexandrina* at different time intervals of treatments.

- **Molluscicidal effect of the separated compound in comparing with niclosamide against *Bulenus truncatus* snails after different time intervals of treatment:**

Results obtained from table (4) revealed the high mortality percentage with increasing the concentrations of rutin for the control of *Bulenus truncatus* snails at all time interval of treatments. The LC_{50}'s registered to be 0.0331, 0.0318, 0.0229 and 0.0347 g/l for rutin, and recorded 0.1414, 0.084, 0.0673 and 0.0517 g/l for niclosamide after 6, 24, 48 and 72 hrs of treatments for all, respectively. In alignment the mortality percentage increased as the niclosamide molluscicide concentration increased at all examined time intervals. In comparing the effectiveness of rutin and the niclosamide molluscicide on the *Bulenus truncatus* snails we observed that, the rutin was found to be more effective on the tested snail than niclosamide molluscicide used. These results revealed that the toxicity index's of rutin were 100% at all time intervals of treatment and was in comparison 23.41, 37.86, 34.03 and 78.61% for niclosamide after 6, 24, 48 and 72 hrs, respectively.

The Ldp-lines of rutin and niclosamide are given in figures 3C and 3D, respectively. The slope values for all were approximately convergent. The slope values for rutin were 1.31, 1.59, 1.63 and 1.66; and for niclosamide were 1.22, 1.29, 1.41 and 1.42 after 6, 24, 48 and 72 hrs of treatments, respectively.

Table (4): Effects of different concentrations of rutin on *Bulenus truncatus* snails in comparison with niclosamide.

		Concentrations (g/l)				LC_{10}	LC_{25}	LC_{50}	LC_{90}	Toxicity Index	slope
		0.05	0.1	0.2	0.4						
6 hrs	Rutin	59.27	73.56	84.73	92.22	0.0035	0.0102	**0.0331**	0.3135	**100**	**1.31**
	Niclosamide	29.15	42.74	57.26	70.86	0.0125	0.0394	**0.1414**	1.6007	23.41	**1.22**
24 hrs	Rutin	62.24	78.51	89.74	95.94	0.0049	0.0119	**0.0318**	0.2043	**100**	**1.59**
	Niclosamide	38.58	53.89	68.64	80.89	0.0085	0.0252	**0.084**	0.8288	37.86	**1.29**
48 hrs	Rutin	71.02	85.2	93.77	97.85	0.0038	0.0088	**0.0229**	0.1396	**100**	**1.63**
	Niclosamide	42.75	59.59	74.81	86.3	0.0084	0.0225	**0.0673**	0.5427	34.03	**1.41**
72 hrs	Rutin	60.4	77.76	89.69	96.11	0.0059	0.0136	**0.0347**	0.2048	**100**	**1.66**
	Niclosamide	49.19	65.81	79.81	89.65	0.0065	0.0173	**0.0517**	0.4126	78.61	**1.42**

Figure (3): The ldp-lines of the rutin (C) and niclosamide (D) against ***Bulenus truncatus*** at different time intervals of treatments.

- **Molluscicidal effect of the separated compound in comparing with niclosamide against *Lymanea* snails after different time intervals of treatment:**

The molluscicidal activities of the rutin and niclosamide against *Lymanea* snails were presented in Table (5). Results obtained from the table revealed the percentage of mortality is directly proportional to the concentrations of rutin and niclosamide when used to treat *Lymanea* snails at all time intervals. The registered LC_{50}'s for rutin were 0.0212, 0.0.0288, 0.0169 and 0.0121 g/l, and recorded 0.01089, 0.0638, 0.0492 and 0.0352 g/l for niclosamide after 6, 24, 48 and 72 hrs of treatments, respectively. In comparing the effectiveness of rutin and the niclosamide molluscicide on the *Lymanea* snails we observed that, the rutin was found to be more effective on the tested snail than niclosamide molluscicide used. These results revealed that the toxicity index's of rutin were 100% at all time intervals of treatment and was in comparison 19.47, 45.14, 34.35 and 34.38% for niclosamide after 6, 24, 48 and 72 hrs, respectively.

The Ldp-lines of rutin and niclosamide are given in figure 4E and 4F, respectively. The slope values for all were also approximately relative to each other. The slope values for rutin were 1.19, 1.09, 1.81 and 1.48 and for niclosamide were 0.9, 1.05, 1.15 and 1.12 after 6, 24, 48 and 72 hrs of treatments, respectively.

Table (5): Effects of different concentrations of rutin on *Lymanea* snails in comparison with niclosamide.

		Concentrations (g/l)				LC_{10}	LC_{25}	LC_{50}	LC_{90}	Toxicity Index	slope
		0.05	**0.1**	**0.2**	**0.4**						
6 hrs	**Rutin**	67.15	78.87	87.68	93.53	0.0018	0.0057	**0.0212**	0.2533	**100**	**1.19**
	Niclosamide	37.92	48.54	59.26	69.33	0.0041	0.0196	**0.1089**	2.9154	19.47	**0.9**
24 hrs	**Rutin**	50.23	72.11	81.93	89.23	0.0019	0.0069	**0.0288**	0.4375	**100**	**1.09**
	Niclosamide	45.56	58.15	69.95	79.96	0.0039	0.0146	**0.0638**	1.0491	45.14	**1.05**
48 hrs	**Rutin**	80.32	91.89	97.36	99.35	0.0033	0.0072	**0.0169**	0.0863	**100**	**1.81**
	Niclosamide	50.31	63.84	75.82	85.24	0.0038	0.0128	**0.0492**	0.64	34.35	**1.15**
72 hrs	**Rutin**	81.87	91.23	96.39	98.71	0.0016	0.0042	**0.0121**	0.0891	**100**	**1.48**
	Niclosamide	56.75	69.36	80.01	88.06	0.0025	0.0088	**0.0352**	0.4953	34.38	**1.12**

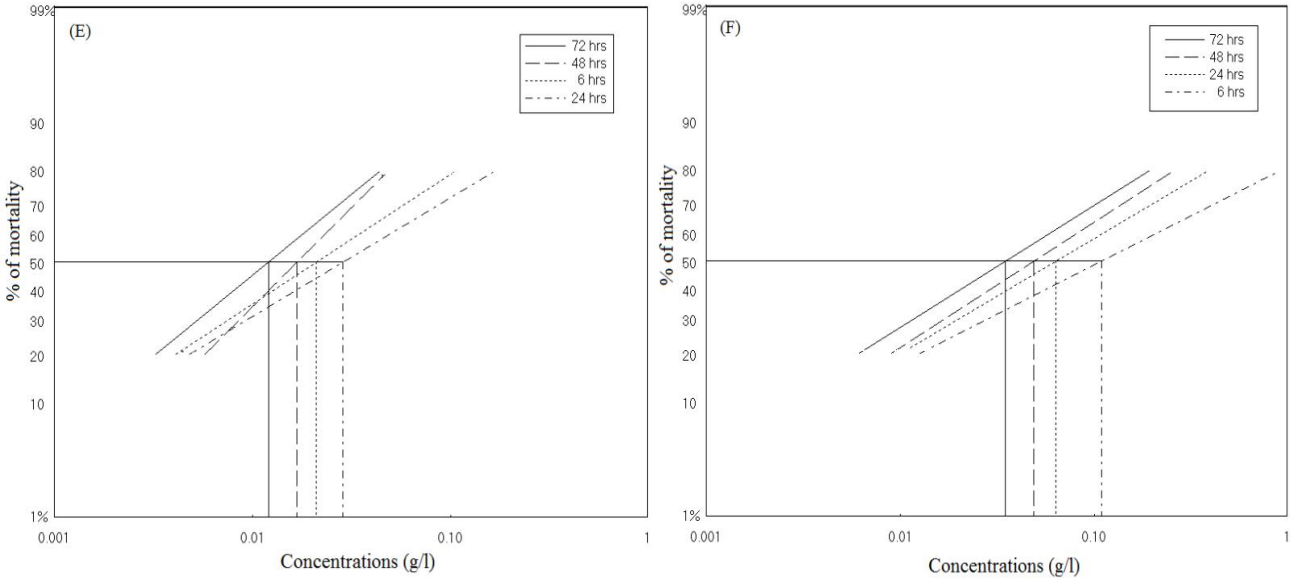


Figure (4): The ldp-lines of the rutin (E) and niclosamide (F) against *Lymanea* at different time intervals of treatments.

DISCUSSION

Medicinal plants are the most important source of drugs for the majority of the world's population. Plant secondary metabolites are important naturally occurring substances from renewable sources, which can be used as drugs, fragrances, pigments, food additives and pesticides **(Khan *et al.*, 2009)**.

C. officinalis L. is known for its biological activity, namely antibacterial, antioxidant, inflammatory etc. **(Amirghofran *et al.*, 2000)** and is widely used in pharmacy. **Bilia *et al.*, (2001)** identified narcissin, rutin, isoquercitrin, quercetin-3-O-rutinosylrhamnoside, isorhamnetin-3-O-rutinosyl-rhamnoside, isorhamnetin-3-O-glucosylglucoside and isorhamnetin-3-O-glucoside in *C. officinalis* flowers.

In the last decade *C. officinalis* is used in tinctures and creams and is valuable for positive effect on skin diseases **(Fonseca *et al.*, 2010; Bernatoniene *et al.*, 2011).** The flowers of *C. officinalis* are used for medicinal purposes. Nevertheless, it is believable that also other parts of plants may possess various biological activities. Methanol and ethanol exhibit similar properties as extraction solvents for phenolic compounds. Aqueous alcohols are used in order to obtain higher recoveries of polyphenols, particularly their glycosides.

These previous results were matching according to **Borai *et al.*, (2005)** who mentioned and proved that eco-friendly extracts of *Calendula officinalis* flowers used to control *Schistosomiasis* snails are safely without any toxic action in the environment as they stated that *Calendula* was tested before for toxicity at Albino rats. So we can apply the optimum concentrations of these highest crude extracts to take them into applied field control of snails.

Also, as shown in bioassay table, we can recommended that application of the used flower extract for finally exposure time up to 72 hours was found to be the best preferred extracts used in comparison niclosamide used. These results matched with **El-Sawy *et al.*, (1991)** who mentioned that exposure of both *Schistosoma haematobium* and *Schistosoma mansoni* to more concentrations of some plant extracts give much efficiency in snails control rather than pesticides used in its control due to the fact that these plants are considered to be used in medical field and they are friendly environmental.

Thus, both *Schistosomiasis* and *Fasciolasis* are diseases which remain the major health problem due to the lack of vaccines, the failure to eradicate the mollusc vector and the recent development of parasite resistance to anti-Schistosome drugs **(Khalife *et al.*, 2000).**

In Egypt, niclosamide has been in use as molluscicides since 1960. At low concentration, it is highly toxic to snails and their egg masses **(Green *et al.*, 1996).** This study aimed to evaluate the biological activity of different extracts of rutin extracted from *Calendula officinalis* flowers in control of each *Biomphalaria alexandrina, Bulenus truncatus* and *lymanea* snails by comparison with the usual molluscicides used (niclosamide).

Thus, Our results showed that high efficiency of Rutin extracted from *Calendula officinalis* flowers in the control of each *Biomphalaria alexandrina, Bulenus truncatus* and *lymnaea* snails in comparison with the niclosamide used. These were in agreement with that mentioned by **Mostafa and Tantawy (2000),** who stated that the survival rate of *B. alexandrina* snails maintained in aqueous solutions of *Calendula micrantha* decreased gradually with time.

Also results obtained from tables (3, 4 and 5) were agreed with those obtained by **Rawi *et al.*, (1996),** who stated that both leaves and flowers of calendula plant exhibited marked potency in killing the snail vectors of *schistosomiasis* and the recorded values showed that *C. officinalis* was more toxic and fetal to these snails.

Results obtained from all previous tables showed also that the various four concentrations and the pesticide applied gave us mortality near to those obtained from medical plants applied but according to each (**EL-Herrawie *et al.*, 1991; Kuumar *et al.*, 1993; Cerone *et al.*, 1995 and Borai *et al.*, 2005)** who all agreed , reported and mentioned the fact that these pesticides are all toxic at their any applied doses due to their residues in our bodies, their accumulations because they are not analyzed in our environment.

In conclusion field application of rutin compound separated from *Calendula officinalis* flowers have the highest ability rather than the applied pesticide in control of each *Biomphalaria alexandrina, Bulenus truncatus* and *lymanea* snails which are responsible for acute or chronic diseases called *Schistosomiasis* and *Fasciolasis* so these extracts can be used safely without any toxicity in environmental use.

These results obtained from the three mentioned tables were confirmed to **(Perez-carron *et al.*, 2002)** who reported that *Calendula* flower extracts are considered to be used in many medical fields such as curing of infectious diseases, inflammations, toxic effects of some drugs, wound healing, antiviral treatment and may be in case of cancer diseases.

So finally field applications of rutin compound separated from *Calendula officinalis* flowers can be used safely without any toxicity in the environment as Calendula was tested before for toxicity at Albino rats, **(Borai *et al.*, 2005)** who mentioned and proved that formulated extracts of *Calendula officinalis* flowers were safe and have no toxic action on albino rats and which are represented to have high potency in control of each *Biomphalaria alexandrina,* and *Bulenus truncatus* snails which are responsible for *Schistosomiasis* in Egypt.

Corresponding author

El-Sheikh, Y. W. A
Pesticide Formulations Department, Central Agricultural Pesticides Laboratory, Agricultural Research Center, Dokki, Egypt
yasserwahied@yahoo.com

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