Pestic. Phytomed. (Belgrade), 33(3-4), 2018, 241–252
DOI: https://doi.org/10.2298/PIF1804241H

UDC 632.937:632.951:594.3
Original scientific paper

Toxicity of *Euphorbia helioscopia* pellets to two phytophagous molluscs, *Theba pisana Müller*, 1774 (*Pulmonata: Helicidae*) and *Arion hortensis Férussac*, 1819 (*Pulmonata: Arionidae*)

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Received: 15 July 2018
Accepted: 10 September 2018

SUMMARY

Harmful land snails and slugs are currently one of the most important threats facing agriculture in many parts of the world. Synthetic molluscicides are the main control method against these gastropods. However, dangers caused by these chemicals to the environment have led scientists to research for environmentally friendly alternatives. The objective of our work was to test and evaluate food pellets containing roots, stems, leaves or flowers of *Euphorbia helioscopia* against *Theba pisana* and *Arion hortensis* adults. Toxicity of the prepared pellets varied depending on plant organ and mollusc species tested. Pellets made of stems (LD_{50} = 1.35 g / 100 ml of agar at 2%) and leaves (LD_{50} = 1.39 g / 100 ml of 2% agar) proved more toxic to adult snails than those made of roots and flowers, which had no significant effects. In the case of slugs, pellets made of leaves (LD_{50} = 1.14 g / 100 ml of 2% agar) were more toxic than those made of stems (LD_{50} = 1.33 g / 100 ml of 2% agar), flowers (LD_{50} = 1.75 g / 100 ml of 2% agar) and roots (LD_{50} = 1.98 g / 100 ml of 2% agar). Compared to a synthetic product containing metaldehyde 5%, the results show that the use of these molluscicides derived from plants as pellets is environment- and health-conscious, targeted and economical. These products can be used in plant protection against phytophagous slugs and snails.

Keywords: Biopesticides; Molluscicides; *Euphorbia helioscopia*; Pellets; Snails; Slugs; Toxicity

INTRODUCTION

Land slugs and snails cause damage and major economic losses to crops everywhere in the world (Hommay & Briard, 1989; South, 1992; Glen & Moens, 2002; Hammond & Byers, 2002; Port & Ester, 2002; Gavin et al., 2012). For example, yield losses across farmlands cost the United Kingdom alone around £10 million (Garthwaite & Thomas, 1996) in 2003, and £30 million were the overall cost of losses to the industry of the same country (Redbond, 2003). Treatment of crops with molluscicides cost about € 45 million in France, known as the largest market for chemical baits for slugs in Europe (Meredith, 2003). Metaldehyde treatments of seeds of grass crops in the Pacific Northwest cost about US $ 14 million. On the other hand, the Australian barley decommissioning due to contamination with *T. pisana* reduced the price paid to farmers from 160 to 120 Australian dollars per ton (Barker, 2002, Howlett, 2012). Although these figures give an indication of damage costs in many countries, we note that such losses are heavier or not assessed in undeveloped countries.

These animals can appear in all wet areas (Singh et al., 2009). They attack leaves, roots, buds, flowers, fruits and even tree trunks, causing damage to cultivated plants (Abdallah et al., 1998). Damage is caused by their feeding, contamination by drooling, faeces and/or sludge, which lead to quality deterioration of products plus financial losses (Iglesias et al., 2003). In addition, they are considered as rotting agents that promote the development of bacteria, viruses and fungi in places where snails feed (Hamdy et al., 2007). In Morocco, the impact severity of land snails and slugs has increased considerably over the past decades; indeed, significant damage can be observed on different crops, such as sugar beet (Rungs, 1962; Jenane & Agbani, 2000), cabbage, salads (Chambre d'agriculture, 2016) and mint (Tanji, 2008; Eddaya et al. 2010).

Marketed synthetic molluscicides consist of niclosamide, metaldehyde and methiocarb. These chemicals are characterized by low solubility in water and slow degradation in soil, thus causing very serious environmental issues (Tadros, 1980; Dai et al., 1998; Oliveira-Filho et al., 2000; Zhang & Jiang, 2002). To cope with problems caused by synthesic molluscicides, several studies of natural herbal products have been conducted. When their active substances are applied in certain concentrations, they can stop snail and slug metabolism, and cause their death. These active substances have been known for a long time. However, out of many products known for their molluscicide activity, only a few of them have so far proved useful in large-scale trials (Strufe, 1968; Hamdy & El-Wakil, 1996; El-Zemity & Radwan, 2001; Hamdy, 2005; El-Zemity, 2006).

Recently, the use of plant products has won an unprecedented boost worldwide. Several countries have encouraged the use of these products owing to their wide range of ideal properties, such as high target toxicity, low toxicity to mammals, relatively low cost, water solubility, biodegradability, abundant growth in endemic areas and safety in use (Kinghorn & Evans, 1975; Marston & Hostettmann, 1985; Singh et al., 2009).

Euphorbiaceae is one of the largest families of Anthophyta with its 300 genera and 5000 species (Uzair et al., 2009). *Euphorbia* is one of the largest genera of Angiosperms with about 2000 species. It has long been admired for its great diversity of forms, including many xerophile species. Despite great diversity, the family is morphologically united and characterized by a cyathium, a very small inflorescence that resembles a single flower (Steinmann & Porter, 2002; Barla et al., 2006; Uzair et al., 2009). Molluscicide activity is widespread in *Euphorbiaceae* family, although the activity varies from one species to another and even between different parts of the same plant. Studies have shown that *Euphorbia helioscopia* has a very interesting molluscicide activity (Shoeb & El-Sayed, 1984; El-Amin & Osman, 1991; Al-Zanbagi, 2000; Al-Zanbagi, 2005).

As far as we are aware, this study was undertaken for the first time in Morocco. Its purpose was to assess the toxicity of roots, stems, leaves and flowers of *E. helioscopia* against two phytophagous molluscs, *Theba pisana* and *Arion hortensis*.

MATERIALS AND METHODS

Euphorbia helioscopia *plant*

Euphorbia helioscopia plants were collected in the Oued Beht region (GPS coordinates: 33° 53'2.538" N; 5° 55' 41.413''W; 190.4msl) near Khemisset-Morocco in February 2015. This region is characterized by a semi-arid climate with cold winter. Annual temperatures range between 15 and 19 °C, depending on altitude and continentality (Administration de l'Hydraulique, 1991; Lakhili et al., 2015). The plant

was identified by the Scientific Institute of Rabat, where specimens were filed under voucher number RAB091057.

The plant drying process was carried out in shade until a stable weight was achieved after twenty days of drying in a well-ventilated place and under temperature not exceeding 35 °C.

Theba pisana *and* Arion hortensis *strains*

Theba pisana adults were collected in the region of Meknes - Morocco (GPS coordinates: 32°17'41.2"N ; 3°59'59.3"W); while *Arion hortensis* was sampled in the region of Dar El Guedari, province of Kenitra - Morocco (GPS coordinates: 34°25'54.4"N ; 6°04'29.8"W). Taxonomic identification was confirmed by the Department of Plant Protection and Environment, National School of Agriculture in Meknes - Morocco.

Preparation of toxic pellets containing Euphorbia helioscopia *powder*

Preparation of pellets used in this work was carried out according to a method used by Singh & Singh (2008). The pellets were composed of a binary combination of carbohydrates (sucrose, starch 10 mM) and amino acids (arginine 20 mM) in 100 ml solution of 2% agart. Carbohydrate and amino acid concentrations used in our test are those specified by Tiwari & Singh, (2004a). The powder of *E. helioscopia* roots, stems, leaves or flowers was added to the solution. Concentrations of 0.25; 0.5; 0.7; 1 or 2 g per 100 ml of agar at 2% from each plant organ were mixed with the previously prepared solution. These concentrations had already been tested by Tiwari, (2012) and showed toxicity against *Lymnaea acuminate* snails. These solutions were then spread with a uniform thickness of 5 mm. After cooling, pellets were cut in bits of 5 mm diameter, and put in the oven to dry at 50 °C for 18 h; these conditions allowed the pellets to keep their intrinsic composition while ensuring a long-lasting use and good nutritional quality.

Biological test

Biological tests were conducted in the Plant Protection and Environment laboratory of the National School of Agriculture of Meknes - Morocco under the following experimentation conditions: T max = 21.36 °C; T min = 8.32 °C; rh = 59.1 ± 2.56 and a natural photoperiod of 10:14 h (L/D). Snail adults (12±4.15 x 21±7.09 mm) (height x diameter ±SD), as well as slugs of the same size (30 ± 6.45 mm in length) were selected for tests and pre-packed before use. Five grams of each concentration were served to 10 individuals/species in plastic boxes (dimensions 30x15x8mm) simultaneously. Meanwhile, two control lots were formed. The first one contained Ariotox (5% metaldehyde) in the form of pellets and it was considered as a positive control used at the recommended dose (20 kg/ha); the other (negative control) contained sucrose, starch 10 mM and amino acids (arginine 20 mM) in a solution of 100 ml of 2% agar formed as pellets. For each bioassay, 3 and 5 repetitions were conducted for slugs and snails, respectively.

Daily observations were made up untill the death of all specimens in each treated batch; dead specimens were counted and removed from boxes. A specimen was considered dead if it did not move after tactile stimulation of seal and body with a brush. Moreover, dead animal body dilated in both species.

Data analysis

To compare the toxicity of different organs of *Euphorbia* to snails and slugs in this study, survival curves were built and compared using Logrank test according to Kaplan & Meier (1958). This test follows χ^2 distribution with one degree of freedom; any treatment χ^2 with a degree of freedom less than 3.841 was considered as not significantly different. Microsoft Excel version 2013 software was used. Lethal doses LD_{50} and LD_{99} (doses required to kill 50% or 99% of the test population after 15 days of testing for slugs and 30 days for snails) and their confidence intervals were determined according to Probit method (Finney, 1971) using Biostat Pro version 2015 software. Lethal times LT_{50} and LT_{99} correspond to the time in which 50 and 99 % of the population died, respectively; they were calculated from the equation of the straight line between the cumulative mortality and duration of molluscs exposure (Harmouzi et al, 2016).

RESULTS

The responses of *T. pisana* and *A. hortensis* adults placed in contact with pellets prepared from roots, stems, leaves or flowers of *E. helioscopia* are summarized in Figures 1 and 2.

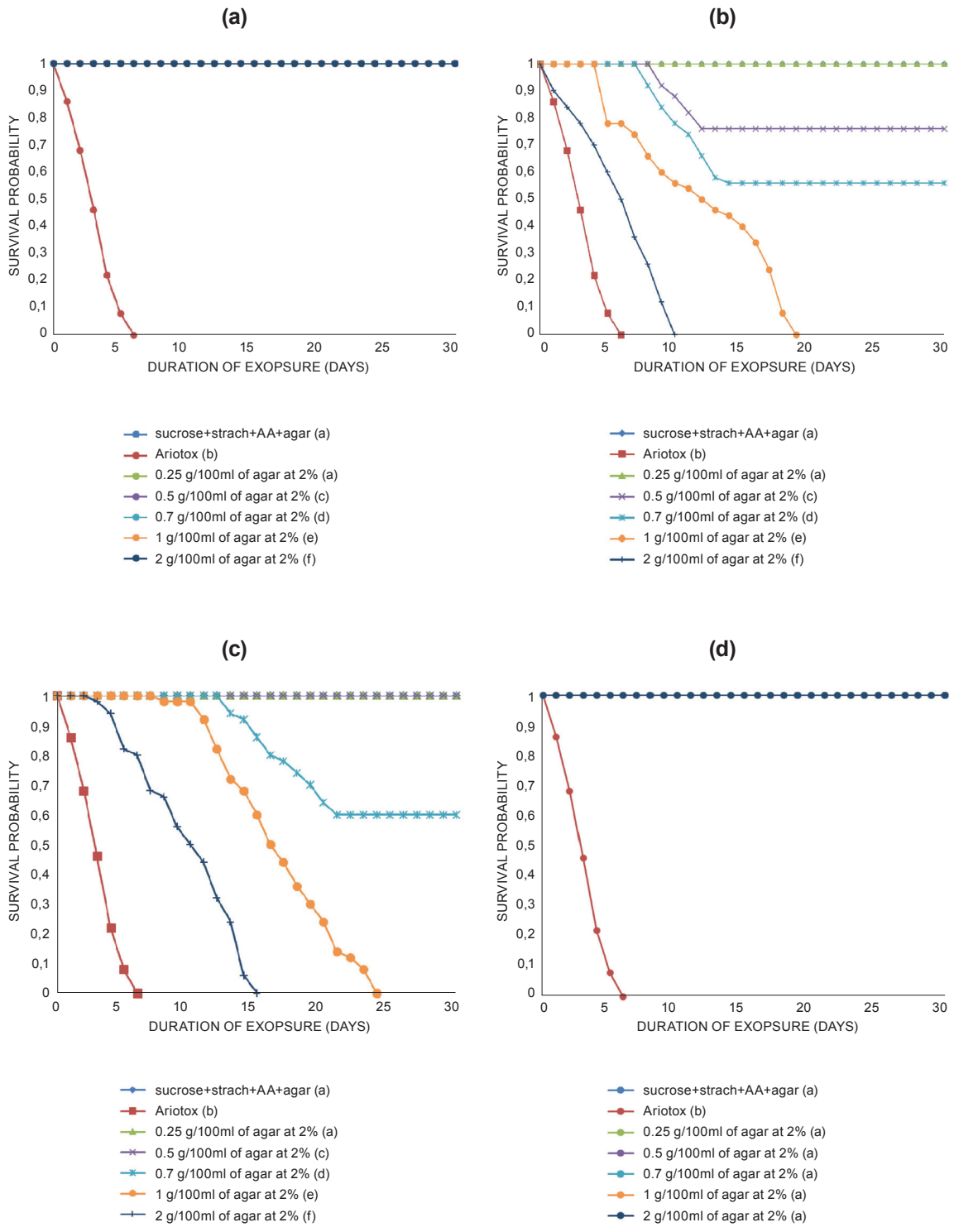

Figure 1: Survival curves of treated *Theba pisana* adults with pellets containing roots, stems, leaves and flowers of *Euphorbia helioscopia*. [Concentrations marked with the same letter are not statistically different (Logrank test at 5%; $\chi^2 > \chi^2_{(0.05;\,1)} = 3.84$)] **a**: *E. helioscopia* roots against *T. pisana* adults, **b**: *E. helioscopia* stems against *T. pisana* adults, **c**: *E. helioscopia* leaves against *T. pisana* adults, **d**: *E. helioscopia* flowers against *T. pisana* adults.

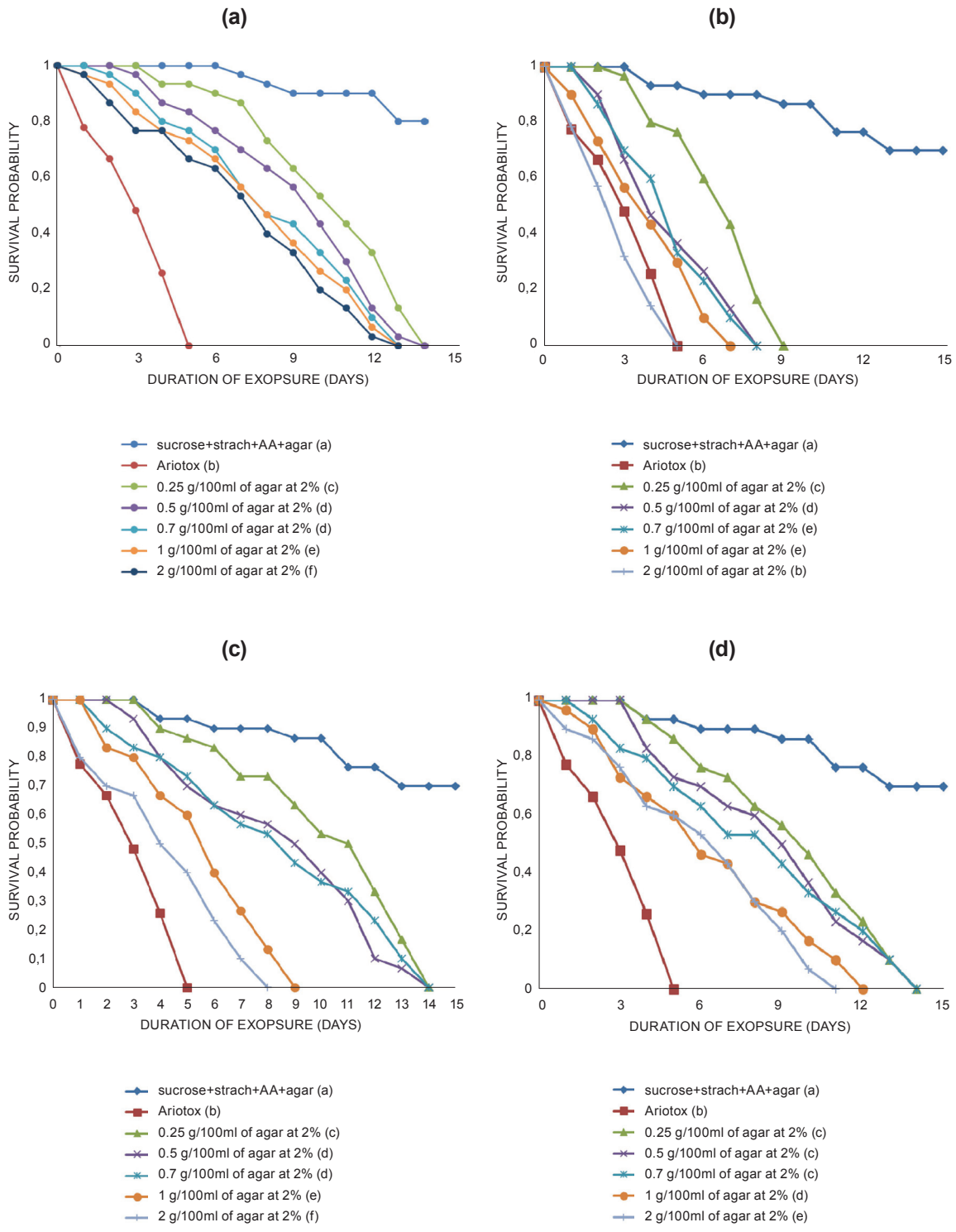

Figure 2: Survival curves of *Arion hortensis* adults treated with pellets containing roots, leaves, stems or flowers of *Euphorbia helioscopia*. [Concentrations marked with the same letter are not statistically different (test Logrank at $P \leq 0.05$; $\chi^2 > \chi^2_{(0.05;1)} = 3.84$)], **a**: *E. helioscopia* roots against *A. hortensis* adults, b: *E. helioscopia* stems against *A. hortensis* adults, c: *E. helioscopia* leaves against *A. hortensis* adults, d: *E. helioscopias* flowers against *A. hortensis* adults.

Regarding snails, pellets made from roots or flowers, showed no toxicity against *T. pisana* adults at any concentration considered. For stem or leaf pellets, no mortality was detected at concentrations of 0.25 g of stems or 0.25 g and 0.5 g of leaves / 100 ml of 2% agar. In contrast, 0.7; 1 or 2 g per 100 ml of 2% agar resulted in statistically higher mortality than in the negative control group (c^2 varied from 13 to 100 > $c^2_{(0.05\,;\,1)}$=3.841), but lower than those found in lots treated with metaldehyde (c^2 varies from 24 to 93 > $c^2_{(0,05\,;\,1)}$=3,841). Then, the toxicity of pellets increased significantly with concentrations, all values of c^2 exceeded $c^2_{(0.05;\,1)}$ =3.841, and ranged from 3.99 to 100.2 for pellets made from stems, and from 24.46 to 103.64 for those made from leaves.

In this trial, pellets made from stems, used at 0.5, 1 or 2 g of stems / 100 ml of 2% agar, proved to be more toxic than those made of leaves. The c^2 values are 63, 13.71 and 24.85, respectively > $c^2_{(0.05\,;\,1)}$=3.841; while 0.7 g of stems or leaves / 100 ml of 2% agar concentration causes a similar snail mortality rate (c^2 =1.47 < $c^2_{(0.05\,;\,1)}$ = 3.841).

Furthermore, during the trial, the time required to kill all or a percentage of test snails varies depending on the applied concentration of each organ. For stems, total mortality of treated adult snails occurred 11 and 20 days after starting treatment with concentrations of 2 and 1 g / 100 ml of 2% agar, respectively; while 0.7 g or 0.5 g 100 ml of 2% agar concentrations caused 44% and 24% mortality after 14 and 12 days of treatment, respectively. For leaves, total mortality was observed after 16 and 25 days of exposure to concentrations of 2 or 1 g/100 ml of 2% agar, respectively; while 0.7 g / 100 ml of 2% agar caused 40% of snail mortality on the 21st day after treatment began (Figure 1). Compared to the positive control, for which the time required to kill 50 and 99% of the treated population was 3 and 6 days, respectively, lethal time for pellets made from *E. heliscopia* used at 1 or 2 g / 100 ml of 2% agar was significantly longer. Indeed, the time required to kill 50% and 99% of snail population treated with 1 or 2 g of pellets made from *E. heliscopia* ranged between 5 and 21 days for stems and between 9 and 26 days for leaves, respectively. Similar to the reference product (methaldehyde), snail mortality evoked by pellets made from stems or leaves of *E. heliscopia* was linearly dependent on the duration of exposure (Table 1).

In the case of slug adults, pellets made from the four organs of *E. helioscopia* caused significantly higher mortality than those recorded in the negative controls (χ^2 varies from 22 to 40 > $\chi^2_{(0.05\,;\,1)}$ = 3.84), but inferior to the reference product (Ariotox) (χ^2 varies from approximately 4.66 to 36 > $\chi^2_{(0.05\,;\,1)}$ = 3.84). However, there is one exception when slugs were treated with pellets made from stems at 2 g/100 ml of 2% agar, which caused a comparable mortality to the reference product ((χ^2 =0.47 < $\chi^2_{(0.05\,;\,1)}$ = 3.84) (Figure 2). For root pellets, slug mortality was statistically comparable for all tested concentrations (χ^2 varied from approximately 0.06 to 2.06 < $\chi^2_{(0.05;\,1)}$ = 3.84). As with snails, slug mortality due to pellets made from stems, leaves or flowers was linearly dependent on concentration and duration of exposure.

In terms of concentrations, the toxicity of pellets made from stems was lower with 0.25 g (8.50 ≥ χ^2 ≤ 32.04 > $\chi^2_{(0.05\,;\,1)}$ = 3.84), similar with 0.5, 0.7 or 1 g (0.03 ≥ χ^2 ≤ 2.01 < $\chi^2_{(0.05\,;\,1)}$ = 3.84), becoming higher with 2 g / 100 ml of 2% agar (6.95 ≥ χ^2 ≤ 32.04 > $\chi^2_{(0.05\,;\,1)}$ = 3.84). Pellets made from leaves, at concentrations of 0.25, 0.5 or 0.7 g / 100 ml of 2% agar caused a similar lethal effect against slugs (0.09 ≥ χ^2 ≤ 2.65 < $\chi^2_{(0.05;\,1)}$ = 3.84), but significantly lower among those treated with 1 or 2 g / 100 ml of 2% agar (9.16 ≥ χ^2 ≤ 27.61 > $\chi^2_{(0.05\,;\,1)}$ = 3.84). These two last concentrations caused statistically comparable mortality (χ^2 = 3.50 < $\chi^2_{(0.05;\,1)}$ = 3.84). The same results emerged with pellets made from flowers and applied at 0.25, 0.5 or 0.7 (0.04 ≥ χ^2 ≤ 3.72 < $\chi^2_{(0.05\,;\,1)}$ = 3.84) and at 1 or 2 g / 100 ml of 2% agar (χ^2 = 0.54 < $\chi^2_{(0.05\,;\,1)}$ = 3.84).

Table 1. LT_{50} and LT_{99} for *Theba pisana* adults treated with pellets made of different *Euphotbia helioscopia* organs

Products	Concentrations	Equations	R^2	LT_{50} (days)	LT_{99} (days)
Metaldehyde	20 kg/ha	-0.18x*+1.01	0.99	2.84	5.57
Stems	1g/100 ml of 2% agar	-0.05x +1.09	0.96	11.57	21.14
	2g/100 ml of 2% agar	-0.10x +1.05	0.98	5.51	10.46
Leaves	1g/100 ml of 2% agar	-0.05x +1.23	0.89	15.76	26.34
	2g/100 ml of 2% agar	-0.07 x+1.15	0.96	9.30	16.34

* - Duration of exposure (days)

Furthermore, by comparing lethal effects of pellets from *E. heliscopia* organs, tested at the same concentrations, those extracted from stems were proved to be the most toxic against *A. hortensis* ($7.29 \geq \chi^2 \leq 27.43 > \chi^2_{(0.05;1)} = 3.84$). For pellets made from leaves or flowers, only 1 or 2 g / 100 ml of 2% agar generated the highest mortality compared to roots ($4.48 \geq \chi^2 \leq 14.02 > \chi^2_{(0.05;1)} = 3.84$), and this is more remarkable with foliar pellets. While 0.25, 0.5 or 0.7 g of roots, leaves or flowers /100 of 2% agar showed mortality rates comparable to those against treated slugs ($0.001 \geq \chi^2 \leq 0.25 < \chi^2_{(0.05;1)} = 3.84$).

All specimens declined 4-9, 8-15, 11-14, and 13-15 days after the beginning of treatment with pellets made of stems, leaves, flowers or roots, respectively. Slugs treated with *E. heliscopia* pellets died much later than those exposed to the reference product (Figure 2).

The time required to kill 50 and 99 % of slug populations depended on plant organs and tested concentrations; it varied from 7 to 9 and 13 to 16 days for roots, from 2 to 6 and 5 to 10 days for stems, from 3 to 10 and from 8 to 17 days for leaves, and from 6 to 9 and 11 to 15 days for flowers. It is negatively correlated with the tested concentrations and generally longer than the time needed for the reference product (Table 2).

The toxicity of pellets made from *E. helioscopia* depends on plant organ and animal species. Sloping values of LD_{50} or LD_{99} show that pellets made from stems or leaves appear to be more toxic than the other two tested organs (Table 3).

Table 2. LT_{50} and LT_{99} for *Arion hortensis* adults treated with pellets made of different *Euphorbia helioscopia* organs

Products	Concentration	Equations	R^2	LT_{50} (days)	r*	LT_{99} (days)	r
Ariotox	20kg/ha	-0.19x* + 1.01	0.99	2.66		5.20	
Roots (g/100 ml of 2% agar)	0.25	- 0.07x + 1.17	0.92	9.20		15.94	
	0.5	- 0.08x + 1.15	0.96	8.23		14.41	
	0.7	- 0.08x + 1.15	0.94	8.69	-0.86	15.21	-0.79
	1	- 0.08x + 1.11	0.98	7.96		14.41	
	2	- 0.08x + 1.09	0.98	7.55		13.87	
Stems (g/100 ml of 2% agar)	0.25	- 0.11x + 1.19	0.89	6.02		10.33	
	0.5	- 0.14x + 1.08	0.98	4.25		7.85	
	0.7	- 0.14x + 1.09	0.98	4.27	-0.92	7.79	-0.94
	1	- 0.15x + 1.02	1.00	3.53		6.84	
	2	- 0.21x + 0.98	0.99	2.35		4.74	
Leaves (g/100 ml of 2% agar)	0.25	- 0.07x + 1.16	0.91	9.68		16.90	
	0.5	- 0.08x + 1.11	0.97	7.96		14.40	
	0.7	- 0.07x + 1.06	0.99	7.91	-0.93	14.86	-0.90
	1	- 0.12x + 1.09	0.98	5.10		9.32	
	2	- 0.12x + 0.98	0.99	3.91		7.93	
Flowers (g/100 ml of 2% agar)	0.25	- 0.08x + 1.17	0.94	8.90		15.42	
	0.5	- 0.08x + 1.13	0.97	8.19		14.54	
	0.7	- 0.07x + 1.06	0.99	7.74	-0.89	14.48	-0.91
	1	- 0.09x + 1.02	0.99	6.09		11.82	
	2	- 0.09x + 1.03	0.99	5.77		11.13	

* : r = Correlation coefficient ; $r_{(0.05;3)} = 0.878$; * : Duration of exposure (days)

Table 3: Parameters of toxicity of pellets containing powders of different organs of *Euphorbia helioscopia* to *Theba pisana* (30 days of treatment) and *Arion hortensis* adults (15 days of treatment).

Treated molusc	Plant organ	Number of treated animal	Slope ± SE	LD_{50} (g/100 ml of 2% agar) [IC]	LD_{99} (g/100 ml of 2% agar) [IC]	χ^2 ($\chi^2_{(0.05;1)} = 3.84$)
Theba pisana	Stems	250	6.30 ± 0.78	1.35 [0.92; 14.11]	3.14 [1.85; 41.01]	5.78
	Leaves	250	8.84 ± 1.40	1.39 [1.13; 1.94]	2.73 [1.84; 14.94]	36.60
Arion hortensis	Roots	150	1.35 ± 0.53	1.98 [0.95; 100.31]	212.56 [16.21; 997.32]	21.11
	Stems	150	3.46 ± 0.61	1.33 [1.01; 1.94]	9.29 [4.87; 64.76]	19.65
	Leaves	150	2.50 ± 0.87	1.14 [0.66; 4.17]	9.23 [4.82; 238.40]	17.90
	Flowers	150	1.42 ± 0.52	1.75 [0.96; 52.17]	146.67 [4.41; 107;02]	20.46

Regarding snails, the values of lethal doses showed that pellets made from *E. helioscopia* stems (LD_{50} = 1.35 g / 100 ml of 2% agar) achieved a toxicity that was near to that of leaves (LD_{50} = 1.39 g / 100 ml of 2% agar). As for slugs, pellets made from *E. helioscopia* leaves showed high toxicity (LD_{50} = 1.14 g / 100 ml of 2% agar) compared to stems (LD_{50} = 1.33 g / 100 ml of 2% agar), flowers (LD50 = 1.75 g / 100 ml of 2% agar) or roots (LD50 = 1.98 g / 100 ml of 2% agar). The LD_{50} and LD_{99} per each of four plant organ decreased with time after ingestion of toxic pellets by molluscs.

DISCUSSION

In the present work, several organs of *E. helioscopia* were tested, and especially stems and leaves were found to have potential molluscicide properties against *T. pisana* and *A. hortensis*. Toxic effects of these plant parts depended on time and dose. Molluscicide properties of diverse species of *Euphorbiaceae* have been widely studied, using different plant organs and different methods of extraction (Liu et al., 1997; Mendes et al., 1997; Al-Zanbagi, 2013). Several studies have shown a considerable specificity of biopesticides against mollusc pests (Crowell, 1967; El-Zemity & Radwan, 1999). Molluscicide activity is common in *Ephorbiaceaes* family although the activity varies considerably from one species to another and even among different parts of the same plant. Chloroform extracted from dried leaves of *Jatropha glauca* showed an LD_{50} of 16.5 ppm, and LD_{90} of 46.8 ppm against *Biomphalaria pfeifferi*. This activity is higher than that reported for extracts of *Jatropha aceroides, J. aethiopica, J. curcas* and *J. gossypifolia* (Singh & Agrawal, 1990; Singh & Agrawal, 1992).

Shoeb and El-Sayed (1984) and El-Amin and Osman (1991) also conducted studies of *Euphorbiaceae* molluscicide activities. Among the extracts of *Euphorbia schimperiana*, those of methanol-rich dry stems and chloroform-rich fresh leaves were the most active plant parts. These activities are similar to those reported for extracts of *Euphorbia pseudocactus* (Shoeb & El Sayed, 1984), *Euphorbia lactea* (Abou El-Hasan et al., 1980; El-Emam et al., 1982) and *Euphobia peplus* (Shoeb & El-Sayed, 1984; Ghandour, 1991). On the other hand, a report by Zani et al. (1993) revealed that *Euphorbia milii* has a molluscicide activity at low concentrations. *Euphorbiaceae* plants have therefore shown sufficient activity to open the door for further investigation of their molluscicide potentials. In addition, the study of natural products of these plants may lead to a discovery of new structures that could be the basis of future molluscicides.

Consistent with reports by Abdel-Hamid (1997) and Tiwari & Singh (2007) on other molluscs, our study showed that the binary combination of carbohydrates (sucrose, starch) mixed with amino acid (arginine) and different parts of *E. helioscopia* form an attractive component for *T. pisana* and *A. hortensis*. Snails and slugs, like many other gastropods, are able to detect their food sources using chemical sense for carbohydrates and amino acids as a sign of food presence (Tiwari & Singh 2004a,b; Singh & Singh, 2008; Kumar & Singh, 2009).

The molluscicide mechanism of action of these natural compounds in molluscs, based on alkaloids, flavonoids or saponins, can have a multiplicity of effects. One is that molluscs may withdraw inside their shells after ejection of haemolymph, or swell and extend out of the shell by breaking the osmotic balance, which is under neurohormonal control (McCullough et al, 1981). For both mollusc species which were the subjects of this study, moluscicide activity resulted in a disruption of their cell membrane and change in its permeability, which is consistent with the studies of Appleton, 1985; Radwan & Zemity, 2007.

CONCLUSION

Toxic pellets formulated from *E. helioscopia* stems and leaves showed molluscicide activity against both tested molluscs, *A. hortensis* and *T. pisana*. The results achieved with these products are very promising, especially those containing stems and leaves of *E. helioscopia*. Our results indicate a positive potential of these products, originally from plants, to be used as biomolluscicides. That enables not only to control these pests, but to protect the environment as well. Molluscicides derived from plants inside food pellets could be environmentally safe, targeted and economic; these biomolluscicides can be considered as safer products for the future, rather than synthetic chemicals. These results can be further developed by integrating these studied concentrations in programs for field treatments, evaluating their effects on non-target animals, and specifying their mode and duration of action.

ACKNOWLEDGEMENTS

We would like particularly to thank the Department of Plant Protection and Environment of the National School of Agriculture - Meknes, Morocco, for material support. Similarly, we thank Mrs. Hayat Belchhab for her tremendous help in the collection and separation of *Euphorbia helioscopia* organs.

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Toksičnost peleta sa *Euphorbia helioscopia* za dve vrste fitofagnih mekušaca, *Theba pisana Müller*, 1774 (*Pulmonata: Helicidae*) i *Arion hortensis Férussac*, 1819 (*Pulmonata: Arionidae*)

REZIME

Puževi i puževi golaći predstvljaju jednu od važnijih pretnji u poljoprivredi u mnogim delovima sveta. Sintetički moluscidi predstavljaju jednu od primarnih metoda odbrane od ovih gastropoda. Ipak, opasnosti koje ove hemikalije predstavljaju u životnoj sredini motivisale su istraživače da istraže alternative koje bi bile bezbedne za životnu sredinu.

Cilj ovog rada bio je da se testira i oceni vrednost peleta sa korenom, stablom, listom ili cvetom biljne vrste *Euphorbia helioscopia* u suzbijanju adulta vrsti *Theba pisana* i *Arion hortensis*. Toksičnost peleta je varirala u zavisnosti od ispitivanih biljnih organa i vrsta mekušca. Pelet od stabla (LD_{50} = 1.35 g / 100 ml agara 2%) i lista (LD_{50} = 1.39 g / 100 ml agara 2%) pokazao se kao toksičniji za adulte puževa nego onaj od korena i cveta, koji nisu pokazali značajan efekat. Kod puževa golaća, pelet od lista (LD_{50} = 1.14 g / 100 ml agara 2%) bio je toksičniji od onog sa stablom (LD_{50} = 1.33 g / 100 ml agara 2%), cvetom (LD_{50} = 1.75 g / 100 ml agara 2%) i korenom (LD_{50} = 1.98 g / 100 ml agara 2%). U poređenju sa sintetičkim proizvodom na bazi metaldehida 5%, rezultati su pokazali da su ovi moluscidi na biljnoj bazi u obliku peleta ekološki i zdravstveno pogodni, ciljani i ekonomični. Ovi proizvodi se mogu koristiti za zaštitu od fitofagnih puževa i puževa golaća.

Keywords: Biopesticidi; Moluscidi; *Euphorbia helioscopia*; Pelet; Puževi; Puževi golaći; Toksičnost