TECHNICAL NOTE

Preliminary Analysis of Alternative Methods for the Elimination of Warehouse Pests

Análisis preliminar de métodos alternativos para la eliminación de plagas de almacén

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ABSTRACT: The work consisted on the evaluation of several alternative methods for the elimination of one type of warehouse pest in grains (*Sitophilus spp*). Six treatments and a control were evaluated with three replicas each one, the grain object of study was sorghum (Sorghum bicolor *Moench*), UDG-110 cultivar. The studied variants were: 6 treatments (3 treatments with different methane dose and 3 treatments with different zeolite dose) and a control, with 3 replicas each one. All the treatments were executed inside test tubes of 50 mL. Methane application was by means of lethal injection with the doses specified in the respective treatments. The tests were carried out in the laboratories of agricultural microbiology, belonging to the Faculty of Agricultural Sciences of the Central University "Marta Abreu" of Las Villas. The objective of the investigation was to evaluate the effect of annihilation of these alternative treatments in the pest studied (*Sitophilus spp*) and its effect in the grains object of valuation (sorghum). The best result was obtained with treatment 6, with which 91.66% of elimination of the investigated pest was reached. The worst behavior was obtained with treatment 3 (0%). Different analysis of the soil utilized to see the possible effects of these treatments in the morphological properties of the grain and in the physical chemical properties of the soil were carried out.

***Keywords*:** Sorghum, Zeolite, Treatment, Sitophilus spp, Physical-Chemical Properties of the Soil.

RESUMEN: El trabajo consistió en la evaluación de varios métodos alternativos para la eliminación de un tipo de plagas de almacén en granos (*Sitophilus spp*). Se evaluaron 6 tratamientos y un control con tres réplicas cada uno, el grano objeto de estudio fue el sorgo (*Sorghum bicolor* Moench, cultivar UDG-110. Las variantes estudiadas fueron: 6 tratamientos (3 tratamientos con diferentes dosis de metano y 3 tratamientos con diferentes dosis de zeolita) y un control, con 3 réplicas cada uno. Todos los tratamientos fueron ejecutados dentro de tubos de ensayo de capacidad igual a 50 mL. La aplicación de metano fue mediante inyección letal con las dosis especificadas en los respectivos tratamientos. Las pruebas se llevaron a efecto en los laboratorios de microbiología agrícola, pertenecientes a la Facultad de Ciencias Agropecuarias de la Universidad Central "Marta Abreu" de Las Villas. El objetivo de la investigación fue evaluar el efecto de aniquilación de estos tratamientos alternativos en la plaga estudiada (*Sitophilus spp.)* y su efecto en los granos objeto de valoración (sorgo). El mejor resultado obtenido de los tratamientos empleados se presentó con el tratamiento 6, con el cual se obtuvo un 91,66% de eliminación de la plaga investigada. El peor comportamiento se presentó con el tratamiento 3 (0%). Se realizaron diferentes análisis del suelo utilizado para ver el posible efecto de estos tratamientos en las propiedades morfofisiológicos del grano; así como en las propiedades físico-químicas del suelo.

Palabras clave: sorgo, zeolita, tratamiento, Sitophilus spp, propiedades físico-químicas del suelo.

INTRODUCTION

The entomofauna associated to warehouses of food has been informed by several investigators like García-Lara *et al.* (2007); García-Leaños *et al.* (2007); Oberbauer *et al.* (2007); García-Leguizamón & Peñuela-Moreno (2022). Among them, they refer as coleopterons: *Sitophylus oryzae (L), Rhizopertha dominica (F), Orizaephilus surinamensis (L), Tribolium castaneum (Hbst), Acantoscelides obtectus (Say), Lasioderma serricorne (F) and as lepidopterons they refer P. interpunctella Hubner, C. cephalonica Stainton*. In Cuba, in Las Tunas Province, during the years since 2006 to 2013, affectations were quantified in rice, pea, bean, lentil, chickpea and other grains stored due to the attack of insects.

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Pests in alimentary products stored, whether they are raw materials, semi-processed products or elaborated food, can cause important damages. On one hand, they can cause the decrease of the organoleptic quality of the product or directly their loss. On the other hand, they add a possible problem of food security, since the presence of these insects or remains of them in the product, can cause allergies to the final consumers.

Most of the affected food are desiccated products of vegetable origin like cereals, vegetables, dry fruits, cocoa or spices. Although these pests can appear in different phases of the elaboration process and food distribution, the main critical points are in the warehouses and raw material silos, in the facilities where food is elaborated and in the warehouses for finished products.

The best way to avoid them is to implement a Program of Integrated Pest Control in the company that, in proactive and holistic way, prioritize the prevention of this problem by means of inspections, monitoring and exclusion and hygiene measures that avoid the development of infestations.

However, besides having the services of pest control professionals, it is important to understand what type of pest can be found in the food stored and to know recognizing the indications of its presence in the facilities. Recently, zeolite has been utilized for different uses, among which the following ones have been reported:

Absorption: Zeolite is used for the absorption of a great variety of materials. This includes applications in drying, purification and separation.

Exchange of ions: This property allows its application like water softeners and the use of zeolite in detergents and soaps (LennteCh, 2021). On the other hand, Romero (2016) reports that diatomeas earth is a form of physical control of insect-pests, because when the pests make contact with this powder, they suffer dehydration and die. Nevertheless, its effectiveness decreases when relative humidity increases. Applications of this product in dry grains after the crop have shown the best results. To store the grain below the 15 °C slows the development and reproduction of the biggest number of pests. Likewise, many insect pests are eliminated at temperatures higher than 35 °C. The great majority of insects in grains stored cannot live with less than 10% of humidity, being 14% or higher humidity, especially appropriate for the activity and reproduction of pest insects.

With the domestic economic situation and before the reinforcement of the blockade of the United States of North America against Cuba in the last 4 years, it has become difficult to import chemical insecticides for most of the basic grains cultivated in the country (corn, bean, soya and sorghum), which are of great importance in human and animal feeding. Before this situation, will it be possible through alternative methods of control of pests and diseases to solve this problem? To answer that question, the objective of this research is to determine the effect of alternative treatments for the control and the elimination of pests in basic grains (corn, bean, soya and sorghum) stored in warehouses.

MATERIALS AND METHODS

The investigation, to laboratory scale (lethal injection of methane) and (zeolite use), was develop at the laboratory of molecular biology of the Faculty of Agricultural Sciences in the Central University “Marta Abreu" of Las Villas. A breeding of weevils was fomented (*Sitophilus spp)* and they were classified by sex, starting in February 2022. Corn was used (*Zea mays L*) for its reproduction in the laboratory of Pathology of Insects of the Center of Agricultural Investigations (CIAP). The materials utilized were natural zeolite coming from the deposit of San Juan de los Yeras, Ranchuelo, Villa Clara. It contains more than 61% of zeolite material, with a grain among 0.5-2.5 mm and average diameter of 1.1 mm. This was collected in the green houses of the Institute of Plant Biotechnology (IBP) of Central University “Marta Abreu" of Las Villas.

Other materials used were:

sorghum grains

test tubes

methane bag

weevils

syringes

Because the zeolite collected possesses an irregular grain, it was ground in a mill and later sifted with sieve No.2.

These materials were taken to the laboratories of Soil and Biofertilizers and Microbiology and Bromatology, belonging to the Center of Agricultural Investigations (CIAP) of Central University "Marta Abreu" of Las Villas where the investigation was carried out. Twenty-one samples divided in 6 treatments and a control with 3 replicas per treatment. In each treatment, 4 couples of weevils were placed (*Sitophilus spp*), with their respective experimental designs to value. In the control treatment (Tc), the weevils remained without methane and zeolite applications.

In treatments T1 (10 mL), T2 (20 mL) and T3 (30 mL), lethal injections of methane were applied in increasing doses. In treatments T4 (16.27 g), T5 (32.20 g) and T6 (35.27 g), increasing volumes of zeolite were applied up to 35.27 g, due to the restrictive of the maxim volumetric capacity of the utilized test tubes (50 mL) that did not allow triplicating the zeolite volume (48.81 g) in treatment

T6. In all the treatments with their respective replicas 12 g of sorghum were placed (*Sorghum bicolor*, variety UDG-110) in the inferior part of the test tubes.

The variables studied were:

- Flow of design of treatments with methane and zeolite to use
- Efficiency of the annihilation of pests by means of lethal injection of methane and by means of zeolite and grain mixtures.
- Germination Tests in the grains tried with the different utilized treatments
- Effect of the treatments on the physical and chemical soil indicators

For the processing of the statistical data the professional application software STATISTICA was used, Version 7 on Windows XP.

Design of Treatments with Methane

To determine the quantity of gas to inject, the necessary calculations were performed maintaining as premise the phosphamine dose that is inoculated in the metallic silos to real scale (3 g of phosphamine per each 181.4 kg of grains to be treated. Methane density considered is presented in expression (1):

$$\gamma(CH_4) = 0.717\ kg/m^3 \quad (1)$$

$\gamma\ (CH_4) = 0.717$ equivalent kg/m^3 to (0.000717 g/ mL).

Knowing the mass of grains inside the test tube (12 g), for simple rule of three, the equivalent mass of phosphamine.

In a metallic silo for grain storage of capacity similar to 181.4 kg (181 400 g), it is well-known that 3 g of phosphamine is used to treat the pest insects, then that quantity of phosphamine will be necessary to treat 12 g of sorghum.

3 g phosphamine? 181 400 g

x phosphamine? 12 g.

From the calculation, it is obtained that:

$$x\ equivalent\ phosphamine = 0.00019\ \text{g}.$$

Substituting in the expression (1) (density of the methane), it is obtained that:

$$\gamma\ CH_4 = m/\text{v};$$

$$v(CH_4) = 0{,}00019\ g/0{,}000717\ g/m\text{L};$$

$$v(CH_4) = 0{,}0003\ m\text{L}.$$

This value represents the minimum dose (volume) of methane that, in theory, should be applied as lethal injection to eliminate the weevils inside the test tubes. It is necessary to reiterate that in the investigation was performed with a minimal dose of 10 mL, a medium dose of 20 mL and a maximum dose of 30 mL, values 3, 5 and 10 times above the minimal dose to apply, respectively.

In Table 1, the efficiency of the treatments by lethal injection of methane are presented.

From the analysis of Table 1, it could be observed that the increase of the dose does not report an increment in the insect mortality.

Corrected mortality, according to Abbott (1925; 1987):

In accordance with expression (2), it is obtained that:

$$Mc1 = \frac{\left(\begin{matrix}Mortality\\ in\ the\ treatment\end{matrix} - \begin{matrix}mortality\\ in\ the\ control\end{matrix}\right)}{(100 - mortality\ in\ the\ control)} . 100 \quad (2)$$

$$Mc1 = \frac{(1-0)}{(100-0)} . 100$$

$$Mc1 = 1.$$

The same procedure is applied in treatments 2 and 3, being:

$$Mc2 = 2$$

$$Mc3 = 0$$

Design of Treatments with Zeolite

To calculate the thickness of zeolite layer to be placed in each test tube, different thicknesses were tested, taking like reference previous studies made by Alemán-Hurtado (2015); Cruz-Lorenzo *et al.* (2013); Castellanos-Alemán *et al.* (2017) with alternative methods of pest control. They develop studies with three zeolite volumes; one minimum (15 mL, equivalent to 16.27 g), one medium (30 mL, equivalent to 32.20 g) and one maximum (35 mL, equivalent to 35.27 g), in all cases zeolite was crushed and sifted looking for uniformity of this material.

RESULTS AND DISCUSSION

Analysis of Efficiency of Treatments by Means of Lethal Injection of Methane

In accordance with Abbot's expression, this means that in treatment 1, this value was an inferior point to

TABLE 1. Efficiency of the treatments by methane injection

Treatment (application dose, mL)	Efficiency (% of elimination)
T1- (10 mL CH_4)	(4.16 %)
T2- (20 mL CH_4)	(8.33 %)
T3- (30 mL CH_4)	(0 %)

the case of treatment 2. In such a sense, it can be summarized that in the replicas of treatment 1, an insect was eliminated and two in those of treatment 2, of 8 (four couples). In treatment 3, dead insects were not reported.

Caballero *et al.* (2005) reported good effectiveness of weevil annihilation with methane in sorghum grains inoculated in periods of evaluation of 3, 12 and 30 days, reporting the best results 30 days after inoculation of methane 91.66%. In this work, a negative effect of methane was not reported on the germinative qualities of the grain (89.66% germination). These authors did not quantify the volume of methane applied to the weevils, but a comparison among different alternative methods of elimination of this type of warehouse pest was carried out. They report good efficiency of the treatment that used methane (91.66% annihilation) compared to the other alternative methods used in green paradise (*Philodendron*), dry paradise (*Philodendron*), green Neem (*Azadirachta indicates*) and dry Neem.

Martínez *et al.* (2021) applied lethal injection of methane in dose of 6, 15 and 30 mL to test tubes with 2,5 g of grains of white beans (*Phaseolus vulgaris L*), cultivar Bat-482 and 5 couples of weevils placed in its interior. They obtained values of efficiency of the annihilation by means of injection of methane of 96,66%, 8 days after inoculation which corroborates the results obtained by Caballero *et al.* (2005); Alemán-Hurtado (2015). These investigations were reproduced in 2022 to verify the possible feasibility of this type of alternative method of elimination of warehouse pests.

The authors of this study consider that in the investigative results obtained, some qualitative aspects influenced. They are:

- Quality of the methane used
- Limited homogeneous distribution of methane gas in the inter granular spaces of sorghum, which are small, causing low effectiveness
- Resistance to methane gas passing through sorghum inter granular spaces, which are small. It allows insect pests to refuge under the sorghum grains and to infest them before their possible annihilation
- Time of exposition of weevils to methane (7 days).

However, these hypotheses are not conclusive, which opens the possibility to continue investigating in this field.

Analysis of Efficiency of Treatments Using Zeolite

In Table 2, the obtained results of zeolite treatment efficiency are presented.

As it can observed in the Table 2, the best behavior was presented with treatment T6, in which 91.66% of the weevils valued was eliminated. In this case, a contradictory result was also presented among treatments T4 and T5, since treatment T5 was inferior to treatment T4. However, in experiments reported by Soca-Nuñez *et al.* (2015), with different doses of zeolite mixed with insects (*Sitophilus spp* and *Prostephanus truncatus*), it was demonstrated that the effectiveness of these annihilation methods depends on the contact the insects have with the zeolite and of the time of exposition to it. On the other hand, according to Gaviria (2021), at the present time, experiments are carried out with mineral salts manufactured in Spain whose main direct and residual effect is to modify the pH in the inter granular space and in the surfaces of the silos and storage cellars. That effect eliminates adults when penetrating their quitine exoskeleton and also inhibits the development of eggs and larva. This author points out that it is necessary to remember that the female of *Sitophilus spp*, deposits her eggs in singular form drilling the grain and leaving there, in the hole, an egg that is protected by a seal that she elaborates. Then, when the larva is born, it finds the grain from which feeding itself, without being noticed in the external surface of the grain. That condition of living inside the grain makes the effect of gaseous insecticides as phosphamine, to be very low and that the mortality of eggs and larva because of the fumigation is minimum. This situation causes that in the following weeks there is a continuity of the infestation, although the adults that are outside the grain have been completely eliminated. Many people say that it is due to the grain is infested again, but it is not this way, it is the same infestation that is developed starting from the eggs and hidden larva inside the grains. Maybe the effect of the gaseous insecticides outlined in the investigation of Gaviria (2021), could have a direct relationship with the low percent of elimination of the pest investigated in the case of the treatments that used methane procedure.

TABLE 2. Efficiency of treatment with zeolite use

Treatment (application dose, g)	Efficiency (% elimination)
T4- (16.27 g zeolite)	(70.83%)
T5- (32.20 g zeolite)	(16.66%)
T6- (35.27 g zeolite)	(91.66%)

Results Referred to the Germination Tests in Sorghum Grains with the Different Treatments Used

The percent of germination obtained in sorghum seeds, at level of Petri dish was of 60%. This value was the reference to contrast with the actual studies at level of flowerpot (polyethylene bags) with 1 kg of soil (dark leached plastic) previously sifted.

In each bag, three irrigations were applied to reason of 200, 100 and 100 mL in intervals of 12 days, with distilled water, on each sowed seed (5 seeds per bag of a 1 kg of soil), for a total of 400 mL of distilled water per bag according to the different treatments and their respective replicas.

In Figure 1, the behavior of the germination percent is observed in the different treatments.

It was observed that treatments T1, T2 and T3 are below the control in the variable germination percent. An increment of this variable in treatments T4, T5 and T6 was evidenced which could be influenced by the use of zeolite in these last ones, although the best result obtained in this indicator (T6), is similar to the value obtained at level of Petri dish. It is important to highlight that from the culmination of the experiment until the stage of sowing the sorghum grains in bags, a period of 60 days lapsed, which could have influenced negatively in the viability of the sorghum grains. In such a sense the obtained result is not conclusive and needs new investigative studies. That coincides with investigations reported by Caballero *et al.* (2005); Alemán-Hurtado (2015); Cuniberti (2020).

Results Referred to the Physical-Chemical Properties of the Soil with the Different Treatments Used

In Table 3, average values of the different indicators of the physical-chemical properties of the soil in the different treatments are presented.

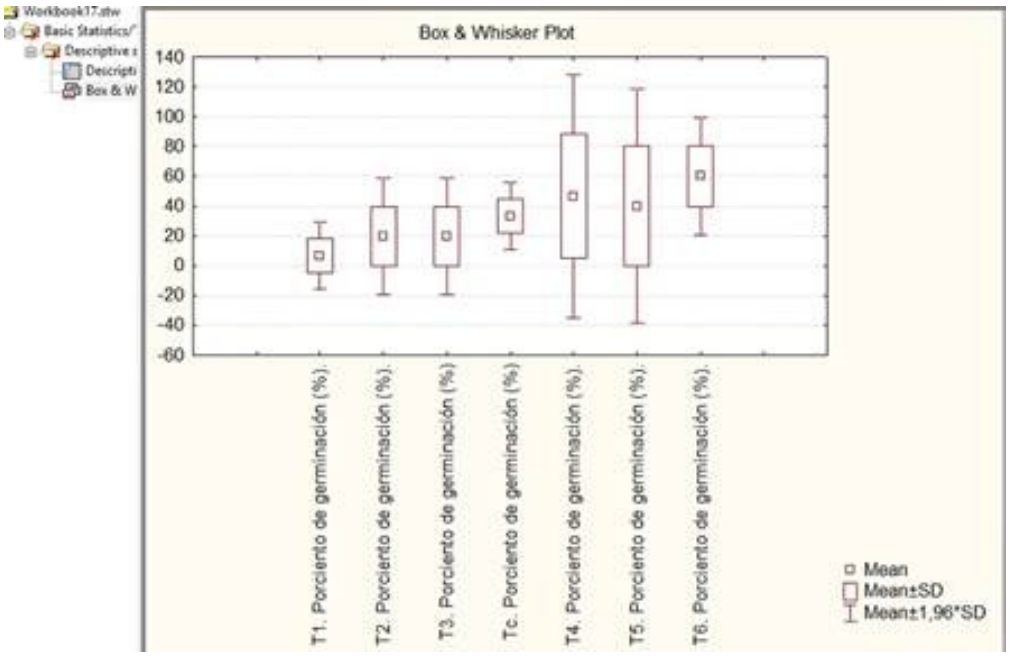

FIGURE 1. Effects of treatments on the germination of the indicator plant (sorgum)

TABLE 3. Physical - chemical properties of the soil per treatment

SOIL. PHYSICAL - CHEMICAL ANALYSIS. (*)							
Treatment	pH		P	MO	FE	AE	Perm.
	KCl	H_2O	mg/100g	%	%	%	Log 10K
1 Control	6.68	7.66	3.02	8.39	70.63	50.58	3.09
2 T 1 10 mL methane	6.66	7.59	3.53	8.60	69.77	53.56	3.12
3 T 2 20 mL methane	6.71	7.53	3.24	8.71	76.79	52.02	3.13
4 T 3 30 mL methane	6.68	7.44	3.07	8.87	69.53	47.80	3.12
5 T 4 16.27g zeolite	6.68	7.45	3.79	8.28	71.90	50.32	3.25
6 T 5 32.20g zeolite	6.70	7.37	4.00	8.50	58.77	57.28	3.10
7 T 6 35.27g zeolite	6.82	7.43	3.45	8.39	77.60	59.06	3.25

(*)-Group of Soils and Biofertilizers, Center of Agricultural Research (CIAP), Faculty of Agricultural Sciences (FCA), Central University “Marta Abreu" of Las Villas. (UCLV). Nov, 2022.

Of the analysis of Table 3, it could be observed that in all the variables valued significant differences were not presented between the control and their respective treatments, either in treatments with methane or in treatments with zeolite. That confirms that these alternative treatments of elimination of warehouse pests did not influence on the physical-chemical properties of the soil studied.

CONCLUSIONS

- The treatments with methane T1 (10 mL), T2 (20 mL) and T3 (30 mL) did not demonstrate good effectiveness in the elimination of the pest insect *Sitophilus spp*.
- The treatments with zeolite registered a satisfactory control of *Sitophilus spp*, especially treatment T6 with 91.66% of effectiveness.
- The treatments studied did not show significant differences regarding their effects on the physical and chemical properties of the soil studied which evidences that the alternative treatments of elimination of warehouse pests do not influence on them.

RECOMMENDATIONS

- To develop investigative studies in the control of *Sitophilus spp*, using the zeolita with inferior grain to 2 mm.
- To value the storage of sorghum grains using zeolita to real scale, as environmental sustainable method for the elimination of *Sitophilus spp*.
- To continue these investigations in the control of warehouse plagues.

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