Evaluation of the effect of essential oil formulations from *Eucalyptus* species against *Arion* spp.

Evaluación del efecto de formulados de aceites esenciales de especies *Eucalyptus* contra *Arion* spp.

DOI: http://doi.org/10.17981/ingecuc.19.2.2023.11

Artículo de Investigación Científica. Fecha de Recepción: 28/01/2022. Fecha de Aceptación: 17/11/2022.

Karen Yulissa Perilla Rueda
Empresa Agrofuturo. Fortul. Arauca (Colombia)
hosneitherm@gmail.com

Leónides Castellanos González
Universidad de Pamplona. Pamplona (Colombia)
lclcastell@gmail.com

Amanda Lucía Chaparro García
Universidad de Pamplona. Pamplona (Colombia)
achaparro@unipamplona.edu.co

To cite this paper
K. Perilla, L. Castellanos & A. Chaparro, "Evaluation of the effect of essential oil formulations from *Eucalyptus* species against *Arion* spp.", *INGE CUC*, vol. 18, no. 2, pp. 131–142, 2023. DOI: http://doi.org/10.17981/ingecuc.19.2.2023.11

Abstract

Introduction— Slugs of the genus Arion are important pests of many crops, causing considerable decreases in yields. Given the need to meet food demand worldwide, it is important to have pest management systems, plant resources are natural alternatives for this purpose.

Objective— Evaluate the effect of formulations of essential oils of Eucalyptus globulus and Eucalyptus cinerea in the control of Arion spp.

Methodology— Two trials were developed with completely randomized designs with 5 treatments and 10 repetitions (one for the effect by contact and the other for ingestion), spraying on Arion spp. slugs, concentrations at 100 and 200 ppm of essential oils of Eucalyptus globulus or Eucalyptus cinerea. The corresponding analyzes of variances were carried out.

Results— The affectation of mobility for Arion spp., was very similar for all treatments. The formulations of both species of Eucalyptus caused mortality of Arion spp by contact since the 9th day and by ingestion on the 4th day, higher than 85% by contact, and 95% by ingestion. The TL50. per contact varied between 10.31 and 10.12 days for the essential oil of E. globulus and between 13.31 and 6.69 days for that of E. cinerea, while by ingestion they ranged between 7.31 and 5.55 days for E. globulus and between 8.22 and 6.6 days for E. cinerea at 100 and 200 ppm respectively.

Conclusions— The formulations of both Eucalyptus species caused mortality by contact since the 9th day and by ingestion since the 4th day, while the TL 50 were relatively lower by ingestion for E. globulus

Keywords— Phytopesticide; garden slugs; contact; ingestion; mortality; lethal dose

Resumen

Introducción— Las babosas del género Arion constituyen plagas importantes de muchos cultivos, ocasionando disminuciones considerables de rendimientos. Ante la necesidad de satisfacer la demanda alimentaria a nivel mundial, es importante contar con sistemas de manejo plagas, siendo los recursos vegetales alternativas naturales para tal fin.

Objetivo— Evaluar el efecto de los formulados de aceites esenciales de Eucalyptus globulus y Eucalyptus cinerea en el control de Arion spp.

Metodología— Se desarrollaron dos ensayos con diseños completamente aleatorizados (uno para el efecto por contacto y otro por ingestión) con 5 tratamientos y 10 repeticiones, asperjando concentraciones a 100 y 200 ppm de formulados de aceites esenciales de E. globulus o E. cinerea para controlar babosas Arion spp. Se realizaron análisis de varianzas.

Resultados— La afectación de la movilidad para Arion spp., fue muy similar para todos los tratamientos. Los formulados de ambas especies de Eucalyptus causaron mortalidad de Arion spp por contacto a partir del noveno día y por ingestión al cuarto día, superiores a 85% por contacto y 95% por ingestión. Los TL50. por contacto variaron entre 10.31 y 10.12 días para el aceite esencial de E. globulus y entre 13.31 y 6.69 días para el de E. cinerea, mientras por ingestión oscilaron entre 7.31 y 5.55 días, para E. globulus y entre 8.22 y 6.6 días para E. cinérea a 100 y 200 ppm respectivamente.

Conclusiones— Los formulados de ambas especies de Eucalyptus causaron mortalidad por contacto a partir del 9no día y por ingestión al 4to día, mientras que los TL 50 fueron relativamente más bajos por ingestión para E. globulus

Palabras clave— Fitoplaguicida; babosa de jardín; contacto; ingestión; mortalidad; dosis letal

I. Introduction

Slugs are a common pest in global horticulture due to the wide range of plant species that serve as food. This plague is favored by the meteorological conditions present in certain areas, such as high relative humidity, constant rainfall, temperatures between 5°C and 25°C, among others, necessary for the proliferation of its species [1]. It is recognized that slugs may have greater activity in places with high relative humidity [2], [3].

In South America, damage by slugs is reported in countries where the aforementioned conditions occur, as is the case in Peru, reporting the species *Agriolimax* spp., *Limax* spp. and *Vaginulus* spp., causing damage to a wide variety of crops [4].

In Colombia, pest molluscs are found in thermal floors that have relative humidity higher than 80 %. It is mentioned that molluscs cause damage to the foliage, tubers and roots of plants, with a wide variety of crops attacked [5]. One of the most important cases occurs in the cultivation of coffee, causing lesions in the fruits until they fall, scratching of stems, total wilting of seedlings due to girdling of the stem, among other damages [6]. Additionally, slug attacks occur in many vegetables, including cabbage, cauliflower, lettuce, spinach and chard, reporting damage by *Deroceras reticulatum Muller, Limax marginatus Muller, and Milax gagates Draparnaut* [5]; these species of slugs also cause damage to black and criolla potato crops [7]. In the case of annual fruit trees for fresh consumption, damage to blackberry crops is reported, which is attributed to *M. gagates* [8].

The municipality of Pamplona, Norte de Santander, has a marked inclination of agricultural production for fruit and vegetable products, as it has adequate agroecological conditions for the production of fruits and vegetables, niches where molluscs that can act as herbivores in a wide variety of crops [9]. A large part of the crops in Pamplona show damage caused by slugs and, in the specific case of strawberries, considerable damage [10], which makes it necessary to carry out treatments with chemical products such as metaldehyde, which, in addition to being expensive, contaminates the harvest and the ambient.

Under the conditions of Pamplona, the damage caused by molluscs in the cultures had been attributed to *M. gagates, D. reticulatus, Arion subfuscus Draparnaud* [11]. Subsequently, the ISER (CO) carried out studies on the slug species *Arion distinctus* (Mabille) considering it to be important in the region and damages are currently reported in orchards and gardens by the garden snail *Helix aspersa Muller* [12], [13].

On the other hand, in a study carried out recently, the following species of slugs have been identified, affecting numerous crops, including vegetables, peas, potatoes, strawberries and fruit trees: from the Agrolimacidae family, *Deroceras reticulatum* and *Deroceras laeve*, from the Limacidae family, the species *Limax* sp. and from the family Arionidae with the species *A. distictus* [14]. In another work, an evaluation of damage caused by slugs in strawberry cultivation in Pamplona was carried out. The incidence levels of fruits damaged by slugs in the plots were significant at low populations (0.1 and 2 individuals/traps) increasing up to 40% when the populations exceeded 6 individuals/traps. Monetary losses were estimated at $50 000 000/ha with rates up to 2 slugs/trap [10].

A. *About Arion spp.*

The order Pulmonata was proposed by Blainville in 1814; reference is made to gastropods that adapted to life on earth, which is why they have pulmonary respiration, although the term is not currently shared by many authors. The anatomy of land slugs is very similar to that of snails and a large number of mollusks. In their lower part they have the foot, the muscular and flat organ that through contractions allows the movement of the animal. To facilitate locomotion, they secrete a mucous substance, which has given them the popular name of slugs. Like snails, they have two antennae on the front of their heads. These antennae have sensory functions: the larger pair has light receptors that function similar to eyes, and the smaller pair detects odors [4].

Within the genus *Arion*, there are species of mollusks in the order *Stylommatophora* of the family Arionidae. For example, the group *A. hortensis* Ferussac species are among the most important slug pests of field crops in the UK, France and other parts of continental Europe. *A. hortensis* and *A. distinctus* are considered sister species that were considered as a species complex under the name *A. hortensis* for a long time but later the complex was divided into three species: *A. hortensis*, *A. distinctus* and *A. owenii* [15]. For Kentuchy USA, six *Arion* species are reported; *A. Rufus*, *A. intermedius*, *A. distinctus*, *A. hortensis*, *A. fasciatus* and *A. susfuscus*, posing difficulties on many occasions for the morphological identification of the species [16].

As previously mentioned, the species of *Arion* initially reported in vegetable crops in the Province of Pamplona was *A. subfuscus* [11]; however, due to the importance of *A. distinctus* in Pamplona, according to other authors, investigations were carried out to control this spice with diatomaceous earth [12]. This slug is similar to the morphological characteristics of *A. subfuscus*, so its precise identification is difficult since it belongs to the *Arion* complex that groups species that are closely similar [16], demanding meticulous identification by an expert on the subject. However, [17] also reported *A. distinctus* as the species of this genus present in Pamplona. In other control works with agricultural lime and diatomaceous earth, results were published, also considering *A. distinctus* as the present species [18].

In a subsequent study, species belonging to the family Arionidae, genus *Arion*, were observed, but it was preferred not to define the species, recommending that molecular studies be carried out to specify the species present. The morphospecies named Arion sp1. was found in the nine crops sampled in Pamplona (peas, bulb onion, strawberry, bean, blackberry, Creole potato, black potato, tree tomato and carrot) and in the five villages studied. Arion sp1. had a size of approximately 4.5 cm, and was characterized by presenting a pneumostome positioned in the anterior zone of the mantle, it did not have the presence of a keel, however, the keel zone was surrounded by a membrane, the posterior end of the body was smoothly rounded and the presence of a caudal gland (shaped like a triangle) was observed [19].

The common garden slug *A. distinctus* is described as yellow-gray in color with a bluish-black head and tentacles. Towards the rear end it has a yellowish stripe in the center of the back and stripes around the sides of the body with a length of 3 cm, it reproduces during almost the whole year and can be an important pest of the gardens since it attacks to cultivated plants, fruits, tubers and bulbs. They come out at night and spend the day in humid places under stones, logs and other objects that protect them from the heat. *Arion* slugs are related to snails, the shell being reduced to a group of calcareous granules below the mantle that appears as a bulge on the upper surface and anterior part of the slug [20].

B. *Alternative Slug Control*

According to the Food and Agriculture Organization of the United Nations, the main objective of world agriculture is to provide a considerable amount of food to be able to satisfy the food demand worldwide, due to the fact that in recent years the population rate has been increasing considerably, which is why a goal has been proposed requiring agriculture to increase its production [21]. These precedents justify the importance of conducting research that seeks results to provide fruit and vegetable growers with alternatives for managing slugs with plant resources, such as eucalyptus trees, abundant in the agroecosystem of the Pamplona villages, [22] since it is stated that among the possibilities of non-chemical alternatives for slug control is the use of plant extracts and essential oils from the eucalyptus tree [23].

Several authors reported that *E globulus* essential oil exhibited antibacterial activity [24]-[26]. On the other hand, the fungicidal effect of the essential oil of *E globulus* has been demonstrated for the prevention of postharvest diseases of strawberries [27]. The essential oil of *E globulus* also presented significant contact toxicity against the insect *S. zeamais*, which was reduced by more than 50% at concentrations of 4 8% and 8%, while toxicity as a fumigant showed mortalities greater than 70% in the treatments. to 35 µL/L [28].

In Brazil, hydrolates and essential oils of different *Eucalyptus* species were evaluated as molluscicides against adult and spawning snails of *Biomphalaria glabrata* and cercariae of *Schistosoma mansoni*. These products were obtained by steam stripping. Of the 21 hydrolates tested, eight were active in snails, nine showed action in spawning, and three showed activity in cercariae by the 1:4 (V/V) dilution. Of the oils studied, 11 had activity for planorbids and spawning at concentrations of 20 ppm (w/v). *Eucalyptus deanei* hydrolate was active on snails, spawns and cercariae at the 1:4 dilution, and the oil at a concentration of 20 ppm [29].

Given this situation, the following scientific question arises: What effect could formulations based on essential oils of two *Eucalyptus* species, abundant in the Pamplona area, have against *Arion* spp slugs under laboratory conditions?

Taking this background into account, the objective of this research was to evaluate the effect of *Eucalyptus globulus* and *Eucalyptus cinerea* essential oil formulations in the control of *Arion* spp.

II. Methodology

The experimental research was carried out in the laboratory of the Center for Biosupplies and Plant Health (CISVEB) on the main campus of the University of Pamplona. The collection of the leaves of *Eucalyptus globulus* and *Eucalyptus cinerea*, was carried out in the main campus of this University and the slugs in a strawberry field near the town of Pamplona. The adult leaves of each eucalyptus species taken from the basal third of the trees were conveniently washed, divided and placed in a hydrodistiller to obtain the essential oil. From these, the formulations of each species were obtained in the Biocalorimetry laboratory of the University of Pamplona.

A. *Population and method*

For the laboratory experiments, individuals of the *Arion* spp. species were used, which were kept in quarantine, fed with lettuce for 15 days. After that time, 50 healthy adult individuals were selected, without strange symptoms, as uniform in size as possible (3 cm to 4 cm) per treatment.

B. *Comparison of the effect by contact and ingestion against the Arion spp. Species*

To compare the efficacy of essential oils on *Arion* spp., two trials were conducted with a completely randomized experimental design with five treatments (four with the formulated ones and one control with 10 repetitions). One trial was conducted to assess the contact effect and another for the ingestion effect, but the treatments were similar.

From the stock solutions of the formulated *Eucalyptus globulus* at 9110.1 ppm and *Eucalyptus cinerea* at 9151.5 ppm delivered by the Natural Resources research group of the University of Pamplona, the 4 treatments listed in the Table 1.

Table 1.
Treatments of the formulations of two species of Eucalyptus, by contact and ingestion in vitro conditions.

Treatments	
1	Eucalyptus globulus formulation at 100 ppm.
2	Eucalyptus globulus formulation at 200 ppm.
3	Eucalyptus cinerea formulation at 100 ppm.
4	Eucalyptus cinerea formulation at 200 ppm.
5	Control without treatment.

Source: Authors.

The spraying to evaluate the formulations according to the treatment 100 ppm and 200 ppm of *Eucalyptus globulus* or *Eucalyptus cinerea* were carried out with a manual sprayer and the slugs were fed in plastic containers with lids of 500 mL.

To evaluate the effect by contact, the preparation was applied to the lid of the 500 mL container, then 5 *Arion* spp. slugs were introduced and fed with fresh food (*Batavia* lettuce) in the 10 vials or repetitions of each treatment. Every three days the lids of the containers were sprayed with the oil concentrations (except in the control), supplying the new untreated food (10 g of lettuce/replication).

To determine the efficacy in the ingestion effect test, the five specimens of *Arion* spp. were deposited in the containers, they were supplied with 10 g of lettuce, which were previously treated with the corresponding formulation according to the respective treatments for three days and every three days this same process was repeated.

These operations were carried out placing new containers every three days for both ingestion and contact to avoid contamination by fungi or other microorganisms.

In the two trials, daily observations were made, evaluating the loss of mobility of the *Arion* spp slugs and their percentage with respect to the total in the jar, in the same way the percentage of daily and accumulated loss of mobility and mortality was determined. For the discrimination of mortality, the death of the individual unable to carry out some type of movement during the observation time of each day was considered. Observations were made up to 15 days after starting each trial.

The data on mobility affectation (%) and mortality (%) for Arion spp., were transformed into 2 arcsen$\sqrt{\%/100}$ and processed by means of a variance analysis for each trial, the means were compared by the test of Tukey ($P < 0.05$), using the statistical package SPSS (Statistical Package for the Social Sciences) version 21 for Windows [33].

With the mortality percentages of slugs per bottle in each concentration of each formulation of each eucalyptus species, dispersion graphs were made over time using the Microsoft Excel application. The linear trend curve was sought using mortality as the dependent variable and time as the independent variable, as well as the linear regression equation and the coefficient of determination (R2). From the equations, the mean lethal time TL_{50} in days was estimated for each concentration of the formulations for *Arion* spp.

III. Results and Analysis

A. *Mobility affectation by contact and ingestion in Arion spp.*

It was observed that the *E. globulus* treatments at 100 and 200 ppm and *E. cinerea* at 200 ppm carried out by contact showed an effect on the mobility of *Arion* spp. slugs from the 4th day after application and *E. cinerea* at 100 ppm on the 6th day. The ANOVA result showed statistically significant difference between the *E. globulus* treatments at 100 and 200 ppm and *E. cinerea* at 200 ppm with *E. cinerea* at 100 ppm and the control, on the 4th and 5th day. On the 6th day, the best treatments were *E. globulus* at 100 and 200 ppm and *E. cinerea* at 200 ppm, since all differ from the control, although no difference was observed between *E. globulus* at 100 ppm and *E. cinerea* at 100 ppm. After the 8th day of starting the experiment, all the treatments showed statistical differences with the control in relation to mobility, obtaining the highest percentages for *E. globulus* at 100 ppm and 200 ppm and *E. cinerea* at 200 ppm (Table 2).

In the treatments by ingestion, affectation of the mobility of the slugs was observed from the third day of the beginning of the trial, however, the ANOVA did not reflect a statistical difference with the control at that moment. For the 4th day, it is observed that all the treatments differ from the control, resulting in the most affected treatments *E. globulus* at 200 ppm and *E. cinerea* at 200 ppm, which differed from *E. globulus* at 100 ppm, but not from *E. cinerea* at 100 ppm, but all treatments differed from the control. However, the 5th, 6th, 7th and 8th day of all treatments differ with the control and not with each other (Table 3).

TABLE 2.
MOBILITY AFFECTATION OF THE ARION SPP WHEN USING THE FORMULATIONS BY CONTACT.

Treatments		Movility (%)				
		D4	D5	D6	D7	D8
1	E. globulus at 100 ppm	12ab	12bc	22ab	22bc	40ab
2	E. globulus at 200 ppm	22a	28a	34a	42ab	54a
3	E. cinerea at 100 ppm	0b	0c	14bc	14cd	24b
4	E. cinerea at 200 ppm	22a	22ab	32a	58a	48a
5	Control	0b	0c	0c	0d	0c
	CV (%)	1.003	0.889	0.611	0.557	0.44
	ET*	3.55	3.48	3.94	4.08	4.62

* Different letters in the columns indicate differences for treatments according to the Tukey test ($P \leq 0.05$).
Source: Authors.

TABLE 3.
MOBILITY AFFECTATION OF THE ARION SPP WHEN USING THE FORMULATIONS BY INGESTION.

Treatments		Movility (%)					
		D3	D4	D5	D6	D7	D8
1	E. globulus at 100 ppm	22a	22b	36a	40a	50a	52a
2	E. globulus at 200 ppm	24a	34a	40a	48a	48a	46a
3	E. cinerea at 100 ppm	16a	30ab	38a	42a	54a	50a
4	E. cinerea at 200 ppm	24a	34a	44a	42a	50a	46a
5	Control	0a	0c	0b	0b	0b	0b
	CV (%)	0.53	0.34	0.32	0.26	0.27	0.29
	ET*	2.93	2.59	3.18	2.87	3.46	3.66

* Different letters in the columns indicate differences for treatments according to the Tukey test ($P \leq 0.05$).
Source: Authors.

B. *Arion spp. Mortality by contact and ingestion*

The ANOVA analysis showed that there was mortality on the 5th day in the treatments based on *E. globulus* at 200 ppm and *E. cinerea* at 200 ppm, but these did not differ from the control. On the 6th, 7th and 8th day, all the treatments did not differ from the control except the *E. cinerea* treatment at 200 ppm. From the 9th day to the 14th day it is observed that all the treatments differ among themselves and from the control. For the 15th day, the treatments show a sustained control of the mortality percentage without statistically differing between them, but with the control (Table 4).

TABLE 4.
ARION SPP. MORTALITY WHEN USING FORMULATIONS BY CONTACT.

Treatments		Mortality (%)										
		D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15
1.	Eucalyptus globulus at 100 ppm	0a	0b	4b	12ab	20bc	44a	62a	70a	86a	94a	98a
2.	Eucalyptus globulus at 200 ppm	2a	6ab	14ab	18ab	32ab	48a	58a	68a	80a	84a	92a
3.	Eucalyptus cinerea at 100 ppm	0a	0b	0b	0b	4cd	18b	28b	40b	58b	66b	88a
4.	Eucalyptus cinerea at 200 ppm	2a	12a	20a	24a	40a	46a	62a	80a	88a	92a	96a
5.	Control	0a	0b	0b	0b	0c	0c	0c	0c	0c	0c	0b
	CV (%)	5	2.1	1.4	1.3	0.79	0.4	0.4	0.3	0.2	0.2	0.1
	ET*	1.2	2.4	3.48	4.51	4.8	4.32	5.6	4.9	4.6	4.4	3.2

* Different letters in the columns indicate differences for treatments according to the Tukey test ($P \leq 0\ 05$).
Source: Authors.

The mortality percentage of *Arion* spp. by contact presented a linear adjustment as a function of time for all treatments, that is, the two concentrations of the two *Eucalyptus* species, obtaining values of determination coefficients greater than 0.75, which allowed the use of the regression equations to estimate the lethal time of each of the treatments (Fig. 1).

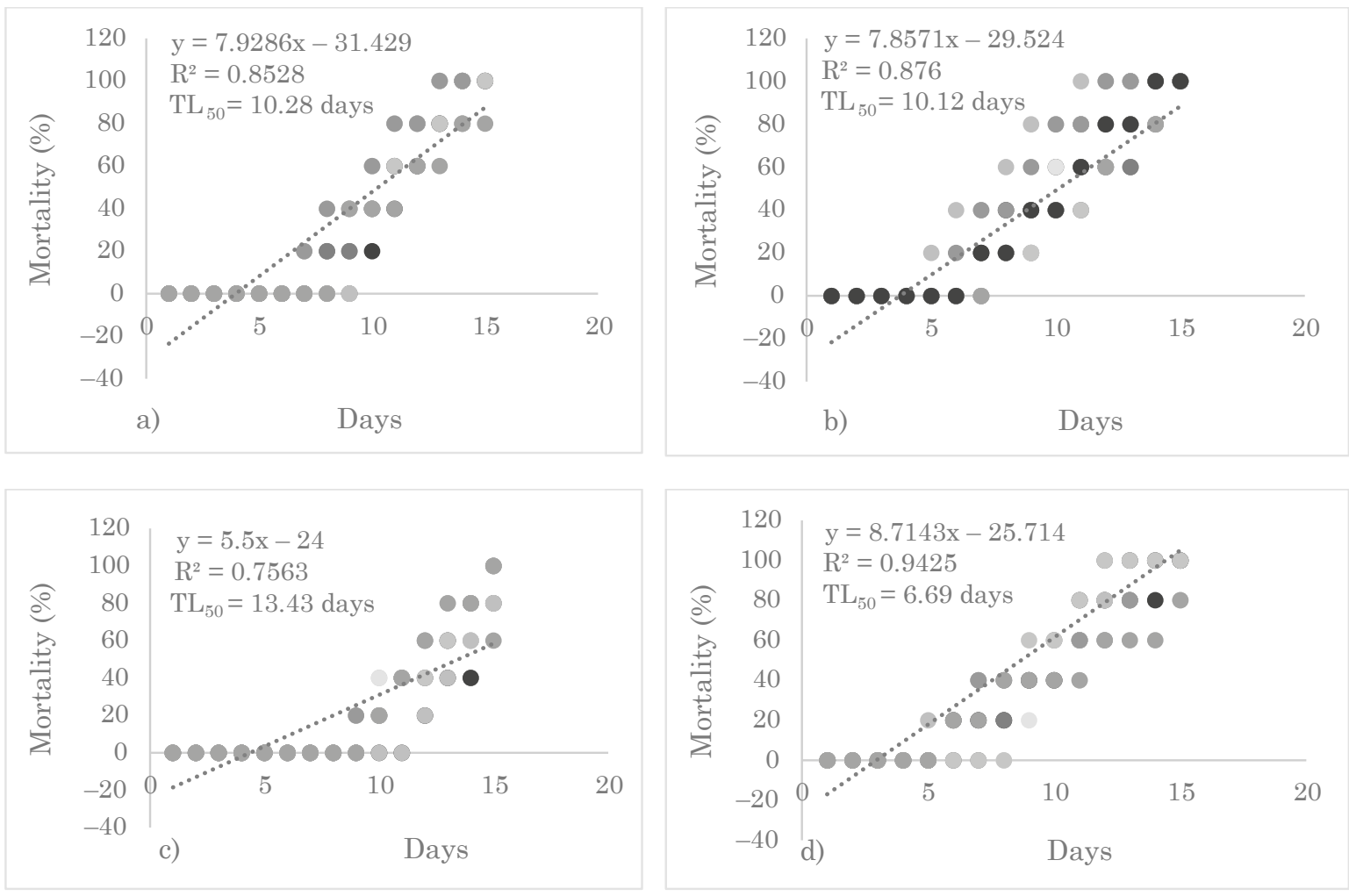

Fig. 1. Lethal Time values in days (TL 50) of the different concentrations of essential oils against Arion spp by contact action: a) E. globulus at 100 ppm, b) E. globulus at 200 ppm, c) E. cinerea at 100 ppm and d) E. cinerea at 200 ppm.
Source: Authors.

E. cinerea at 200 ppm showed the lowest relative values of TL_{50}, of 6.69 days, it means that at this time a 50% mortality of the *Arion* spp. slug population is achieved. The rest of the treatments had a more discreet behavior with respect to the TL_{50}, although it should be noted that the TL_{50} were similar for *E. globulus* at 100 and 200 ppm, while the TL_{50} for *E. cinerea* at 100 ppm was relatively high 13.43 days. It was interesting that in the case of *E. globulus* the TL_{50} for the two concentrations of the formulations were similar.

In the ANOVA it was possible to observe that the *E. globulus* treatments at 100 ppm and 200 ppm and in *E. cinerea* at 100 ppm and 200 ppm carried out by ingestion showed an effect on the slugs showing mortality of *Arion* spp. on day 2 after the application, but they did not differ from the control. However, on day 3 it is observed that the *E. cinerea* treatment at 200 ppm differs from the other treatments and from the control, however, on the 5th, 6th and 7th day all the treatments differ with the control and not with each other. On the 8th and 9th day the best treatment was *E. cinerea* at 200 ppm, although on the 8th day it did not differ from *E. globulus* at 200 ppm and on the ninth from this and from *E. globulus* at 100 ppm. From the tenth to the 15th day, the treatments show a sustained growth in the mortality percentage without statistically differing between them, but with the control (Table 5).

Table 5.
Arion spp. mortality when using formulations by ingestion.

Treatments		Mortality (%)													
		D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15
1.	E. globulus at 100 ppm	0a	0b	8b	26a	30a	30a	36b	46ab	60a	64a	80a	86a	92a	94a
2.	E. globulus at 200 ppm	2a	10a	10b	28a	34a	38a	44ab	56ab	68a	78a	88a	92a	94a	98a
3.	E. cinerea at 100 ppm	0a	0b	4b	18a	28a	30a	36b	42b	54a	60a	74a	80a	90a	94a
4.	E. cinerea at 200 ppm	6a	14a	22a	30a	36a	44a	52a	62a	68a	76a	84a	90a	94a	96a
5.	Control	0a	0b	0b	0b	0b	0b	0c	0c	0b	0b	0b	0b	0b	0b
	CV (%)	3.22	1.33	1.03	0.68	0.41	0.41	0.4	0.36	0.38	0.32	0.23	0.18	0.13	0.1
	ET*	1.63	2.02	289	4.41	3.34	3.71	3.9	4.76	6.02	5.66	4.81	4.1	3.14	2.4

* Different letters in the columns indicate differences for treatments according to the Tukey test ($P \leq 0.05$).
Source: Authors.

The percentage of mortality of *Arion* spp. by ingestion presented a linear adjustment as a function of time for all treatments, that is, the two concentrations of the two *Eucalyptus* species, obtaining values of determination coefficients greater than 0.90, which allowed the regression equations to be used to estimate the lethal time of each of the treatments (Fig. 2).

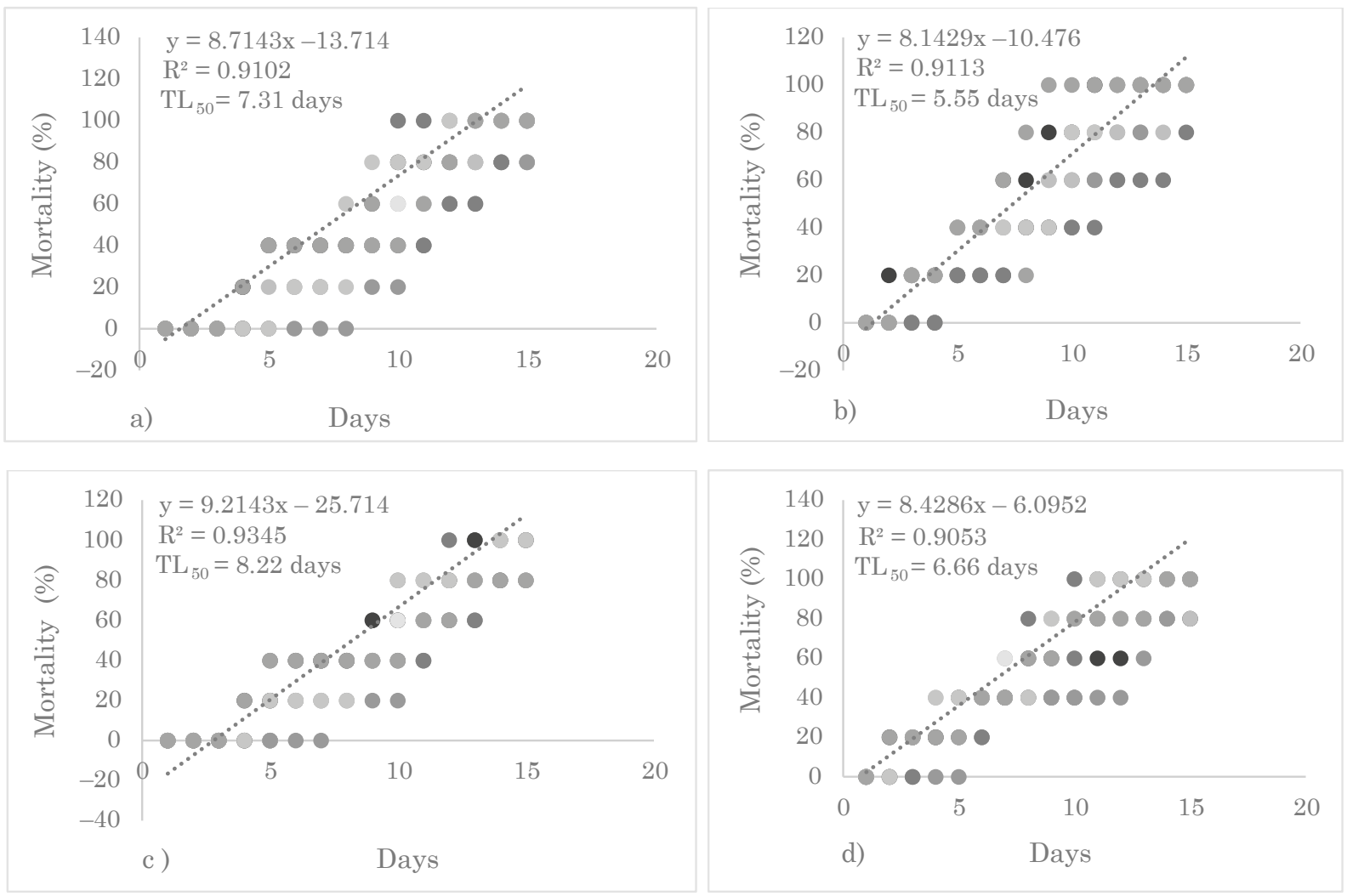

Fig. 2. Lethal Time values in days (TL 50) of the different concentrations of essential oils against Arion spp by ingestion action: a) E. globulus at 100 ppm, b) E. globulus at 200 ppm, c) E. cinerea at 100 ppm and d) E. cinerea at 200 ppm.
Source: Authors.

E. cinerea at 100 ppm showed the highest relative values of lethal time (TL_{50}) of 8.22 days, that is, 50% mortality of the *Arion* spp. slug population is achieved at that time. For the *E. globulus* treatment at 100 ppm, it obtained 50% of the mortality of the population at 7.3 days and for *E. globulus* at 200 ppm it was very similar with 6.6 days, therefore, the treatment that showed lower relative values of lethal time (TL *50*) was *E. globulus* at 200 ppm with 5.5 days to reach a mortality of 50% of the slug population. Interestingly, for both *E. globulus* and *E. cinerea* the TL_{50} were relatively lower for the highest concentrations (200 ppm).

IV. Discussion

It was already known that the essential oil of *Eucalyptus* species manifested antibacterial activity in general [24]-[26], fungicidal activity against strawberry postharvest fungi [27], and also insecticidal activity against *S. zeamais* [28], and that in a review [23], refers to the use of plant extracts and essential oils from the eucalyptus tree among the possible non-chemical alternatives for slug control, since eight species of *Eucalyptus* different from those studied in this research were reported in Brazil. with a molluscicidal effect [29], however, slugs of the genus *Arion* were not studied.

Also in a recent review on the possibilities of using essential oils and their components as promising for the control of gastropod molluscs, the use of *E camabndulenses* and its metabolites 4-terpineol (+)-4-Carene, γ- terpinene, p-Menth-1-en-8-ol, for its action on the terrestrial mollusk *Theba pisana* and *E. globulus* and its metabolites 1,8-Cineole, α-Pinene on the aquatic mollusk *Bulinus truncatus* [30].

With the present results, the potentiality of the essential oils of two eucalyptus species (*E. globulus and E. cinerea*) for the control of molluscs is confirmed, since in preliminary results the reduction of slug populations in strawberry had been verified from of rustic baits made with extracts of *E. globololus* and *E camandulensis* under the conditions of Pamplona [31].

It has been reported that metaldehyde-based formulations work mainly by ingestion and do not always have the desired efficacy against the small gray slug *D. reticulatum*, which depends on the concentration of the product, its attractants, and soil moisture [32], which is why it recommends the search for other alternatives to control pest slugs.

These results show that the essential oils of eucalyptus species are candidates for the control of slugs in crops in Pamplona where *E. globulus and E. cinerea* are abundant [22], and are added to the alternatives of other authors such as soil of diatoms [18], which are not always available to farmers because high doses are recommended, which is why it is an expensive option in practice.

The investigations should continue under field conditions based on the promising results obtained in laboratory conditions, evaluating those formulated in different crops, for which the links with the chemistry laboratory of the University of Pamplona must be maintained, with a view to working in the field, improvement of the formulations that allow to define the effectiveness and its persistence in field conditions.

As we have been able to appreciate the relative values of the TL_{50} in the case of *Arion* spp. they were lower by ingestion than by contact for the two formulations of the two Eucalyptus species (*E. globulus and E. cinerea*), an aspect to take into account to decide the type of formulation to apply and the way to carry out the treatments in the field.

V. Conclusions

1) The affectation of the mobility for *Arion* spp., was observed in a very similar way for all the treatments of *E. globulus* and *E. cinerea* at concentrations of 100 ppm and 200 ppm.

2) The formulations of both Eucalyptus species at the two concentrations (100 ppm and 200 ppm) caused mortality of Arion spp by contact from the 9th day and by ingestion on the 4th day, reaching mortalities higher than 85% on the 15th day by contact, and 95% by ingestion on the 14^{th} day.
3) Linear regression equations were obtained with significant coefficients of determination between slug mortality and exposure time, which allowed estimating TL_{50} values by contact between 10.31 and 10.12 days for the essential oil of *E. globulus* and between 13.31 and 6.69 days for *E. cinerea*, depending on the concentration, while by ingestion the TL_{50} were relatively lower, varying between 7.31 and 5.55 days, for the essential oil of *E. globulus* and between 8.22 and 6.6 days for *E. cinerea* at 100 and 200 ppm respectively.

References

[1] M. Serre, Manejo de babosas en el cultivo de girasol en siembra directa, Pioneer Arg. S.A., 2005. Recuperado de https://www.pioneer.com/CMRoot/International/Argentina_Intl/AGRONOMIA/con_agric_inv_lotes/IL_Manejobabosa_girasol_05.pdf

[2] N. Herrera y L. Castellanos, "Informe sobre la incidencia de moluscos plaga en organopónicos del municipio de Cienfuegos, Cuba", *Cent. Agrícol.*, vol.40, no. 1, pp. 89–90, Ene. 2013.

[3] N. Herrera, B. López, L. Castellanos y I. Pérez, "Incidencia de los moluscos plagas en los organopónicos del municipio de Cienfuegos", *Cent. Agrícol.*, vol. 40, no. 4, pp. 49–55, Oct. 2013.

[4] V. Cañedo, A. Alfaro y J. Kroschel, *Manejo integrado de plagas de insectos en hortalizas. Principios y referencias técnicas para la Sierra Central de Perú*, LIM, PE: CIP, 2011. https://doi.org/10.4160/9789290604075

[5] C. Vásquez, Manejo fitosanitario del cultivo de hortalizas. Medidas para temporada invernal, BO, CO: ICA, 2012. Recuperado de https://www.ica.gov.co/getattachment/bb883b42-80da-4ae5-851f-4db05edf581b/Manejo-fitosanitario-del-cultivo-de-hortalizas.aspx

[6] L. Constantino, S. Gomes, P. Benavides, "Descripción y daños causados por las babosas Colosius pulcher y Sarasinula plebeia en el cultivo de café en Colombia", *Avanc. Tecn.*, vol. 392, pp. 1–8, Feb. 2010. Recuperado de https://www.cenicafe.org/es/publications/avt0392.pdf

[7] C. Vásquez, *Manejo fitosanitario del cultivo de la papa (Solanum tuberosum subsp. andigena y S. phureja). Medidas para la temporada invernal*, BO, CO: ICA, 2011. Recuperado de https://www.ica.gov.co/getattachment/b2645c33-d4b4-4d9d-84ac-197c55e7d3d0/Manejo-fitosanitario-del-cultiva-de-la-papa-nbsp;-.aspx

[8] C. Vásquez, *Manejo fitosanitario del cultivo de la mora (Rubus glaucus Benth). Medidas para la temporada invernal*, BO, CO: ICA, 2011. Recuperado de https://www.ica.gov.co/getattachment/b7e061eb-ebd3-4f80-9518-c771712405eb/-nbsp3bmanejo-fitosanitario-delcultivo-de-la-mora.aspx

[9] G. Gualdrón, B. Maldonado, D. Espitia y J. García, "Aproximación al caso de desarrollo local de la zona rural del municipio de Pamplona", *FACE*, vol. 17, no. 2, pp. 142–154, Abr. 2017. https://doi.org/10.24054/face.v17i2.543

[10] L. Castellanos y J. Serrano, "Pérdidas económicas por babosas en fresa (Fragaria × ananassa, Duch) bajo las condiciones de Pamplona, Norte de Santander", *FACE*, vol. 20, no. 1, pp. 49–60, Jun. 2020. https://doi.org/10.24054/01204211.v1.n1.2020.4010

[11] C. Pabuence y M. Sanabria, *Caracterización de babosas en hortalizas de la Provincia de Pamplona*, CO, PMP: ISER, 2004.

[12] B. Hernández, N. Guerrero y M. Sierra, "Determinación de los daños en babosas (Arion distinctus) causado por la tierra diatomea a diferentes concentraciones bajo condiciones de laboratorio en el ISER, Pamplona", *Rev. Dist. Al Día*, vol. 1, no. 1. pp. 1–9, May. 2015. Recuperado de http://www.iser.edu.co/iser/hermesoft/portalIG/home_1/recursos/documentos_generales/16102015/inv_revista_al_dia_mayo_2015.pdf

[13] A. Méndez y L. Castellanos, "Eficacia de la tierra de diatomeas contra Helix aspersa en condiciones in vitro en Pamplona, Norte de Santander", *JONNPR*, vol. 2, no. 12, pp. 659–666, Oct. 2017. https://doi.org/10.19230/jonnpr.1698

[14] M. Rizzo, M. Cobos, L. Castellanos & W. Becerra, "Influence of soil conditions on eight crops on the incidence of slugs in the Monte Adentro village, Pamplona, Norte de Santander", *BISTUA*, vol. 17, no. 3, pp. 166–178, Feb. 2019. https://doi.org/10.24054/01204211.v3.n3.2019.3575

[15] J. Iglesias & B. Speiser, "Distribution of Arion hortensis s.s. and Arion distinctus in Northern Switzerland", *J. Molluscan Stud.*, vol. 67, no. 2, pp. 209–214, May. 2000. https://doi.org/10.1093/mollus/67.2.209

[16] A. Thomas, R. Mc Donnell, T. Paine & J. Harwood, *A Field Guide to the Slugs of Kentucky*, LEX, KY: UK, 2010. Available in: https://entomology.ca.uky.edu/files/efpdf4/sr103.pdf

[17] R. Cobos., W. Becerra–Rozo y L. Castellanos, "Riqueza y abundancia de las babosas en cuatro cultivos de Pamplona, Norte de Santander, Colombia", *BISTUA*, vol. 17, no. 2, pp. 229–233, Ene. 2019. https://doi.org/10.24054/bistua.v17i2.251

[18] A. Méndez & C. Castellanos, "Effectiveness of diatomaceous earth and lime on arionids and agriolimacids", *Cienc. y Tecnol. Agropecu.*, vol. 20, no. 3, pp. 579–594, Sep. 2019. https://doi.org/10.21930/rcta.vol20_num3_art:1587

[19] L. Castellanos, J. Serrano y W. Becerra, "Preferencia por morfoespecies de babosas en diferentes cultivos y ambientes del municipio Pamplona, Norte de Santander", *Rev. amb. agua, aire suelo*, vol. 1, no. 1, pp. 1–10, Ene. 2020. https://doi.org/10.24054/aaas.v11i1.356

[20] BIOPEDIA, *Babosa común de jardín (Arion distinctus)*, Julio 4, 2023. [En linea]. Disponible en https://www.biopedia.com/babosa-comun-de-jardin-arion-distinctus/

[21] S. Gliessman, "Transforming food systems with agroecology", *Agroecol. Sustain. Food Sys.*, vol. 40, no. 3, pp. 187–189, Jan. 2016. https://doi.org/10.1080/21683565.2015.1130765

[22] X. Yáñez y O. Cuadro, "Composición química y actividad antibacteriana del aceite esencial de las especies Eucalyptus globulus y E. camaldulensis de tres zonas de Pamplona (Colombia)", *Bistua*, vol. 10, no. 1, pp. 52–61, Ene. 2012. https://doi.org/10.24054/01204211.v1.n1.2012.48

[23] C. Quintero, L. Castellanos & W. Becerra-Rozo, "Possibilities of the alternative management of mollusk pests in agricultural crops. A Review", *INGE CUC*, vol. 18 no. 1, pp. 1–13, Oct. 2021. https://doi.org/10.17981/ingecuc.18.1.2022.01

[24] A. Ponce, R. Fritz, C. Del Valle y S. Roura, "Antimicrobial activity of essential oils on the native microflora of organic Swiss chard", *LWT Food Sci. Technol.*, vol. 36, no. 7, pp. 679–684, Nov. 2003. https://doi.org/10.1016/S0023-6438(03)00088-4

[25] M. Salari, G. Amine, M. Shirazi, R. Hafezi & M. Mohammadypour, "Antibacterial effects of Eucalyptus globulus leaf extract on pathogenic bacteria isolated from specimens of patients with respiratory tract disorders", *Clin. Microbiol. Infect.*, vol. 12 no. 2, pp. 194–196, Feb. 2006. https://doi.org/10.1111/j.1469-0691.2005.01284.x

[26] A. Mossi, V. Astolfi, G. Kubiak, L. Lerin, C. Zanella, G. Toniazzo, D. de Oliveira, H. Treichel, I. Devilla, R. Cansian & R. Restello, "Insecticidal and repellency activity of essential oil of Eucalyptus sp. against Sitophilus zeamais Motschulsky (Coleoptera, Curculionidae)", *J. Sci. Food Agric.*, vol. 91, no. 2, pp. 273–277, Jan. 2011. https://doi.org/10.1002/jsfa.4181

[27] V. Trejo-Ramírez, M. Trejo-Márquez, S. Pascual-Bustamante y A. Lira-Vargas, "Extracción de aceite esencial de eucalipto y su aplicación como agente antifúngico en un envase activo para conservación de frambuesa", *Rev. Iber. Tecnolog. Postcosecha*, vol. 16, no. 2, pp. 228–233, Jan. 2015. Recuperado de http://www.fcb.uanl.mx/IDCyTA/files/volume1/1/3/79.pdf

[28] R. González-Guiñez, G. Silva-Aguayo, A. Urbina-Parra y M. Gerding-González, "Aceite esencial de Eucalyptus globulus Labill y Eucalyptus nitens H. Deane & Maiden (Myrtaceae) para el control de Sitophilus zeamais Motschulsky", *Chilean J. Agric. Anim. Sci.*, vol. 32, no. 3, pp. 204–216, Nov. 2016. https://doi.org/10.4067/S0719-38902016005000005

[29] N. Mendes, N. Araújo, C. de Souza, J. Pereira e N. Katz, "Atividade moluscicida e cercaricida de diferentes espécies de Eucalyptus", *Rev. Soc. Bras. Med. Trop.*, vol. 23, no. 4, pp. 197–199, May. 1990. https://doi.org/10.1590/S0037-86821990000400002

[30] M. Radwan & A. Gad, "Essential oils and their components as promising approach for gastropod mollusc control: a review", *J. Plant Dis. Prot.*, vol. 128, pp. 923–949, Jun. 2021. https://doi.org/10.1007/s41348-021-00484-5

[31] L. Castellanos & E. Mora, "Preliminaries on the use of baits with eucalyptus extracts for the control of slugs in Pamplona, Colombia", *Inf. Process. Lett.*, vol. 3, no. 1, pp. 1–7, Sep. 2020.

[32] C. Salvio, A. Faberi, A. López, P. Manetti & P. Clemente, "The efficacy of three metaldehyde pellets marketed in Argentina, on the control of Deroceras reticulatum (Müller) (Pulmonata: Stylommatophora)", *Span. J. Agric. Res.*, vol. 6 no. 1, pp. 70–77, Jan. 2008. http://dx.doi.org/10.5424/sjar/2008061-295

[33] *SPSS* (version 21). IBM. Disponible en https://www.ibm.com/support/pages/spss-statistics-210-available-download

Karen Yulissa Perilla Rueda. Empresa Agrofuturo. Fortul (Arauca, Colombia).

Leónides Castellanos González. Universidad de Pamplona (Pamplona, Colombia). https://orcid.org/0000-0001-9285-4879

Amanda Lucía Chaparro García. Universidad de Pamplona (Pamplona, Colombia). https://orcid.org/0000-0001-9728-0931