Phytochemical screening and molluscicidal effects of polyphenols and saponins extracted from different organs of *Euphorbia helioscopia*.

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ABSTRACT

Molluscs, such as snails and slugs, can be detrimental to various crops and plants, causing significant economic losses. The control of these phytophagous molluscs often relies on the use of synthetic molluscicides, which can have adverse effects on the environment and human health. Therefore, there is a growing interest in exploring natural alternatives for mollusc control. This study aimed to investigate the active molluscicidal components present in various organs of *Euphorbia helioscopia* from Morocco. A preliminary phytochemical screening was conducted, revealing the presence of polyphenols in leaves, flowers, and stems, while saponins were found exclusively in the roots. Alkaloids were absent in all plant parts. The highest yield of polyphenols was obtained from the stems, followed by flowers and leaves. The extraction of saponins resulted in a lower percentage yield. The molluscicidal activity of the extracts was evaluated against two terrestrial phytophagous molluscs, *Theba pisana* and *Arion hortensis*, following World Health Organization (WHO, 1965) guidelines. The results showed that polyphenols from *E. helioscopia* stems exhibited the highest toxicity against *Arion hortensis* slugs, followed by polyphenols from flowers, saponins from roots, and polyphenols from leaves. Similarly, for *Theba pisana* snails, the most toxic extracts were polyphenols from stems, followed by polyphenols from flowers, polyphenols from leaves, and saponins from roots. These findings highlight the potential of *E. helioscopia* extracts as natural molluscicides, demonstrating their effectiveness against the tested molluscs.

Key words: *Euphorbia helioscopia*, phytochemistry, biocontrol, polyphenols, saponins.

1. Introduction

Weeds are commonly regarded as undesirable plants that compete with cultivated crops for resources, space, and light. However, in recent years, there has been growing interest in exploring the potential of weeds for various scientific applications, particularly in the field of biopesticides. Biopesticides are a type of pesticide derived from natural sources such as plants, animals, bacteria, and fungi. They offer an environmentally friendly alternative to conventional chemical pesticides, with reduced harmful effects on non-target organisms and the ecosystem as a whole [1].

As a result, the quest for safer and more environmentally friendly tools and products, both in agriculture and for human use, has emerged as a significant field of scientific research. Increasing numbers of scientists are now actively engaged in exploring alternatives to synthetic pesticides available in the market. These synthetic pesticides are often characterized by their poor solubility in water and slow degradation in soil, leading to potential environmental issues [2,3].

The *Euphorbiaceae* family exhibits widespread molluscicidal activity, although the specific activity can vary among different species and even different parts of the same plant. Extensive studies have demonstrated the remarkable molluscicidal activity of Euphorbia helioscopia, making it a highly interesting candidate for further investigation [2,3, 4-5].

The *Theba pisana* snail is considered a serious pest that causes damage to crops. It has the ability to gather in large numbers, with up to 3000 snails found on a single tree [6]. This snail is capable of defoliating large trees, including citrus and ornamental plants. It also feeds on garden crops, seedlings, and grains such as wheat, barley, oilseeds, carrots, and legumes [7]. In cereal production areas, this species causes both direct and indirect losses. Direct losses include waterlogging of machinery and direct consumption of the crop. Indirect losses manifest through grain contamination and the possibility of infestation by secondary fungal pathogens due to the additional moisture they provide [8].

On the other hand, *Arion hortensis* is a highly destructive slug that affects winter wheat, rapeseed, sugar beet, and potatoes worldwide, leading to significant losses. However, in central Europe, these same crops are attacked less frequently or with less severity [9-10].

In this work, results of phytochemical screening of *E. heliocopia*, extraction of its polyphynols and saponins as well as toxicity of these extracts against to two phytophagous molluscs, *Theba pisana* and *Arion hortensis* will be presented.

2. Methodology

2.1. *Euphorbia helioscopia* plant

Euphorbia helioscopia plants were collected from the Oued Beht region near Khemisset, Morocco, in February 2015. The precise GPS coordinates of the collection site are 33° 53’2.538" N; 5° 55’ 41.413’’W, with an elevation of 190.4 meters above sea level. The region is characterized by a semi-arid climate, with cold winters. The annual temperatures in the area range between 15 and 19 °C, depending on the altitude and continentality factors (Administration de l’Hydraulique, 1991; Lakhili et al., 2015).

The plant specimens were identified by the Scientific Institute of Rabat, and a voucher specimen was filed under the number RAB091057.

For the plant drying process, the collected plants were dried in the shade until a stable weight was achieved. This drying process took approximately twenty days and was conducted in a well-ventilated area, ensuring that the temperature did not exceed 35 °C.

2.2.*Theba pisana* and *Arion hortensis* strains

Adults of *Theba pisana* were collected in the Meknes region of Morocco, with the precise GPS coordinates being 32°17'41.2"N; 3°59'59.3"W. On the other hand, specimens of Arion hortensis were sampled in the Dar El Guedari province of Kenitra, Morocco, with GPS coordinates of 34°25'54.4"N; 6°04'29.8"W. The taxonomic identification of both species was confirmed by the Department of Plant Protection and Environment at the National School of Agriculture in Meknes, Morocco.

2.3.Phytochemical Screening

Phytochemical screening was conducted to perform various chemical analyses, including the detection of alkaloids, tannins, flavonoids, anthracenitic derivatives, sterols and triterpenes, saponins, reducing compounds, oses, holosides, and mucilages. This qualitative analysis relies on coloration and/or precipitation reactions. The screening was carried out using both

dried and fresh plant materials, following the methodology described by [11]. Table 1 provides an overview of the chemical groups investigated and the specific reagents employed.

Based on the results of the phytochemical studies, the extraction of polyphenols was performed from the leaves, flowers, and stems, while saponins were extracted from the roots of *Euphorbia helioscopia*.

Tableau 1: Specific reagents and reactions of phytochemical screening [11]

Chemical groups		**Specific reagents**	**Specific reactions**
Alkaloids		Dragendorff. (Potassium tetraiodobismuthate**)**	Orange coloration with the appearance of precipitate.
Polyphenolic compounds	Tannins	Stiasny reaction ($FeCl_3$)	A greenish or bluish-black coloration
	Flavono ids	reaction with cyanidin	Orange-pink coloration; pink-violet or red.
Quinonic compounds	Coumarins	Bornträger- UV reaction	Intense inflorescence
Saponins		Determination of Foam Index (FI*)	Positive test if FI > 100 intense foam.
Sterols and triterpenes		Libermann-Burchard (Acetic anhydride - H_2SO_4)	The appearance at the interphase of a purple or violet ring, changing to blue and then green
Reducing compounds		Fehling's solution test	Burgundy red precipitate
Anthracenic derivatives	free anthraquinones	**(**Chloroform-NH_4OH)	More or less red coloration
	O-heterosides	(HCL Concentrated - NH_4OH)	Red coloration, more or less dark
	C-heterosides	**(**$FeCl_3$ - NH_4OH)	More or less intense red coloration
Oses and holosides		(H_2SO_4, Saturated ethanol with thymol)	Red coloration
Mucilages		Adding absolute ethanol to the 10% decoction.	Formation of a fluffy precipitate by mixing

2.4. Polyphenols extraction

Extraction was performed following the method described by [12]. Powdered leaves, flowers, and stems of Euphorbia helioscopia (100 grams for each plant part) were initially subjected to hexane extraction using a Soxhlet apparatus for 6 hours at 65 °C to remove fats. Subsequently, 25 grams of defatted powder from each plant part were individually subjected to a second extraction using a Soxhlet apparatus for 12 hours at 60 °C with 250 ml of solvent (methanol or ethanol). After extraction, the solvent was evaporated using a vacuum rotary evaporator at 40 °C. The crude extracts were collected in small dark sterile flasks and stored at 4 °C.

2.5. Saponins extraction

Saponins were extracted using the method developed by [13]. The ground material of *Euphorbia helioscopia* roots was delipidated for two hours with 250 ml of pure n-hexane. After removing the organic phase, the resulting precipitate was macerated in 300 ml of absolute ethanol with magnetic stirring at room temperature for 24 hours. The ethanolic phase was then evaporated to dryness under vacuum at 40 °C using a rotary evaporator. The dry residue was extracted three times with a mixture of 100 ml distilled water and petroleum ether (v:v) heated at 50 °C in a water bath for 30 minutes. The aqueous phases were combined and transferred into 150 ml of n-butanol for analysis, allowing it to sit for 30 minutes. The organic phase was evaporated to dryness at 40 °C using a rotary evaporator, weighed, and dissolved in 1% ethanol for biological tests.

After each purification step, the extracts intended for the biological tests were evaporated to dryness, and the obtained residue was weighed. The yield, expressed as a percentage relative to the weight of the starting material, was determined using the following equation: $Y = (Wc - We) * 100 / Q$, where:

Y: Yield (in %)

Wc: Weight of the balloon with contents (in g)

We: Weight of the empty balloon (in g)

Q: Weight of the starting plant material (in g) (25g for saponins and 60g for polyphenols)

2.6. Molluscicidal activity

Toxicity evaluation of *Euphorbia helioscopia* extracts was conducted following the guidelines set by the World Health Organization [14]. Adult snails and slugs of similar age and size were selected for the experiments, using a test procedure based on the study by [15].

Homogeneous lettuce leaf discs were soaked in a series of concentrations (25, 50, 100, and 200 ppm) of the saponin/polyphenol solutions for 30 minutes and then allowed to dry. These treated lettuce discs were then placed in boxes, with each box containing 10 snails or 10 slugs. Three replicates were performed for each concentration. Untreated lettuce discs were used as a control treatment. The experiments were conducted with an exposure period of 48 hours at temperatures ranging from 20°C to 25°C.

Percentage mortalities were recorded 48 hours after the treatments. Daily observations were made until all individuals in the treated groups had died. Dead individuals were counted and removed from the boxes. An individual was considered dead if it did not move upon tactile stimulation of the operculum and body with a brush. Additionally, for both species, the animal's body dilated after death.

2.7.Data analysis

To assess the toxicity of different extracts of *E. helioscopia* to snails and slugs in this study, survival curves were constructed and compared using the Logrank test, as described by [16]. The Logrank test follows a chi-square distribution with one degree of freedom. Any treatment with a chi-square value less than 3.841 was considered not significantly different. Microsoft Excel version 2013 software was used for data analysis.

Lethal doses LD50 and LD99, which represent the doses required to kill 50% or 99% of the tested population after 15 days for slugs and 30 days for snails, were determined using the Probit method developed by [17]. Confidence intervals for these lethal doses were also calculated. Biostat Pro version 2015 software was utilized for this analysis.

Lethal times LT50 and LT99 were calculated as the time at which 50% and 99% of the population died, respectively. These values were derived from the equation of the straight line fitted to the cumulative mortality data plotted against the duration of exposure to molluscs, following the approach described by [18].

3. Results and discussion

3.1.**Phytochemical screening**

Phytochemical screening reactions have allowed us to identify the presence of certain chemical substances. Based on our results, the following observations were made:

- Alkaloids were absent in all parts of the *Euphorbia helioscopia* plant.
- Tannins were present in the leaves, stems, and flowers, with varying levels in the roots.
- Flavonoids, particularly flavones, were found only in the stems and roots. Flavanols and flavanonols were detected in leaves and flowers.
- Lencoanthocyans were present in all parts of the plant.
- C-heterosides were identified in all four organs of *Euphorbia helioscopia*.
- O-heterosides were absent in roots, and free anthraquinones were not detected in any part of the plant.
- Sterols and tri-terpenes were present in all plant parts examined.
- However, reducing compounds and mucilages were found to be absent.
- Saponosides were present exclusively in the roots of Euphorbia helioscopia (Table 2).

These findings provide insights into the chemical composition of *Euphorbia helioscopia* and the distribution of various chemical compounds across its different organs.

Table 2: Summary of phytochemical screening results of leaves, stems, flowers and roots of *E. helioscopia*.

Chemical Group			Roots	Leaves	Stems	Flowers
Alkaloids			**-**	**-**	**-**	**-**
Polyphenolic compounds	**Tannins**		**+**	**+**	**+**	**+**
		Catechetical Tannins	**-**	**+**	**+**	**+**
		Gallic Tannins	**+**	**+**	**+**	**+**
Flavonoids	**Anthocyanins**		**-**	**-**	**-**	**-**
	Flavones		**+**	**-**	**+**	**-**

	Flavanols et flavanonols	**-**	**+**	**-**	**+**
	Lencoanthocyans	**+**	**+**	**+**	**+**
Anthracene derivatives	**Free anthraquinones**	**-**	**-**	**-**	**-**
	O-heterosides	**-**	**+**	**+**	**+**
	C-heterosides	**+**	**+**	**+**	**+**
Sterols and terpenes		**+**	**+**	**+**	**+**
Saponosides		**+**	**-**	**-**	**-**
Reducing compounds		**-**	**-**	**-**	**-**
Mucilages		**-**	**-**	**-**	**-**

According to [19], *Euphorbia helioscopia* contains diterpenoid esters of jatrophan, specifically helioscopianoides A - Q, as well as euphornin N [20, 21, 22, 23- 24]. Chemical analysis of polyphenols in all parts of *E. helioscopia* has identified the presence of four hydrolysable tannins known as helioscopins A and B [25, 26- 27]. Other studies have reported the presence of flavonoids and tannins [22, 28- 29], glycosides such as quercetin-3-pglucoside, quercetin-3-β-galactoside, quercetin-3-β-galactoside-2"-galla [30], and aryl glycoside, 300-O-galloyl-benzyl-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside [31, 32- 33]. Steroids, lipids [23- 31], and other secondary metabolites such as 24-methylene cycloartanol, 24-methylenecycloart-3-one, cycloartanol, and stigmast-4-ene-3-one have also been identified [34].

The diversity of secondary metabolites in *E. helioscopia* may explain its various applications in different fields, particularly its effects on plant pests, including phytophagous molluscs. Phytochemical screening, utilizing specific assays, has allowed for the characterization of polyphenols, flavonoids, and saponins in the various parts of E. helioscopia. These secondary metabolites exhibit significant toxicological effects at certain doses. These findings are still preliminary, highlighting the importance of further studies involving the extraction of these active compounds and evaluating their toxicity against molluscan pests.

3.2.**Extraction of polyphenols and saponins**

According to statistical analysis, the type of plant material had a significant impact on the yield of polyphenols (**Table 3**). The highest yield was observed in *E. helioscopia* stems (22.9 ± 0.004%), followed by flowers (21.9 ± 0.009%) and leaves (19.8 ± 0.012%). Saponin yield was the lowest, with a percentage of 0.63 ± 0.48%.

Table 3: Polyphenol and saponins yield extracted from *E. helioscopia*.

	yield (%)	**E-type**	**ES**	**IC**
P**olyphenols**				
E. helioscopia leaves	19.8	0.012	0.007	0.013
E. helioscopia flowers	21.7	0.009	0.005	0.010
E. helioscopia stems	22.9	0.004	0.002	0.004
Saponines				
E. helioscopia roots	0.63	0.477	0.276	0.540

According to [35], the extraction yields of polyphenols from *E. helioscopia* were found to be 10.64%, 19.20%, and 13.68% for stems, flowers, and leaves, respectively. Some studies have reported higher levels of polyphenols in the leaves compared to other plant organs [36]. Additionally, the extraction of galactoside from quercetin, later known as tithymalin, resulted in a yield of 46.93% [28-33]. Other compounds such as hydrocarbons yielded 9.5%, aldehydes 8.9%, and sterols 1.4% [37].

Polyphenol yields are significantly influenced by various factors, including genetic factors such as plant species, plant organs, phenological stage, and environmental factors such as soil and climate conditions. Biotic and/or abiotic stresses during plant growth can also impact polyphenol content [38, 39, 40, 41-42]

3.3.Molluscicidal activity

Responses of individual snails and slugs to the various concentrations of polyphenols and saponins studied are summarized in the form of survival curves (Figure 1 and 2). All tested polyphenols resulted in significantly higher mortality rates compared to the control groups. In fact, the toxicity exhibited by these molluscs increased with higher concentrations of the applied products, leading to shorter survival times with prolonged exposure.

In the treated groups, at a concentration of 200 ppm, snail longevity ranged from 1 to 3 days for polyphenols extracted from leaves, 1 to 2 days for stems, and 1 to 4 days for both polyphenols extracted from flowers and saponins extracted from the roots of *E. helioscopia*.

In contrast, the control groups showed stable longevity throughout the experiment. Overall, the longevity of snail individuals in all groups was statistically comparable (Figure 1).

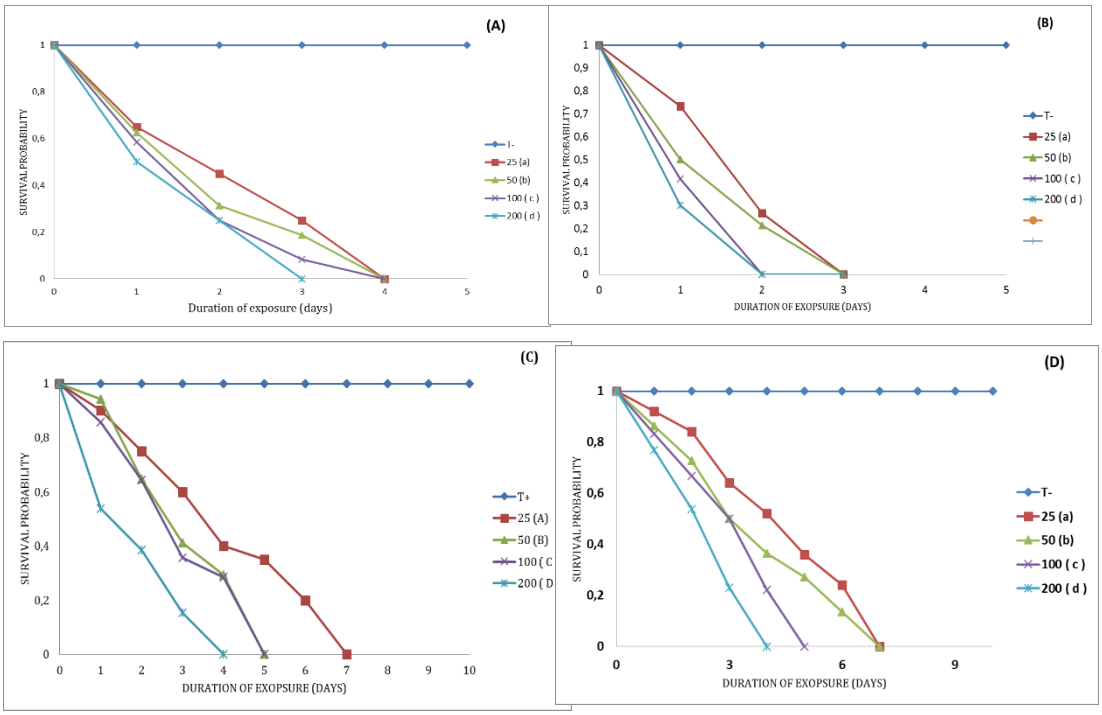

Figure 1: Survival curve of adults of *Theba pisana* treated with polyphenols extracted from leaves, stems and flowers and saponins extracted from roots of *Euphorbia helioscopia*. (Concentrations affected by the same letter do not show statistically significant differences between them (Logrank test; $P \leq 0{,}05$; $\chi^{2} > \chi^{2}_{(0,\,05\,;\,1)} = 3{,}84$)). (**A**): Polyphenols extracted from leaves of *E. helioscopia* against *T. pisana* (adult), (**B**): Polyphenols extracted from stems of *E. helioscopia* against *T. pisana* (adult), (**C**): Polyphenols extracted from flowers of *E. helioscopia* against *T. pisana* (adult), (**D**): Saponins extracted from roots of *E. helioscopia* against *T. pisana* (adult)

In the case of adult slugs, the application of polyphenols extracted from the leaves, stems, and flowers of *E. helioscopia*, as well as saponins extracted from its roots, resulted in

significantly higher mortalities compared to the negative control group (Figure 2).

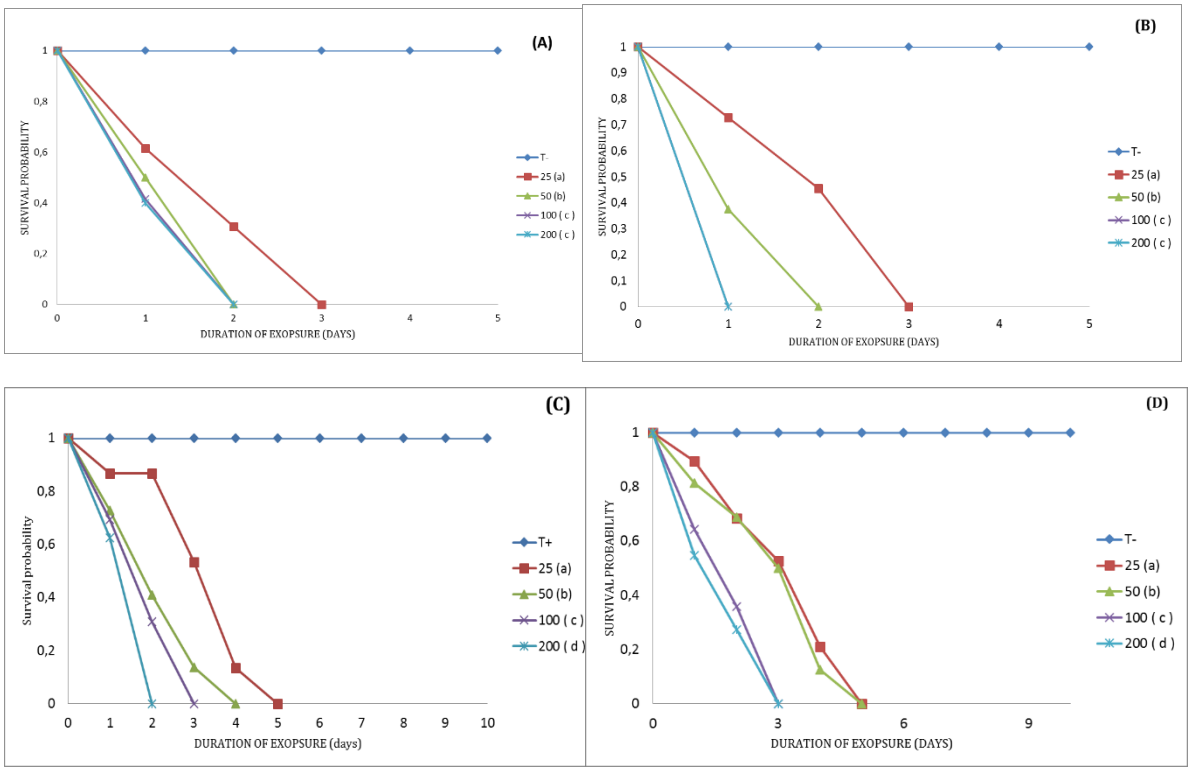

Figure 2: Survival curve of A*rion hortensis* adults treated with polyphenols extracted from leaves, stems and flowers and saponins extracted from roots of *E. helioscopia*. (Concentrations affected by the same letter do not show statistically significant differences between them (Logrank test at $P \leq 0.05$; $\chi^2 > \chi^2$ (0, 05; 1) = 3.84). (A): Polyphenols extracted from leaves of *E. helioscopia* against *A. hortensis* (adult); (B): Polyphenols extracted from stems of *E. helioscopia* against *A. hortensis* (adult); (C): Polyphenols extracted from flowers of *E. helioscopia* against *A. hortensis* (adult); (D): Saponins extracted from roots of *E. helioscopia* against *A. hortensis* (adult).

Survival times for 50% of adult snails and slugs exposed to different concentrations of polyphenols ranged from less than 24 hours to about 8 days for snails and from less than 24 hours to about 4 days for slugs, depending on the concentration and the specific mollusc species considered. In the control group, adult snails survived throughout the entire duration of the test period (Tables 4 and 5).

Table 4: LT_{50} and LT_{99} of *T. pisana* adults treated with polyphenols extracted from leaves, stems, flowers and saponins extracted from roots of *E. helioscopia*.

Product	Concentration (ppm)	Equation	R^2	LT_{50} (days)	r	LT_{99} (days)	r
Polyphenols extracted from leaves of *E. helioscopia*	25	18,95x + 9,84	0,96	2,45	0,91	5,43	0,90
	50	18,47x + 18,25	0,89	1,72		4,37	
	100	17,81x + 26,03	0,79	1,35		4,10	
	200	23x + 20	0,84	1,30		3,43	
Polyphenols extracted from stems of *E. helioscopia*	25	23,66x + 12,67	0,93	1,58	0,92	3,65	0,89
	50	23,67x + 16,67	0,88	1,41		3,48	
	100	32,33x + 12,33	0,91	1,17		2,68	
	200	32,33x + 15,67	0,86	1,06		2,58	
Polyphenols extracted from flowers of *E. helioscopia*	25	11,78x + 12,26	0,93	3,20	0,91	7,36	0,91
	50	14,64x + 15,12	0,93	2,38		5,73	
	100	13,93x + 22,98	0,84	1,94		5,46	
	200	17,62x + 24,28	0,81	1,46		4,24	
Saponins extracted from roots of *E. helioscopia*	25	11,83x - 0,296	0,99	4,25	0,95	8,39	0,97
	50	11,72x + 10,52	0,97	3,37		7,55	
	100	14,76x + 13,81	0,94	2,45		5,77	
	200	17,43x + 21,43	0,85	1,64		4,45	

In the case of polyphenols and saponins extracts, the lethal time (LT_{50} and LT_{99}) decreases as the concentration increases. These two parameters are negatively correlated, as shown in Tables 4 and 5. For snails, the LT_{50} decreased from 2.45 to 1.30, 1.58 to 1.06, 3.20 to 1.46, or 4.25 to 1.64 days, and the LT_{99} decreased from 5.43 to 3.43, 3.65 to 2.58, 7.36 to 4.24, or 8.39 to 4.45 days, respectively, for polyphenols extracted from leaves, stems, flowers, and saponins extracted from roots of *E. helioscopia* (Table 4).

On the other hand, for slugs, the LT_{50} increased from 1.42 to 1.30, from 1.36 to 0.76, from 2.59 to 1.04, or from 3.99 to 1.27 days, and the LT_{99} increased from 3.55 to 2.46, from 3.59 to 1.74, from 5.58 to 2.62, or from 7.23 to 3.43 days, respectively, for polyphenols extracted from leaves, stems, flowers, and saponins extracted from roots of *E. helioscopia* (Table 5).

Table 5: LT_{50} and LT_{99} of *Arion hortensis* adults treated with polyphenols extracted from leaves, stems and flowers and saponins extracted from roots of *E. helioscopia*.

Product	Concentration (%)	Equations	R^2	LT_{50} (days)	r	LT_{99} (days)	r
Polyphenols extracted from leaves of *E. helioscopia*	25	23x + 17,33	0,88	1,42	0,91	3,55	0,73
	50	32,33x + 12,33	0,91	1,17		2,68	
	100	31,33x + 18	0,82	1,02		2,59	
	200	31x + 22,67	0,74	0,88		2,46	
Polyphenols extracted from stems of *E. helioscopia*	25	22x + 20	0,83	1,36	0,84	3,59	0,80
	50	31,67x + 18,33	0,82	1,00		2,55	
	100	50x + 11,11	0,87	1,17		1,76	
	200	50x + 12,22	0,85	0,76		1,74	
Polyphenols extracted from flowers of *E. helioscopia*	25	16,43x + 7,38	0,98	2,59	0,92	5,58	0,96
	50	18,09x + 15,87	0,91	1,89		4,59	
	100	23x + 16,67	0,88	1,45		3,58	
	200	31x + 17,67	0,82	1,04		2,62	
Saponins extracted from roots of *E. helioscopia*	25	15,12x + 10,36	0,96	3,99	0,78	7,23	0,72
	50	14,64x + 17,98	0,90	3,12		5,53	
	100	23x + 15,33	0,90	1,51		3,64	
	200	22,67x + 21,33	0,82	1,27		3,43	

To assess the toxicity levels of the four tested extracts on the two cohorts of *T. pisana* and *A. hortensis*, lethal concentrations were calculated. The toxicological parameters of these extracts are summarized in Tables 6 and 7. The LC_{50} values ranged from approximately 20.98 to 39.66 ppm, while the LC_{99} values ranged from approximately 115.50 to 374.41 ppm, depending on the plant parts from which the extracts were obtained.

Table 6: Toxicity parameters of polyphenols extracted from leaves, stems, flowers and saponins extracted from roots of *E. helioscopia* against *Theba pisana* snail.

Extact from *E. helioscopia* organ/animal	Slope ± SE	LD_{50} (g/100ml) [IC]	LD_{99} (g/100ml) [IC]	χ^2 ($\chi^2_{(0.05;1)}$ = 3.84)
Polyphenols from leaves/ *T. pisana*	1.04 ± 0.45	24.45 [4.89; 67.92]	374.41 [-]	0.11
Polyphenols from stems/ *T. pisana*	1.17 ± 0.53	15.83 [1.83; 63.88]	115.50 [-]	0.04
Polyphenols from flowers/ *T. pisana*	1.80 ± 0.56	20.98 [4.89; 36.98]	193.56 [49.92; 201.07]	0.06
Saponins from roots/ *T. pisana*	1.80 ± 0.56	39.66 [20.79; 67.04]	186.42 [73.27; 651.26]	0.18

On the other hand, *for the A. hortensis* population, the LD_{50} values ranged from approximately 12.50 to 57.79 ppm, while the LD_{99} values ranged from about 85.48 to 449.01 ppm, depending on the extracts from different parts of *E. helioscopia*.

Various extracts from different organs of *E. helioscopia*, particularly polyphenols extracted from stems and flowers, demonstrated potential molluscicidal properties against both *T. pisana* and *A. hortensis*. The toxic effects of these plant parts were found to be dependent on both the dosage and duration of exposure.

In terms of LD_{50} values and slope of the dose-response curves, snails generally exhibited higher tolerance to polyphenols and saponins compared to slugs (refer to Table 6 and 7).

Tableau 7: Toxicity parameters of polyphenols extracted from leaves, stems, flowers and saponins extracted from roots of *Euphorbia helioscopia* against *A. hortensis slug*.

Extact from *E. helioscopia* organ/animal	Slope ± SE	LD_{50} (g/100ml) [IC]	LD_{99} (g/100ml) [IC]	χ^2 ($\chi^2_{(0.05;1)}$ = 3.84)
Polyphenols from leaves/ *T. pisana*	4.00 ± 1.33	57.79 [11.19; 218.95]	449.01 [-]	6.86
Polyphenols from stems/ *T. pisana*	2.25 ± 0.74	12.50 [1.88; 23.39]	85.48 [55.73; 395.03]	0.03
Polyphenols from flowers/ *T. pisana*	1.85 ± 0.54	34.30 [15.38; 53.61]	212.75 [116.79; 595.03]	0.12
Saponins from roots/ *T. pisana*	1.43 ± 0.44	36.76 [08.31; 144.63]	160.05 [-]	0.30

For the two molluscs, when comparing the LD_{50} values of the four applied extracts (Tables 6 & 7), it is evident that polyphenols extracted from *E. helioscopia* stems (12.50 ppm) exhibit the highest toxicity against slugs, followed by polyphenols extracted from flowers (34.30 ppm), saponins extracted from roots (36.76 ppm), and polyphenols extracted from leaves

(57.79 ppm). Similarly, for snails, polyphenols extracted from *E. helioscopia* stems (15.83 ppm) also demonstrate the highest toxicity, followed by polyphenols extracted from flowers (20.98 ppm), polyphenols extracted from leaves (24.45 ppm), and saponins extracted from roots (39.66 ppm).

All tested concentrations of *E. helioscopia* extracts resulted in significantly higher mortality compared to the negative control. Therefore, the polyphenols and saponins studied exhibited acute toxicity against the two targeted mollusc pests. The molluscicidal properties of various species of *Euphorbiaceae* have indeed been extensively investigated, utilizing different parts of the plants and employing different extraction processes [4, 43-44].

This study confirms the findings reported in our previous study in 2018 [45], which examined the adult snail population of Theba pisana. Pellets based on stems of E. helioscopia (LD50 = 1.35 g/100ml) and leaves (LD_{50} = 1.39 g/100ml at 2% agar) were found to be more toxic compared to those based on roots and flowers, which had no noticeable effects. In the case of *A. hortensis* slugs, pellets based on E. helioscopia leaves (LC_{50} = 1.14 g/100 ml at 2% agar) exhibited higher toxicity than those based on stems (LC_{50} = 1.33 g/100 ml at 2% agar), flowers (LC_{50} = 1.75 g/100 ml at 2% agar), and roots (LC_{50} = 1.98 g/100 ml at 2% agar).

Furthermore, extracts from both *E. helioscopia* and *E. Schimperiana* showed promising results as molluscicides. The methanol extract of dry leaves from *E. helioscopia* demonstrated an LD_{50} of 50.8 ppm and an LD_{99} of 68.2 ppm [4]. [46-47] reported higher activity than [4] using acetone extracts from the same plant.

A study by [3] demonstrated that extracts of *Euphorbia schimperiana* and *Euphorbia helioscopia* exhibit strong molluscicidal activity against the snail *Bulinus wrighti*. Furthermore, [48-49] reported that aqueous extracts of *Euphorbia lactea cristata*, *E. Royleana*, *E. Antisyphlitica*, and *Jatropha gossypifolia* were toxic to snails, specifically *Lymnaea acuminata* and *Indoplanorbis exustus.*

Research conducted by [50] focused on the aqueous extracts of *Euphorbia myrsinites L.* (*Euphorbiaceae*) and their molluscicidal activity against *Biomphalaria glabrata*. The stem and leaf extracts exhibited LD_{50} values of 15.1 and 8.9 ppm, respectively, which fall within the effective molluscicide limits set by the WHO. There is a wide range of plants containing

compounds that are toxic to both targeted and non-targeted organisms, often at lower doses than synthetic pesticides [51, 52, 53, 54, 55, 56, 58, 59, 60, 61].

The advantage of using such products is that they may contain biodegradable compounds, reducing the likelihood of environmental contamination. We strongly believe that if these *Euphorbiaceae* products were employed as molluscicides, they would not only effectively control gastropod pests but also offer the advantages of accessibility, affordability, rapid biodegradability, and safety.

Active ingredients of biomolluscicides are secondary metabolites extracted from plants, such as saponins, alkaloids, and polyphenols including tannins and flavonoids. A study by [62] reported the presence of a flavonoid called quercetin in Polygonum senegalense leaves, which exhibited significant molluscicidal activity at 10 ppm, resulting in 100% mortality of three snail species (*Lymnaea natalensis, Biomphalaria peifferi*, and *B. glabrata*) within 24 hours. Similarly, the compound eupatorine isolated from the Baccharis timera plant was lethal to *Biomphalaria glabrata* at 100 ppm. However, other glycosides derived from Asparagus plumosus were found to have no harmful effects on snails [53-59].

In Ethiopia, the molluscicidal activity of saponins was first observed by [63]. It was noted that areas of the river where these berries were used for washing clothes had significantly reduced snail populations. Based on this observation, a five-year pilot snail control program was initiated in Ethiopia, resulting in a significant reduction in *Schistosoma mansoni* infection rates. The compounds responsible for the molluscicidal activity are triterpenoid saponins, with LD_{100} values as low as 2 ppm [59-64]. However, a disadvantage of Euphorbia extracts is that they can be highly toxic to both vertebrates and invertebrates [3]. Nevertheless, further research on natural products derived from these plants may lead to the discovery of new compounds that could serve as the basis for future molluscicides [3].

4. Conclusion

Polyphenols and saponins derived from *E. helioscopia* demonstrate potential as an environmentally friendly alternative to synthetic molluscicides in agriculture, as they have shown oral toxicity to two common terrestrial mollusc pests, *T. pisana* and *A. hortensis*. Implementing these natural compounds could help reduce the reliance on synthetic agents, leading to a decrease in issues such as residue accumulation, resistance development, and environmental pollution.

However, further research is necessary to explore the modes of action, optimal application methods, and the influence of various physical factors on the degradation of these botanical

compounds. Additionally, it is crucial to identify and characterize these active ingredients and assess their toxicity towards the tested mollusc pests. Although our study provides promising preliminary results, further investigation is warranted to expand our understanding and practical application of these natural molluscicides.

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Références

[1] Al-Zanbagi, N,A., Banaja A.A, & Barrett, J. (2000). Molluscicidal activity of some Saudi Arabian Euphorbiales against the snail Biomphalaria pfeifferi. Journal of Ethnopharmacology. 70(2), 119-125. doi:10.1016/s0378-8741(99)00155-5

[2] El-Amin, S.M., & Osman, N.S. (1991). Squalene and Urs 12-en-28 01 from the Molluscicidal plant, Euphorbia helioscopia. Egyptian Journal Bilharzia, 13, 181-187.

[3] Al-Zanbagi, N,A., Banaja A.A, & Barrett, J. (2000). Molluscicidal activity of some Saudi Arabian Euphorbiales against the snail Biomphalaria pfeifferi. Journal of Ethnopharmacology. 70(2), 119-125. doi:10.1016/s0378-8741(99)00155-5

[4] Al-Zanbagi, N.A. (2005). Two molluscicides from Saudi Arabian Euphorbiales against Bulinus wrighti. Journal of King Abdulaziz University-Science, 17, 11-19.

[5] Harmouzi, A., Boughdad, A., El ammari, Y. & Chaouch, A. (2018). Toxicity of Euphorbia helioscopia pellets to two phytophagous molluscs, Theba pisana Müller, 1774 (Pulmonata: Helicidae) and Arion hortensis Férussac, 1819 (Pulmonata: Arionidae). Pesticidi i fitomedicina, 33(3-4), 241-252.

[6] Baker, G. H., (2002). Gastropods as pests in New Zealand pastoral agriculture, with emphasis on Agriolimacidae, Arionidae and Milacidae. Book chapter 18: Molluscs as crop pests 2002 pp.193-215.

[7] Cowie, R.H., Dillon Jr., R.T., Robinson, D.G., Smith, J.W., 2009. Alien non-marine snails and slugs of priority quarantine importance in the United States: a preliminary risk assessment. Am. Malacol Bull. 27, 113–132. https://doi.org/10.4003/006.027.0210.

[8] Rumi, A., J. Sánchez, and N. Ferrando. 2010. Theba pisana (Müller, 1774) (Gastropoda, Helicidae) and other alien land molluscs species in Argentina. Biological Invasions 12: 2985–2990.

[9] South, A. (1992). Terrestrial slugs: Biology, ecology, and control. London, UK: Chapman and Hall.

[10] Port, G., & Ester, A. (2002). Gastropods as pests in vegetable and ornamental crops in Western Europe. In: G.M. Barker (Ed.). Molluscs as crop pests (pp 337-351).Wallingford, UK: CABI Publishing.

[11] Houghton, P. & Raman, A. (1998). Laboratory Handbook for the Fractionation of Natural Extracts. Edition Number 1, Springer US. DOI 10.1007/978-1-4615-5809-5

[12] Maoulainine, B. M., Salem, M., & Boukhari, O. (2012). Antioxidant proprieties of methanolic and ethanolic extracts of Euphorbia helioscopia , (L .) aerial parts, 19(3), 1125–1130.

[13] S.W. Applebaum et al. Physiological aspects of host specificity in the bruchidae- IV. Developmental incompatibility of soybeans for Callosobruchus. Journal of Insect Physiology (1965)

[14] WHO, "Molluscicide screening and evaluation," Bulletin of the World Health Organization, vol. 33, pp. 567–581, 1965.

[15] Eshra, E. H. (2014). Toxicity of methomyl , copper hydroxide and urea fertilizer on some land snails. Annals of Agricultural Sciences, 59(2), 281–284. https://doi.org/10.1016/j.aoas.2014.11.017

[16] Kaplan, E.L., & Meier, P. (1958). Nonparametric estimation from incomplete observations. Journal of the American Statistical Association, 53(282), 457- 481. doi:10.1080/01621459.1958.10501452

[17] Finney, D.J. (1971). Probit analysis. (3rd edition) (p 333). New York, NY: Cambridge University Press.

[18] Harmouzi, A., Boughdad, A., El Ammari, Y. et al. Chemical composition and toxicity of Moroccan Tetraclinis articulata and Juniperus phoenicea essential oils against Aphis citricola Goot, 1912 (Homoptera, Aphididae). Res Chem Intermed 42, 7185–7197 (2016). https://doi.org/10.1007/s11164-016-2528-5

[19] Mai, Z., Ni, G., Liu, Y., Zhang, Z., Li, L., & Chen, N. (2018). Helioscopianoids A – Q , bioactive jatrophane diterpenoid esters from Euphorbia helioscopia, 8(5), 805–817. https://doi.org/10.1016/j.apsb.2018.03.011

[20] Geng, D., Shi, Y., Min, Z. Da, & Liang, J. Y. (2010). A new diterpenoid from Euphorbia helioscopia, 21, 73–75. https://doi.org/10.1016/j.cclet.2009.09.013

[21] Tao, H., Hao, X., Liu, P., & Zhu, W. (2008). Cytotoxic Macrocyclic Diterpenoids from Euphorbia helioscopia, 31(12), 1547–1551. https://doi.org/10.1007/s12272-001-2149-3

[22] Chen, H., Wang, H., Yang, B., Jin, D., Yang, S., Wang, M., … Guo, Y. (2014). Fitoterapia Diterpenes inhibiting NO production from Euphorbia helioscopia. Fitoterapia, 95, 133–138. https://doi.org/10.1016/j.fitote.2014.03.010

[23] Kosemura, S. (1989). From euphorbia helioscopia, 28(12), 3421–3436.

[24] Di, G., Li-tao, Y. I., Yao, S. H. I., & Zhi-da, M. I. N. (2015). Structure and antibacterial property of a new diterpenoid from Euphorbia helioscopia. Chinese Journal of Natural Medicines, 13(9), 704–706. https://doi.org/10.1016/S1875-5364(15)30069-8

[25] Lee, S.H., Tanaka, T., Nonaka, G. I. and Nishioka, I. 1990. Tannins and related compounds. XCV. Isolation and characterization of helioscopinins and helioscopins, four new hydrolyzable tannins from Euphorbia helioscopia L. (1). Chem. Pharm. Bull. 38(6), 1518-1523.

[26] Abdulladzhanova, N.G., Mavlyanov, S.M. & Dalimov, D.N. (2003a). Chemistry of Natural Compounds 39: 399. https://doi.org/10.1023/B:CONC.0000003425.70068.85

[27] Abdulladzhanova, N. G., Mavlyanov, S. M., & Dalimov, D. N. (2003b). POLYPHENOLS OF CERTAIN PLANTS OF THE EUPHORBIACEAE FAMILY, 39(4), 322–323.

[28] Kawase, A., & Kutani, N. (2014). Some Properties of a New Flavonoid , Tithymalin ~ Isolated from the Herbs of Euphorbia Helioscopia Linnaeous, 1369, 4–6. https://doi.org/10.1080/00021369.1968.10859027

[29] Ramezani, M., Behravan, J., Arab, M., and Amel Farzad, S. 2008. Antiviral activity of Euphorbia helioscopia extracts. Journal of Biological Sciences 8 (4): 809-813.

[30] Nahrstedt, A. (2014). [Flavonol glycosides from Euphorbia helioscopia , E . stricta , E . verrucosa and E . dulcis (author ' s transl)], (February), 4–7. https://doi.org/10.1055/s-0028-1097806

[31] Hang, W. Z., & Uo, Y. G. (2006). Chemical Studies on the Constituents of the Chinese Medicinal Herb Euphorbia helioscopia L ., 54(July), 1037–1039.

[32] Sheng, W., Gao, L., Ke, X., Zhi, Y., & Chen, H. (2010). A new lathyrane diterpene glycoside from Euphorbia helioscopia L ., 21, 191–193. https://doi.org/10.1016/j.cclet.2009.10.002

[33] Peng, H., Xiao, L., Shi, F., & Cheng, Y. (2011). Quantitative Analysis of Quercetin in Euphorbia helioscopia L by RP-HPLC, 59–64. https://doi.org/10.1007/s12013-011-9161-0

[34] Chem, T. J. (2006). Secondary Metabolites from Euphorbia helioscopia and Their Vasodepressor Activity, 30, 325–332.

[35] Maoulainine, B. M., Salem, M., & Boukhari, O. (2012). Antioxidant proprieties of methanolic and ethanolic extracts of Euphorbia helioscopia , (L .) aerial parts, 19(3), 1125–1130.

[36] Basma, A.A., B., Zakaria, Z., Yoga Latha, L. and Sasidharan, S. 2011. Antioxidant activity and phytochemical screening of the methanol extracts of Euphorbia hirta L. Asian Pasific Journal of Tropical Medicine 4(5): 386-390.

[37] Ahm, W., Rabi, N. A., & Ahm, S. (1993). Epicuticular Leaf Wax of Euphorbia helioscopia L . (Euphorbiaceae), 9, 5–9.

[38] Woodhead, S. 1981. Environmental and biotic factors affecting the phenolic content of different cultivars of Sorghum bicolor. Journal of chemical ecology. 7(6) 1035-1047

[39] Conor, A.M., Luby, J. J., Tong, C.B.S., Finn, C. E. and hancock, J. F. 2002. Genotype and environmental variation in antioxidant activity, total phenolic content and anthocyanin content among blueberry cultivars. Soc.Hort.Sci. 127(1) 89-97.

[40] Fujita, M., K., Takeda, N., Kohyama, Y., Doi and Matsunaka, H. 2002. Genotypic variation in polyphenol content of barley grain. Euphytica 124: 55-58.

[41] Bystricka, U., Vollmannova, A., Margitanova, E. and Cicova, I. 2010. Dynamics of polyphenolics formation in different plant parts and different growth phases of selected buckwheat cultivars. Acta Agriculturae Slovenica 95 (3): 225 - 229.

[42] Stine, K.S., Rinnan, A., Mortensen, A. G., Laurse , B., De Troiani, R. M., Noellemeyer, E. J., Janovska, D., Dusek, K., Délano-Frier, J., Taberner, A., Christophersen, C. and Fomsgaard, I. S. 2011. Variations in the polyphenol content of seeds of field grown Amaranthus genotypes. Food Chemistry 129:131-138.

[43] Liu, S.Y., Sporer, F., Wink, M., Jourdanes, J., Henning, R., Li, Y.L., & Ruppel, A. (1997). Anthraquinones in Rheum palmatum and Rumex dentatus (Polygonaceae) and phorbol esters in Jatropha curcas (Euphorbiaceae) with molluscicidal activity against the schistosome vector snails Oncomelania, Biomphalaria and Bulinus. Tropical Medicine & International Health. 2(2), 188-189. doi:10.1046/j.1365-3156.1997.d01-242.x

[44] Mendes, N.M., Vasconcellos, M.C., Baptista, D.F., Rocha, R.S., & Schall, V.T. (1997). Evaluation of the molluscicidal properties of Euphorbia splendens var. hislopii (N.E. B.) latex: experimental test in an endemic area in the state of Minas Gerais, Brazil. Memórias do Instituto Oswaldo Cruz. 92(5), 719-724. doi:10.1590/s0074-02761997000500029

[45] Anjoud, H., Boughdad, A., Yassine, E. A., & Abdelaziz, C. [2018]. Toxicity of Euphorbia helioscopia pellets to two phytophagous molluscs, Theba pisana Müller, 1774 (Pulmonata: Helicidae) and Arion hortensis Férussac, 1819 (Pulmonata: Arionidae). Pesticidi i fitomedicina, 33(3-4), 241-252.

[46] Shoeb, H.A., & El-Sayed, M.M. (1984). A short communication on the molluscicidal properties of some plants from Euphorbiaceae and Agavaceae. Helminthologia, 21, 33-54.

[47] El-Amin, S.M., & Osman, N.S. (1991). Squalene and Urs 12-en-28 01 from the Molluscicidal plant, Euphorbia helioscopia. Egyptian Journal Bilharzia, 13, 181-187.

[48] Singh A, Agarwal RA. 1988a. Possibility of using latex of euphorbiales for snail control. Sci. Total Environ. 77: 231–236.

[49] Singh A. 1991. Molluscicides of plant origin against harmful snails. PhD thesis, Gorakhpur University, Gorakhpur (UP), India.

[50] Patel, A. V., Wright, D. , Blunden, G. , Sumner, S. and Rice, J. (2011), Stable Molluscicide Formulation of an Aqueous Extract of Euphorbia myrsinites. Phytother. Res., 25: 1412-1414. doi:10.1002/ptr.3467

[51] Amusan OOG, Msonthi JD, Makhuba LP. 1997. Molluscicidal activity of Urginia epigea. Fitot 68: 185–186.

[52] Schall VT, Vasconcellos MC, Souza CP, Baptista DF. 1988. The molluscicidalactivity of crown of Christ (Euphorbia spelendens var, hisloppi) latex on snails acting as intermediate hosts of Schistosoma mansoni and Schistosoma haemotobium. J. Trop. Med. Hyg. 58: 7–10.

[53] Marston A, Dudan G, Gupta MP, Sois PN, Correa MD, Hostettman K. 1996. Screening of Panamanian plants for molluscicidal activity. Int. J. Pharmacol. 34: 15–18.

[54] Singh SK, Yadav RP, Singh A. 2009. The toxicity of Thevetia peruviana and Alstonia scholaris plants to target and non-target aquatic organism. Proceedings of the Symposium on Functional Biodynamics and Ecophysiology of Animals; 301–307.

[55] Singh SK, Yadav RP, Singh D, Singh A. 2004a. Toxicological eff ects of stem bark of Thevetia peruviana and Alstonia scholaris on the freshwater snail Lymnaea acuminata. Malaysian Appl. Biol. 33(1): 61–68.

[56] Singh SK, Yadav RP, Tiwari S, Singh A. 2005. Toxic eff ect of stem bark and leaf of Euphorbia hirta plant against freshwater vector snail Lymnaea acuminata. Chemosphere 59(2): 263–270.

[57] Singh SK. 2000. Studies on molluscicidal properties of some local plants of eastern Uttar Pradesh against harmful snails. PhD thesis, DDU Gorakhpur University, Gorakhpur (UP), India.

[58] Singh SK. 2000. Studies on molluscicidal properties of some local plants of eastern Uttar Pradesh against harmful snails. PhD thesis, DDU Gorakhpur University, Gorakhpur (UP), India.

[59] Singh, S. K., Yadav, R. P. and Singh, A. (2010), Molluscicides from some common medicinal plants of eastern Uttar Pradesh, India. J. Appl. Toxicol., 30: 1-7. doi:10.1002/jat.1498

[60] Yadav RP, Singh SK, Singh A. 2009. Toxic eff ects of euphorbious plants against freshwater vector snails in ponds. Proceedings of the Symposium on Functional Biodynamics and Ecophysics of Animals; 293–300.

[61] Yadav RP, Tiwari S, Singh A. 2005. Toxic eff ects of taraxerol extracted from Codiaeum variegatum stem bark on target vector snail Lymnaea acuminate and non-target fi sh. Iberus 23(1): 1–13.

[62] Dossaji S, Kubo I 1980. Quercetin 3-(2"-galloyl glycoside), a molluscicidal fl avonoid from Polygonum senegalense. Phytochemistry 19: 482.

[63] Lemma A. 1965. A preliminary report on the molluscicidal properties of Endod (Phytolacca dodecandra). Ethiopian Med. J. 3: 187–190.

[64] Hostettmann K, Kizu H. Tomimori T. 1982. Molluscicidal properties of various saponins. Planta Med. 44: 34–35.