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Effectiveness of diatomaceous earth and lime on arionids and agriolimacids

Eficacia de la tierra de diatomeas y la cal sobre ariónidos y agriolimácidos

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Abstract

The aim of this research is to evaluate the effect of d iatomaceous earth (DE) and lime on *Arion distinctus* under laboratory conditions and on arionids and agriolimacids in the field. Dusting and spraying treatments were carried out to evaluate in the laboratory the effect by ingestion and by contact on the mobility and mortality of the specimens. In the laboratory, three doses of diatomaceous earth (1, 2 and 4 kg/ha), one of agricultural lime (2 kg/ha), and one control were used; and in the field, only the spraying method was evaluated with the same number of variants, but in all cases, doses were doubled. A random block design was used in a 5 x 4 arrangement, and the evaluation was carried out through the trap method, and 20 refuge traps per treatments were placed. Slug populations and the efficiency were established three and seven days after the applications. Tukey's mean comparison test was used for the populations in the treated plots and in the control. A higher effect was obtained by contact compared to ingestion. In both field and laboratory applications, the highest mortalities were observed after seven days, without significant differences between diatomaceous earth and lime. The results obtained show that DE is useful in the control of these mollusk species, and better results are obtained with two applications.

Keywords: *Arion distinctus*, disease surveillance, mortality, noxious molluscs, slugs

Resumen

El objetivo de la presente investigación fue evaluar el efecto de la tierra de diatomeas (TD) y la cal sobre *Arion distinctus* en condiciones de laboratorio, y sobre ariónidos y agriolimácidos en campo. Se realizaron tratamientos por espolvoreo y aspersión para evaluar su efecto por ingestión y contacto en el laboratorio, y se registró la movilidad y la mortalidad de los ejemplares. En laboratorio se emplearon tres dosis de tierra de diatomeas: 1, 2 y 4 kg/ha, una de cal agrícola a 2 kg/ha, y un testigo; en campo solo se evaluó el método por aspersión con la misma cantidad de variantes, pero en todos los casos se duplicaron las dosis. Se utilizó un diseño de bloque azar en arreglo 5 × 4 y la evaluación se llevó a cabo a través del método de trampas; para ello, se colocaron 20 trampas de refugio por tratamientos. Se determinaron las poblaciones de babosas y la eficacia a los tres y siete días después las aplicaciones. Se aplicó una prueba de comparación de medias de Tukey para las poblaciones en las parcelas tratadas y el testigo. Se obtuvo mayor efecto por contacto que de ingestión. Tanto en las aplicaciones en campo como en laboratorio, las mayores mortalidades se observaron a los siete días, sin diferencias significativas entre la TD y la cal. Los resultados obtenidos muestran que la TD es útil en el control de estas especies de moluscos y su resultado es mejor con dos aplicaciones.

Palabras clave: *Arion distinctus*, babosas, moluscos nocivos, mortalidad, vigilancia de enfermedades

Introduction

After insects and mites, snails are the group of invertebrates against which biological control programs direct their attention (Nicholls, 2008). However, studies on population levels and damages due to pest mollusks and their bioregulators have not been established in many geographical areas.

Some of these mollusks are intermediate hosts of intestinal parasites, such as the trematodes of the genera *Schistosoma* Weinland (Trematoda: *Diplostomida: Schistosomatidae*) and Fasciola Linnaeus (Trematoda: Plagiorchiida: Fasciolidae), and nematodes such a*s Angiostrongylus* Kaminsky (Nematoda: Metastrongyloidea) (Núñez, 2006).

In Colombia, two species of mollusks have been identified attacking coffee plantations: *Colosius pulcher* (Colosi) and *Sarasinula plebeia* (Fischer) (Constantino, Gomes, & Benavides, 2010). In potato cultivation, there are reports of the small slug *Deroceras reticulatum* (Müller), the striped brown slug *Limax marginatus* (Müller), the gray slug *Milax gagates* (Draparnaud) and the flat slug *Veronicella* spp. (Instituto Colombiano Agropecuario [ICA], 2011). In vegetables, *Deroceras reticulatum*, *Limax marginatus*, and *M. gagates* have been reported (Instituto Colombiano Agropecuario [ICA], 2012). Although in the conditions of Pamplona, department of Norte de Santander, a great number of crops are damaged by slugs, few articles are found regarding this plague, including a work published by Hernández, Guerrero and Sierra (2015) on the slug species *Arion distinctus* (Mabille) that thrives in the region, and the study of Méndez and Castellanos (2017) on the garden snail *Helix aspersa* Muller.

In agriculture, the control of terrestrial gastropod pests is carried out almost exclusively using chemical pesticides found as powder and baits (pellets) containing between 2% and 8% of metaldehyde or carbamates (Bailey, 2002). On the contrary, in Cuba, natural products have been proposed, e.g., Solasol obtained from *Solanum globiferum* Dunal (Solanaceae) (Alfonso et al., 2000).

Sustainable agriculture requires new control alternatives to reduce or eliminate the application of traditional pesticides based on the use of fumigants and persistent chemicals with high environmental impact. The current trends in integrated pest management (IPM) are oriented towards the preservation of the environment together with the use of low impact methods and natural pesticides of low toxicity, among which diatomaceous earth (DE) is highlighted (Dal Bello, Padín, Juárez, Pedrini, & De Giusto, 2006). The insecticidal activity of DE depends on its physicochemical characteristics, such as the SiO_2 content, the particle size, the lipid adsorption capacity and the presence of impurities (clays), among others (Bilbao, Mañá, & Murúa, 2007).

Although there is much publicity on the effects and insecticide and molluscicide efficiency of DE, scientific information is scarce, so it is necessary to carry out research to verify their effects on different mollusk species, the level of control obtained, the dosage and the form of use, as well as to verify the usage recommendations of 2 kg/ha given by some commercial companies such as Agropulí (2016). Accordingly, the aim of this research was to evaluate the effect of DE and lime on *A. distinctus* under laboratory conditions and arionids and agriolimacids in the field.

Materials and methods

This research was developed in the laboratory of Faculty of Agricultural Sciences, Centro de Bioinsumos y Sanidad Vegetal (Cisveb) of Universidad de Pamplona, Pamplona municipality, department of Norte de Santander, Colombia.

For the laboratory experiments, individuals of the species *Arion distinctus* Mabille (Mollusca: Gastropoda: Arionidae) were used, because it is one of the most abundant mollusks found in several crops in Pamplona, and because it has already been used in laboratory tests (Hernández et al., 2015). The specimens were obtained from the farm Quebrada Seca located in the Monteadentro settlement (07°21.676' N; 072°39.829' W), at an altitude

of 2,573 m above the sea level in a strawberry (*Fragaria* var. Albion (Rosaceae)) plantation; the individuals were kept under quarantine conditions and fed with lettuce for 15 days. After that time, healthy adult individuals as uniform in size as possible, i.e., between 3.5 and 4.5 cm, were selected.

Four laboratory experiments were carried out: two employing the spraying method and two using the dusting method; in each treatment, the action mode, i.e., by ingestion and by contact, was evaluated on the mobility and the mortality of the slug. Three doses of DE (1, 2, and 4 kg/ha), one of agricultural lime (2 kg/ha), and one control without treatment were used to assess the effectiveness of the spraying and dusting treatments by contact and by ingestion against *A. distinctus* under *in vitro* conditions.

The treatments of the four experiments were arranged in a completely randomized design with five treatments and eight repetitions. The experimental unit (repetition) was composed of a 500 mL plastic container, in which five specimens of *A. distinctus* were placed.

The spraying treatments were carried out with a manual sprayer at the doses mentioned with suspensions of agricultural lime and DE depending on the inner surface of the containers, estimating a final solution of 200 L/ha. The five treatments established were applied, and the control was left untreated.

After spraying the containers and their covers, a waiting period of 30 minutes was left for the treatments to dry. After that period, five specimens of *A. distinctus* were placed with tweezers, staying in contact with the product for one hour. Then, they were given untreated lettuce (*Lactuca sativa* L., Asteraceae) leaves, enough for two-days of feeding to establish the effect by contact.

To establish the effect by ingestion, five specimens of *A. distinctus* were placed in clean containers with tweezers, supplying them for two days with lettuce leaves that were sprayed with DE and agricultural lime according to the corresponding treatments. Spraying and food (lettuce) supply were carried out every two days, passing the individuals to new containers to avoid contamination by fungi or other microorganisms caused by the excrement of the mollusks (Herrera, López, Castellanos, & Pérez, 2013).

For the dusting experiments, the treatments were carried out manually by spraying the containers with converted doses according to calculations obtained *in vitro* from the surface of the container, to guarantee the expected treatments, i.e., 1, 2 and 4 kg/ha of DE, one of agricultural lime (2 kg/ha), and a control without treatment.

To establish the effectiveness of the effect by ingestion, five specimens of *A. distinctus* were placed with tweezers in the containers, feeding them with lettuce leaves treated or sprayed by hand for two days with DE and agricultural lime according to the corresponding treatments.

To determine the effectiveness of the effect by contact, the containers and their covers were sprayed, leaving the containers to dry for 15 minutes. Then, five specimens of *A. distinctus* were placed with tweezers in the containers. After 30 minutes, enough food was given for two days (untreated lettuce leaves) to the individuals in each container. These operations were performed with new containers every two days for both the ingestion and the contact treatments, to avoid contamination by fungi or other microorganisms.

To establish the effectiveness by contact and by ingestion with the spraying and dusting treatments, daily observations were made, establishing for *A. distinctus* the simple percentage of mortality and the effect on mobility. For mortality discrimination, the individuals incapable of performing any movement specifically concerning the cephalic tentacles during the observation days were considered dead. For mobility, slow movements and lack of appetite were considered as effects.

The two experiments were evaluated both for spraying and dusting as well as for the effect by contact and

by ingestion in each plastic container, three, seven, 10, and 15 days after the start of the tests. In each moment, alive and dead individuals were counted, extracting all the dead individuals of each container to carry out further evaluations correctly. The data in percentage of mortality and mobility for *A. distinctus* were transformed in 2 arcsen$\sqrt{}$%/100, and they were processed employing an analysis of variance for each test, whose means were compared by Tukey´s test ($p < 0.05$).

Verification of the effectiveness of diatomaceous earth on slugs under field conditions (strawberry plantation)

For the evaluation of the effectiveness of DE, an experiment was conducted in a strawberry plantation (*Fragaria* var. Albion) of three months of age, arranged in a double row plantation frame without plastification. This plantation was located in the farm Quebrada Seca (07°21.676" N; 72°39.829"W) at an altitude of 2.573 m above the sea level, situated in the Monteadentro settlement, municipality of Pamplona, Norte de Santander, Colombia.

A random block design in a 5 ×4 arrangement with five treatments and four replications (plots) was used. The plots consisted of four double 7 m long furrows and a surface of 28 m^2. Before starting the trial, a sampling with refuge traps was carried out to identify the slug species present and establish the initial slug populations.

Sampling

To quantify the population of the mollusks, five traps were placed per replica (plot) for a total of 20 traps/refuges per treatment, which were elaborated manually from 25 mm thick cardboard boxes. Each trap consisted of a square of this material of 25 × 25 cm fastened with a metal rod of 0.30 cm in diameter and 40 cm in length forming an "L," to avoid possible displacements by the wind or by animals in the strawberry plantations (figure 1). This trap design intended to create a cool, humid, and suitable environment for slugs; the traps were labeled and placed randomly on the soil of each plot.

The traps were placed in the afternoon, and the counting was carried out the following morning. Each trap was reviewed, recording the number of living individuals present in each trap according to respective treatments. The species present were identified according to the key published by Thomas, McDonnell, Paine and Harwood (2010).

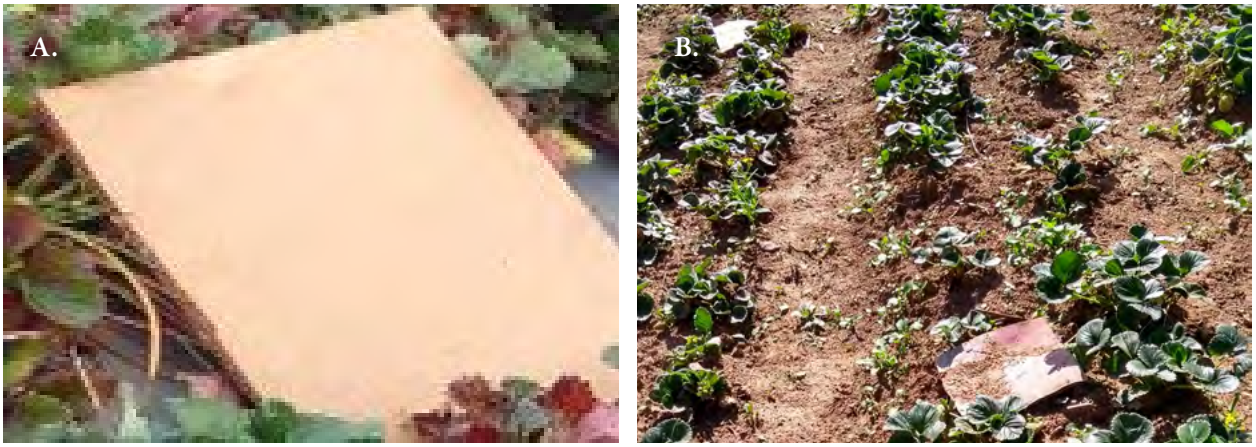

Figure 1. Slug trap. a. Cardboard or refuge trap of 25 × 25 cm; b. Trap in the strawberry field.

Three doses of DE and one dose of agricultural lime were evaluated, which were duplicated concerning the ones employed *in vitro*, including the results obtained and also the impossibility of applying these through dusting, where the best *in vitro* mortalities were obtained. Treatment application was carried out with a motor pack STHIL SR 430 of 14 L capacity, with a final solution of 200 L/ha.

The treatments were carried out in the afternoon hours, guaranteeing that there was moisture in the soil. Two applications were carried out, the second eight days after the first. In this second application, the agricultural adhesive PEGAL 18 CS (ethoxylated fatty alcohol) was added at 767 g/L. Slug population sampling was done three and seven days after each treatment application.

The effectiveness of each treatment per plot was established three and seven days after each application, for which the Abbott formula was used (Püntener & Zahner, 1981):

$$\textit{Effectiveness (\%):}\ (C\text{-}T)\ /C\ *100 \qquad (1)$$

Where *C* is the population level in the control treatment, and *T* is the population level in the treatment.

An analysis of variance was performed with the initial sampling population, and the means were compared using Tukey´s test ($p < 0.05$). The population data of the slugs after the treatments were also processed using an analysis of variance for each test, whose means were also compared by Tukey's test ($p < 0.05$). For all statistical analyses, the statistical package SPSS (Statistical Package for the Social Sciences) version 21 for Windows (International Business Machines [IBM], 2012) was used.

Results and discussion

In vitro evaluation of diatomaceous earth and lime applied through spraying

Only DE treatments with 4 kg/ha and agricultural lime applied by *in vitro* spraying showed effects by ingestion on the mobility of *A. distinctus* after three days; however, after five and seven days of the trial start, all the treatments showed an effect on the mobility of the slug. The highest value of affectation was observed after seven days and, although the Anova showed statistical differences among all the treatments compared to the control, the greatest affectation was observed when DE was applied at 4 kg/ha; however, it only reached 45 % (table 1).

Only slug mortality was observed after five and seven days after the start of the treatment. After five days (120 h), the treatments with agricultural lime and DE at 4 kg/ha caused mollusk mortality, but without statistical difference with the control. After seven days, the highest mortality was caused by DE at 4 kg/ha and agricultural lime with 25 % and 20 %, respectively, with statistical differences from the rest of the treatments and the control, and further, these last two with the control.

The mollusks affected moved slower and also manifested loss of appetite, concentrating on the upper part of the vessels or containers. By contact, there was an effect on the mobility of the slugs in the treatments with DE at 4 kg/ha and agricultural lime after three days; however, after five and seven days, all the treatments showed an effect on the mobility of the slug. DE at 4 kg/ha and agricultural lime caused the highest levels of affectation after five days, which differed from the rest of the treatments and the control, while after seven days, the highest affectation (55 %) was caused by DE at 4 kg/ha. Agricultural lime caused more effect compared to DE at 1 and 2 kg/ha, but these differed from the control (table 2).

Table 1. Effect by ingestion of the treatments applied by *in vitro* spraying on individuals of *Arion distinctus*

Treatment	Affected in mobility (%)			Mortality (%)		
	Three days	Five days	Seven days	Three days	Five days	Seven days
Control	0 b	0 b	0 d	0 a	0 a	0 c
DE 1 kg/ha	0 b	2.5 b	20 c	0 a	0 a	10 b
DE 2 kg/ha	0 b	7.5 b	22.5 c	0 a	0 a	12.5 b
DE 4 kg/ha	7.5 a	25 a	45 a	0 a	5 a	25 a
Agricultural lime 2 kg/ha	2.5 ab	22.5 a	35 b	0 a	2.5 a	20 a
C.V. (%)	7.78	4.59	8.10	0	9.56	0.96
Typical error *	0.07	0,08	0.04	0	0.08	0.04

* Uneven letters in the columns differ for $p \leq 0.05$ by Tukey's test.
Source: Elaborated by the authors

Table 2. Effect by contact of the treatments applied by *in vitro* spraying to individuals of *Arion distinctus*

Treatment	Affected in mobility (%)			Mortality (%)		
	Three days	Five days	Seven days	Three days	Five days	Seven days
Control	0 b	0 c	0 d	0 a	0 b	0 d
DE 1 kg/ha	0 b	5 bc	22.5 c	0 a	0 b	10 c
DE 2 kg/ha	0 b	10 b	27.5 c	0 a	0 b	15 bc
DE 4 kg/ha	10 a	32.5 a	55 a	0 a	10 a	42.5 a
Agricultural lime 2 kg/ha	5 ab	30 a	40 b	0 a	5 ab	25 b
C.V. (%)	4.37	3.22	1.29	0	4.37	1.90
Typical error *	0.06	0.07	0.05	0	0.06	0.06

* Uneven letters in the columns differ for $p \leq 0.05$ by Tukey's test.
Source: Elaborated by the authors

There was no slug mortality by contact with the spraying treatments after three days, but there was mortality in some treatments after five days and in all treatments after seven days, although these were very low, i.e., not exceeding 10 % mortality with DE at 4 kg/ha after seven days. Furthermore, a tendency was observed in both variables (impairment in mobility and mortality) regarding higher relative values in the effect by contact compared to the effect by ingestion.

In vitro evaluation of diatomaceous earth and lime applied through dusting

All treatments showed significant differences compared to the control, but those with the highest mortality were DE at 4 kg/ha, agricultural lime at 2 kg/ha and DE at 2 kg/ha (table 3).

Mortality was observed when slugs ingested the treatment with DE at 4 kg/ha and agricultural lime applied by dusting after three days, with statistical differences compared to the rest of the treatments and the control, with superiority for DE. After five days, the highest mortality values were observed with DE at 4 kg/ha and agricultural lime. After seven days, the DE at 4 kg/ha caused 100 % mortality showing a statistical difference with the agricultural lime treatment, and this last differed from the DE at 2 kg/ha, and this with DE at 1kg/ha; further, all differed with the control. All dusting treatments affected the mobility of slugs by ingestion after three days. At that time, the DE at 4 kg/ha caused the greatest affectation of the mollusks (85 %), followed statistically by the treatment with agricultural lime; then, the treatment that followed was the one with DE at 2 kg/ha that differed from the one with DE at 1 kg/ha, and this with the control. After five days, the greatest impact on mobility, i.e., 100 %, was caused by DE at 4 kg/ha and agricultural lime, showing a statistical difference with DE at 2 kg/ha, which differed from the DE treatment at 1 kg/ha, and this with the control. The DE at 2 and 4 kg/ha and the agricultural lime caused 100 % impairment in mobility after seven days, with no statistical difference with the DE treatment at 1 kg/ha; however, all the treatments differed from the control (table 4).

Table 3. Effect by the ingestion of the treatments applied by dusting to individuals of *Arion distinctus*

Treatment	Affected in mobility (%)			Mortality (%)		
	Three days	Five days	Seven days	Three days	Five days	Seven days
Control	0 c	0 e	0 c	0 c	0 d	0 e
DE 1 kg/ha	0 c	35 d	77.5 b	0 c	15 c	55 d
DE 2 kg/ha	15 b	52.5 c	95 a	0 c	27.5 b	67.5 c
DE 4 kg/ha	45 a	85 a	100 a	17.5 a	55 a	100 a
Agricultural lime 2 kg/ha	32.5 a	70 b	100 a	7.5 b	47.5 a	90 b
C.V. (%)	1.91	1.16	1.07	2.75	1.08	0.25
Typical error *	0.06	0.06	0.07	0.05	0.05	0.03

* Uneven letters in the columns differ for $p \leq 0.05$ by Tukey's test.
Source: Elaborated by the authors

Table 4. Effect by contact of the treatments applied by dusting to individuals of *Arion distinctus*

Treatment	Affected in mobility (%)			Mortality (%)		
	Three days	Five days	Seven days	Three days	Five days	Seven days
Control	0 e	0 d	0 b	0 c	0 d	0 c
DE 1 kg/ha	20 d	57.5 c	97.5 a	0 c	32.5 c	72.5 b
DE 2 kg/ha	37.5 c	80 b	100 a	0 c	45 c	95 a
DE 4 kg/ha	85 a	100 a	100 a	45 a	92.5 a	100 a
Agricultural lime 2 kg/ha	55 b	100 a	100 a	27.5 b	72.5 b	100 a
C.V. (%)	0.81	0.09	0.62	0.71	3.72	2.38
Typical error *	0.05	0.02	0.06	0.03	0.12	0.11

* Uneven letters in the columns differ for $p \leq 0.05$ by the Tukey test.
Source: Elaborated by the authors

Slug mortality by contact was only observed after three days with the DE at 4 kg/ha and agricultural lime treatments applied by dusting, with superiority for the former. After five days, the highest values were observed with DE at 4 kg/ha (92.5 %); meanwhile agricultural lime caused 72.5 % mortality with a statistical difference compared to DE at 1 and 2 kg/ha, i.e., these treatments differed from the control but not from each other. After seven days DE at 4 kg/ha and agricultural lime caused 100 % mortality without statistical differences compared to the DE treatment at 2 kg/ha. These three treatments exceeded the values obtained with DE at 1 kg/ha, and this, in turn, exceeded the values found with the control.

Field evaluation of diatomaceous earth and lime in strawberry fields

Samples taken before the start of the trial revealed the presence of three slug species in the traps. The majority of the specimens belonged to the species *Arion distinctus*, but other species of slugs corresponded to *A. hortensis* (Arionidae) and *Deroceras reticulatum* (Agriolimacidae) (figure 2).

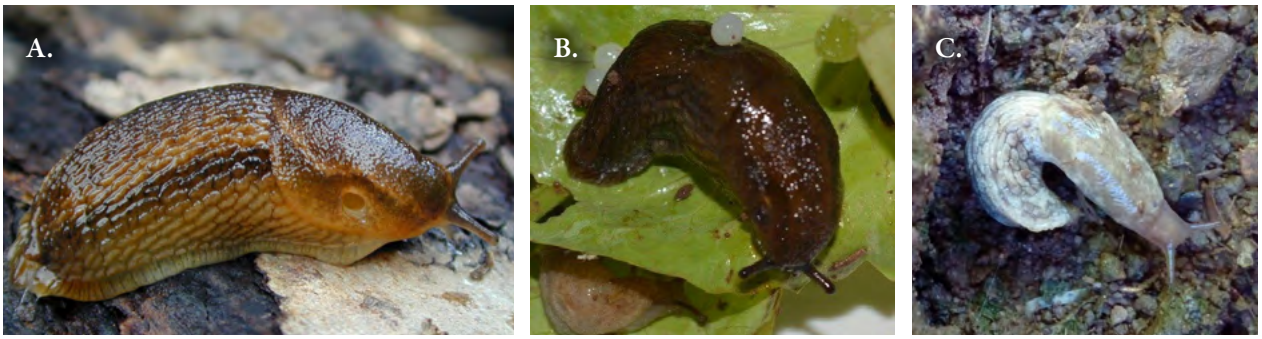

Figure 2. Slugs identified in the field. a. *Arion distinctus* Mabille, 1868; b. *Arion hortensis* Férussac, 1819; c. *Deroceras reticulatum* (Müller, 1774).

The samplings made before starting the trial showed slug populations between 9 and 11.25 individuals per trap, without showing a statistical difference between the plots where the treatments were going to be carried out. So the study started with a uniform population (table 3).

Three days after the applications were carried out, all the treatments caused slug mortality. The dead specimens were dissected on the ground (figure 3a), both inside and outside the traps. The affectation of the mollusks both by the action of the DE and by the lime prevented counting the dead specimens by species, even in the laboratory, due to the dark and almost black coloration they acquired (figure 3b).

On the third day after starting the trial, the lowest population values (with mortality of 45.65 %) were observed with DE at 8 kg/ha, but without showing statistical differences with the populations of the rest of the treatments, except with the control. Seven days after the first application, a similar situation was observed; the slug populations in the plots with the treatments did not differ in these, except with the control; however, the highest relative mortality was observed with DE at 4 kg/ha which caused 43.48 % mortality, and with agricultural lime that caused 45.65 % mortality (table 6).

Table 5. Number of individuals (slugs) found in the traps placed initially in the strawberry fields according to the evaluated treatments

Treatments evaluated	Number of individuals/trap
DE 8 kg/ha	11.15
DE 4 kg/ha	10.15
DE 2 kg/ha	9
Agricultural lime 2 kg/ha	11.25
Control without treatment	11.15

Source: Elaborated by the authors

Figure 3. Slug sampling. a. Slugs dissected by the action of DE and lime in the field; b. Slugs collected and taken to the laboratory.

Table 6. The efficiency of diatomaceous earth against slugs under field conditions after the first application

Treatments	Population		Mortality	
	Three days	Seven days	Three days	Seven days
Control	11.15 a	11.15 a	0	0
DE 2 kg/ha	6.25 b	6.5 b	31.52	35.22
DE 4 kg/ha	6.45 b	6.4 b	43.91	44.35
DE 8 kg/ha	6.5 b	7.45 b	45.65	43.48
Agricultural lime 4 kg/ha	7.7 b	6.25 b	33.04	45.65
C.V. (%)	16.26	23.00	0	0
Typical error*	0.55	0.65	0	0

* Uneven letters in the columns differ for $p \leq 0.05$ by Tukey's test.
Source: Elaborated by the authors

Although mortality was obtained in both moments (after three and seven days) and the treatments showed statistical differences, the effectiveness was very similar in both moments; however, it did not reach 60 % of effectiveness that is the percentage expected with alternative products under field conditions (Tarquí, 2007).

Something that could have contributed to these results was the frequent rainfall that occurred in Pamplona and its surrounding areas after the application, as can be seen in figure 4.

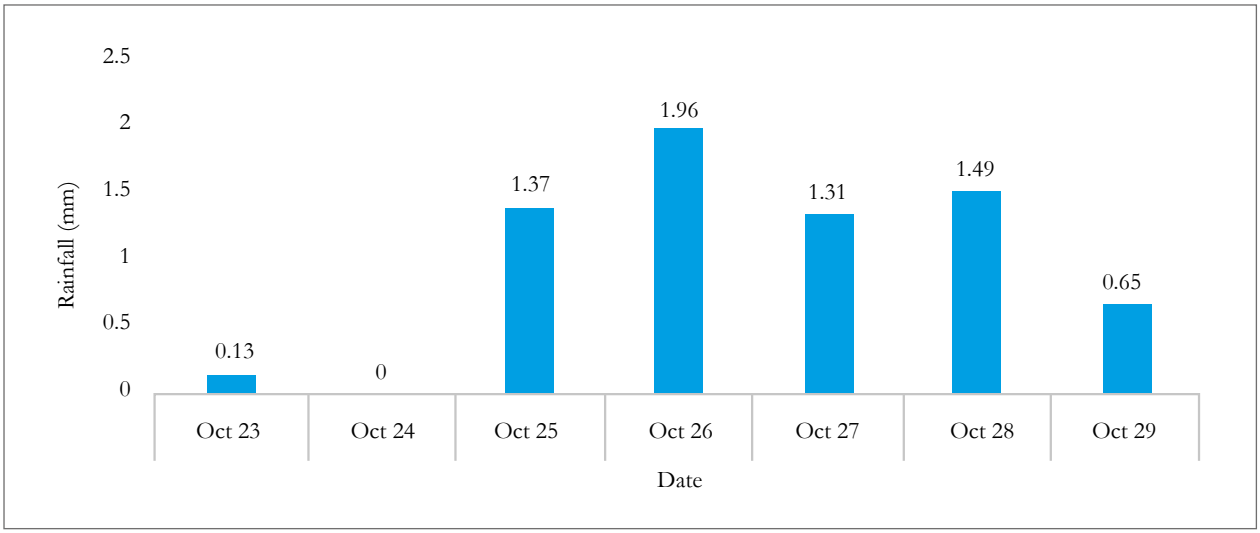

Figure 4. Total daily rainfall after the first application.
Source: Elaborated by the authors based on data obtained from the Meteorological Station of Unipamplona (2017)

Three days after the second rainfall, the highest population levels were found in the control, with statistical differences compared to the populations of the treatments; however, there were no differences among treatments; nevertheless, the mortality levels fluctuated between 83.04 and 93.48 %. After seven days, the populations of the treatments differed from the control, but the treatment of DE at 8 kg/ha showed the lowest populations (with a mortality of 95.24 %), although without statistical difference with the DE treatment at 4 kg/ha and the one with agricultural lime (table 7).

Table 7. Efecto por contacto de los tratamientos realizados contra babosa por aspersión después de la segunda aplicación

Treatments	Population		Mortality	
	Three days	Seven days	Three days	Seven days
Control	11.15 a	10.25 a	0	0
DE 2 kg/ha	1.9 b	2.15 b	83.48	79.52
DE 4 kg/ha	1.55 b	1.4 bc	86.67	86.52
DE 8 kg/ha	0.75 b	0.5 c	93.48	95.24
Agricultural lime 4 kg/ha	1.95 b	1.85 bc	83.04	82.38
C.V. (%)	26.21	13.46	0	0
Typical error*	0.47	0.32	0	0

* Uneven letters in the columns differ for $p \leq 0.05$ by Tukey´s test.
Source: Elaborated by the authors

The results show an interesting fact concerning the persistence of DE in time, as apparently, there is a cumulative effect of the DE in the second application with the first. Further, the positive effect that PEGAL could have played as an adherent should be considered because during the evaluation week or later, there were also rains (figure 5).

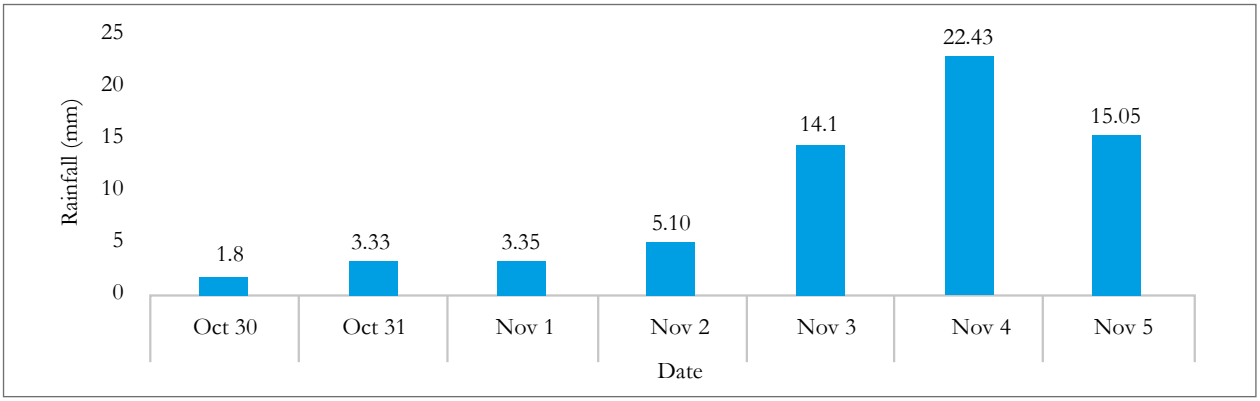

Figure 5. Total daily rainfall after the second application.
Source: Elaborated by the authors based on data obtained from the Meteorological Station of Unipamplona (2017)

Considering the *in vitro* treatments applied by dusting, there was a tendency to cause more mobility effects and mortality and to show higher relative values in the effects caused by contact compared to the ones obtained by ingestion.

In general, higher relative mobility impairment values, as well as mortality values caused by spraying compared to dusting, were observed. Only the treatment with DE at 4 kg/ha by dusting reached 100 % mortality in the tests, both considering the effect by ingestion as by contact. On the contrary, by spraying, the best results were observed with DE at 4 kg/ha by contact, indicating that the dose should be increased with this type of treatment under field conditions as stated by Ciomperlik et al. (2013). These authors mention that in other studies, tests had been carried out with higher doses compared to the ones used under laboratory conditions, i.e., DE of 30 g/m² equivalent to 300 kg/ha (Ciomperlik et al., 2013). Despite this, the mortality levels obtained *in vitro* at the doses studied were high compared to those used by other researchers concerning the effect of DE on *A. distinctus* (Hernández et al., 2015).

In the field trials, high percentages of affected slugs were observed with DE at 8 kg/ha when an adherent was added (higher than 90 %), so the current results allowed verifying the recommendation made by the company Agropulí (Agropulí, 2016) in relation to the DE as a molluscicide for the control of slugs that attack strawberry crops in the conditions of the Pamplona area. Nonetheless, doses should be higher.

In other trials, DE was not effective for the control of any phase of the giant African snail *Lissachatina fulica* (Férussac) (Mollusca: Gastropoda: Achatinidae), neither under laboratory nor under field conditions at the dose of 30 g/m², but it was effective against other associated snails (Ciomperlik et al., 2013). This is consistent with the results of Méndez and Castellanos (2017), who also did not find DE efficacy at doses similar to those used in the *in vitro* trial for the control of the snail *Helix aspersa* Müller (Mollusca: Gastropoda: Helicidae), which is also large.

In the case of insects, it has also been found that different species of weevils have responded differentially to the application of DE. For example, *Sitophilus oryzae* (L.) and *Oryzaephilus surinamensis* (L.) (Insect: Coleoptera: Curculionidae) showed 100 % mortality after 72 hours of exposure to a dose of 7 g/kg of flour, meanwhile *Tribolium castaneum* (Herbst) (Insecta: Coleoptera: Tenebrionidae) reached this mortality level also after 72 hours but with doses four times higher (i.e., 32 g/kg of flour) (Amiri-Besheli, Doustdtar, & Raeis, 2017).

Several authors have also made reference to the fact that the relative humidity and temperature influence the effectiveness of DE on insects, such as on the moth *Ephestia kuehniella* Zeller (Insect: Lepidoptera: Pyralidae) (Athanassiou, Kavallieratos, Tsakiri, Xyrafidis, & Vayuas, 2006) and on weevils such as *Rhyzopertha dominica* (F.) (Insecta: Coleoptera: Bostrichidae), *Tribolium castaneum* (Herbst), *S. oryzae* and *O. surinamensis* (Athanassiou et al., 2016).

Other authors also refer to the need to make a second application with plant juices of the family Agavaceae rich in saponins to achieve higher effectiveness in treatments against mollusks (Nodarse, Castellanos, Pérez, & Becerra, 2015).

Furthermore, when researchers such as Martín, Pérez, Castellanos and Soto (2017) conducted the evaluations under field conditions against *Praticolella griseola* with alternative molluscicides from extracts of plant species as *Solanum globiferum* (Dunal) and *Capsicum frutescens* L. (Solanaceae), these only achieved mortality effectiveness values of 60 %. Therefore, our results are considered adequate to be used as an alternative molluscicide.

Nevertheless, it is also necessary to have DE formulations duly certified concerning the percentage of silicon, since Bilbao et al. (2007) did not obtain effectiveness against *Triatoma infestans* Klug (Insecta: Hemiptera: Reduviidae), with a DE from a deposit in San Juan, Argentina. Meanwhile, Zúñiga and Caro (2012) recommended the application of DE against this insect species, as it offers protection for prolonged periods.

Conclusions

The diatomaceous earth applied *in vitro* causes an effect on the mobility and the mortality of *A. distinctus*, with relative superiority of the effect by contact compared to the one by ingestion, and only achieved 100 % mortality with the treatments applied by dusting at the dose of 4 kg/ha. This product has an effectiveness in the field for the control of slugs in strawberry crops with two foliar sprayings at a dose of 8 kg/ha and the addition of 18.767 g/L of PEGAL CS pH as adherent.

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Disclaimers

The authors agree with the publication of this article and declare that there are no conflicts of interest.

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