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Agroecological strategies for *Sitophilus zeamais* handling in maize stored

Estratégias agroecológicas para el manejo de *Sitophilus zeamais* en maíz almacenado

Estratégias agroecológicas para o manejo de *Sitophilus zeamais* em milho armazenado

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Rev. Fac. Agron. (LUZ). 2022, 39(1): e223921
ISSN 2477-9407
DOI: https://doi.org/10.47280/RevFacAgron(LUZ).v39.n1.21

Crop Production
Associate editor: Dra. Lilia Urdaneta

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Received: 17-06-2021
Accepted: 05-12-2021
Published: 28-02-2022

Keywords:
Physical control
Natural control
Corn weevil
Damage

Abstract

Sitophilus zeamais is an economically important pest because it feeds on stored corn, since its control is carried out with increasingly toxic insecticides, the objective of the effect of more ecological alternatives such as the application of CaO (quicklime 2 %), *Beauveria bassiana* (1.10^9 espores.mL^{-1}), *Ricinus communis* powder aqueous (1.5 %), modified atmosphere storage (20 L silo) and airtight environment (in barrel of 20 L) on the incidence and damage of *S. zeamais* in stored corn, in the municipality of Tlacotepec de Benito Juarez, Puebla. The experiment was carried out under a completely randomized block design with five repetitions, where the five control methods mentioned above were tested and compared with a control treatment (plastic bag). Quicklime, storage in a modified atmosphere and storage in a hermetic environment significantly decreased (Tukey, $p < 0.05$) the number of weevils in corn grains during 247 days of storage and the percentage of damage to the grains remained below from 1 %. Therefore, these results showed that the application of quicklime, airtight environment and storage in a modified atmosphere controlled the damage caused by *S. zeamais* and are highly favorable for the conservation and protection of stored corn grains.

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Resumen

Sitophilus zeamais es una plaga de importancia económica, debido a que se alimenta de maíz almacenado, ya que su control se realiza con insecticidas cada vez más tóxicos, el objetivo de este estudio fue evaluar el efecto de alternativas más ecológicas como la aplicación de CaO (cal viva al 2 %), *Beauveria bassiana* (1.10^9 esporas.mL^{-1}), extracto de polvo de *Ricinus communis* (1,5 %), almacenamiento en atmósfera modificada (silo de 20 L) y ambiente hermético (garrafas de 20 L) sobre la incidencia y daños de *S. zeamais* en maíz almacenado, en el municipio de Tlacotepec de Benito Juárez, Puebla. El experimento se realizó bajo un diseño de bloques completamente al azar con cinco repeticiones, donde fueron probados los cinco métodos de control antes mencionados, y comparados con un tratamiento testigo (costal de plástico). La cal viva, almacenamiento en atmósfera modificada y almacenamiento en ambiente hermético disminuyeron significativamente (Tukey, $p < 0,05$) el número de gorgojos en los granos de maíz durante 247 días de almacenamiento y el porcentaje de daños de los granos se mantuvo por debajo del 1 %. Por tanto, estos resultados demostraron que la aplicación de cal, ambiente hermético y el almacenamiento en atmósfera modificada controlaron los daños ocasionados por *S. zeamais* y son altamente favorables para la conservación y protección de los granos de maíz almacenados.

Palabras clave: control físico, control natural, gorgojo del maíz, daño.

Resumo

Sitophilus zeamais é uma praga de importância econômica, pois se alimenta de milho armazenado, uma vez que seu controle é feito com inseticidas cada vez mais tóxicos, o objetivo deste trabalho foi avaliar o efeito de alternativas mais ecológicas como a aplicação de CaO (2 % cal virgem), *Beauveria bassiana* (1.10^9esporos.mL^{-1}), extrato em pó de *Ricinus communis* (1,5 %), armazenamento em atmosfera modificada (silo de 20 L) e ambiente hermético (garrafas de 20 L), na incidência e danos de *S. zeamais* em milho armazenado, no município de Tlacotepec de Benito Juárez, Puebla. O experimento foi conduzido em delineamento de blocos inteiramente casualizados com cinco repetições, onde os cinco métodos de controle mencionados acima foram testados e comparados com um tratamento controle (sacola plástica). A cal viva, o armazenamento em atmosfera modificada e o armazenamento em ambiente hermético diminuíram significativamente (Tukey, $p < 0,05$) o número de gorgulhos nos grãos de milho durante 247 dias de armazenamento e a porcentagem de danos aos grãos permaneceu abaixo de 1 %. Portanto, esses resultados mostraram que a aplicação de calcário, ambiente hermético e armazenamento em atmosfera modificada controlam os danos causados por *S. zeamais* e são altamente favoráveis a conservação e proteção dos grãos de milho armazenados.

Palavras-chave: controle físico, controle natural, gorgulho do milho, danos.

Introduction

Mexico is considered the center of origin and biodiversity of corn (Barrera-Guzmán *et al.*, 2020); its production and conservation is fundamental, providing many benefits as human and animal food (Gul *et al.*, 2021). The Agenda for Services for the Marketing and Development of Agricultural Markets (ASERCA, 2019) indicated that in Mexico the capacity of agricultural warehouses was 39.56 million tons in 2019, of which 83.7 % are stored indoors and 16.3 % outdoors, allowing small producers to guarantee the conservation of grains (Vázquez & Moreno, 2016), and a source of food (Turrent, 2013).

Unfortunately, this activity is hampered by insect pests of stored grains, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) is considered the most important postharvest pest of corn (Da Silva *et al.*, 2020; De Assis *et al.*, 2014), causing losses of 4 to 25 % of its grains (Ortiz *et al.*, 2015). To reduce losses and protect the seed against this pest, synthetic insecticides are used (Isman, 2015); whose intensive application causes damage to the environment and wild biota, contaminates soil and water, and causes resistance of some pests, in addition its use represents a risk to the health of living beings, affecting the neurological, reproductive, respiratory, cardiovascular and respiratory systems and thyroid damage (Silveira *et al.*, 2018). One option is to apply adequate management through the use of plant species that have an insecticidal effect and are found in each region, to evaluate in different formulations (extracts, oils and powders), which has the advantage of being degradable in stored grains and crops, causing a low impact on human being, environment and the economic income of the producers (Lanza *et al.*, 2020; Pérez-Torres *et al.*, 2017). In addition, the use of physical methods that help conservation and storage of grains is proposed, to generate sustainable and environmentally friendly alternatives, in addition to being profitable for the benefit of low-income producers and proposing practical solutions to the problem of warehouse pests, the objective of this research was to evaluate the effect of the application of CaO (quicklime), *Beauveria bassiana* (Bals) Vuill, *Ricinus communis* L. powder extract, storage in a modified atmosphere and storage in an airtight environment for the agroecological management of *Sitophilus zeamais* in stored corn.

Materials and methods

The experiment was held in the municipality of Tlacotepec de Benito Juárez, Puebla; Mexico (18º 14’ 12’’ and 18º 21’ 00’’ north latitude and meridians 96º 55’ 24’’ and 96º 43’ 00’’ west longitude), where the predominant climate is temperate semi-dry (National Institute of Statistics and Geography [INEGI], 2009). The treatments were established in a corn warehouse (4 m long by 3 m wide) made of concrete block with an access door that remained closed.

The corn that was used was white creole, the cob was left to dry in the open field for two months (October-November) harvesting in December 2014, it was shelled and placed in 50-kilo raffia bags for storage. Before establishing the experiment, a sampling was made, taking two kilos of corn from each sack, it was checked and was checked and the presence or absence of insects was verified. The experiment began on January 30, 2015, six treatments were applied for the agroecological management of *S. zeamais* in stored corn (table 1). The methodology and concentrations of the treatments that were adopted in this research were proposed by Castillo (2014), using quicklime powder at 2 % per kilo of corn grains and *B. bassiana*, and

by experience in previous studies, *R. communis* powder was used at a concentration of 1.5 % per kilo of corn.

Table 1. Treatments used for the agroecological management of *Sitophilus zeamais* in stored corn.

Treatment	Code assigned to treatments	Concentration (%)	Container
Quicklime (CaO)	cv	2.0	Plastic sack (raffia)
Beauveria bassiana	Bb	1.10^9	
Ricinus communis	Rc	1.5	
Storage in modified atmosphere	am	----	25k silos
Storage in hermetic environment	ah	----	20 L plastic bottle
Control	tg	----	Plastic sack (raffia)

Calcium oxide or quicklime (CaO) (limestone), was obtained in the municipality of Tecali de Herrera, Puebla from the company "Oxical de Tecali S. A. de C. V." dedicated to the production of calcium oxide, specialized in pulverized high purity quicklime. It was manually sprinkled at a concentration of 2 % p/p per kilo of corn in a homogeneous way before being stored in raffia sacks with a single dose.

Thirty-two g p/p of *Beauveria bassiana* product (Organic Vel) were applied at a concentration of 1.10^9 spores.mL^{-1}.

The treatment with *R. communis* was made from the mature fruit of the plant that was collected in the study area in its fruiting stage, the taxonomic determination was made in the herbarium of the Benemérita Universidad Autónoma de Puebla (BUAP). The material was dried on brown paper under shade for 25 days until constant weight was obtained. It was crushed in a NIXTAMATIC brand electric mill for grain, until obtaining a fine powder that was applied to the corresponding experimental units, at a concentration of 1.5 % p/p per kilo of corn, manually sprinkling the corn seeds homogeneously. These concentrations were applied for each experimental unit. In the case of Bb and Rc, the applications were made every two months according to the amount of grain contained in the experimental unit, the sacks were opened and the extract powder was applied manually with a plastic spoon.

For modified atmosphere storage treatment, galvanized sheet containers (silos) of 25 kg capacity were used, inside which, 16 kg of corn and a lighted candle were placed on top of the grain with the purpose of generating a reaction of combustion, sealing the container to prevent the possible access of air inside. This procedure was repeated each time a corn sample was taken.

The purpose of storage in an airtight environment was to limit the respiration of the grain. Twenty liter jugs with 16 kg of corn were used, which remained closed to prevent the entry of air and were placed in a horizontal position to ensure that the exit of the corn samples was always through the lowest part of the container.

The control was the grain stored in raffia sacks (16 kg), without application of any treatment (what the farmer normally does to store it).

Sampling was carried out every 20 days, for which they were collected from the top of each storage container using 250 g of corn seeds and the following variables were evaluated: 1) number of individuals: the number of adult weevils was quantified, which were identified in the Laboratorio de Diagnóstico y Sistemática de Plagas of Centro de Agroecología of Instituto de Ciencias of BUAP, and 2) percentage of damage: the grains affected by the weevil were placed against the light to estimate the amount of damaged surface in each grain. The assessment of the percentage of damage in the grain of the sample was obtained with the following formula:

$$\text{PDG} = \frac{\sum_{i=0}^{n} Pi}{n} x \quad 100$$

Where:

PDG: Percentage of damage in the grains of the sample

n: # of grains in the sample

Pi: Proportion of damage in the i-th grain with *i*=1,2,3…,*n*

A randomized complete block design with six treatments (table 1) and five repetitions per treatment (6x5) was used, generating a total of 30 experimental units, consisted each of 16 kg of corn.

The producer stores his corn for eight months, based on this, the experiment ended at 247 days, so the data obtained on the number of adult individuals, and percentage of damage were processed at 127 and 247 days (half and end of maize storage time). The data obtained were subjected to an analysis of variance and Tukey's test of means, with a significance of $p < 0.05$ to see differences between treatments, prior to this, the hypothesis of homogeneous variances was verified by means of the Barttlet test, using the statistical software STATGRAPHICS Centurion XVII (StatPoint, 2014).

Results and Discussion

Number of adult individuals

The ANOVA results indicated that there were significant differences between the treatments on the two evaluation dates ($p < 0.05$) and the comparison of means determined that, for day 127, the two types of storage, the use of quicklime and *B. bassiana* exerted a decrease in individuals in relation to the treatments of *R. communis* and the control, where the largest number of adults were present. At 247 days, the application of *R. communis* and *B. bassiana* was not effective for the control of the insect pest when comparing the number of individuals with the control, while in the treatments storage in an airtight environment, quicklime and storage in an modified atmosphere, the lowest number of weevils were collected with 0.6, 1.2 and 3.4 individuals, respectively (table 2).

Table 2. Number of adult individuals of *Sitophius zeamais* for each treatment at 127 and 247 days of evaluation.

Treatment	Number of adult individuals 127 d	Treatment	Number of adult individuals 247 d
Control	46.8 ± 11.1^{b}	*B. bassiana*	78.4 ± 6.1^{c}
R. communis	38.4 ± 8.9^{b}	Control	66.0 ± 5.0^{bc}
B. bassiana	8.4 ± 3.1^{a}	*R. communis*	55.0 ± 2.5^{b}
Storage in modified atmosphere	8.2 ± 2.6^{a}	Storage in hermetic environment	3.4 ± 1.5^{a}
Quicklime	0.2 ± 0.2^{a}	Quicklime	1.2 ± 0.2^{a}
Storage in hermetic environment	0.2 ± 0.2^{a}	Storage in modified atmosphere	0.6 ± 0.8^{a}

[a, b, c]Mean value ± standard error followed by different lowercase letters between columns indicate significant differences ($p < 0.05$), by Tukey's test.

In the case of the hermetic environment, if the grain is stored without weevils as in this investigation, they cannot enter the storage container because it is kept closed, however, the fact that weevils were found in this treatment (0.2 and 0.6), indicates that some corn seeds could be infested by the insect during the drying process in the field, according to Patiño (2019), the females open a hole in the grain to oviposit the egg in their interior (one egg per grain), it seals the hole with a secretion that hardens, the larva hatches and feeds, developing inside the grain until pupating; it usually leaves the kernel completely hollow until it becomes an adult. Despite this possibility, as long as the infestation in the field is low, the hermetic environment is still a good method to avoid the proliferation of *S. zeamais* in stored maize.

Regarding CaO powder, the results coincide with the evaluations carried out by Silva *et al.* (2004), who pointed out that calcium carbonate powders maintained their effectiveness for prolonged periods, avoiding the infestation of *S. zeamais* in stored corn grains, achieving percentages of 70 and 84 % mortality. This way of acting is due to the fact that calcium oxide or quicklime, due to its caustic, dehydrating, lipoid-dissolving and albuminoid-destroying action, destroys the chitinous substances that form part of the envelope of the scabies parasites (mites) and makes its eggs sterile (Bietti, 2019), in addition to contact with water, an exothermic reaction is caused causing the death of the insect (Osorno, 2012). This response was corroborated by collecting some adults in grains where quicklime powder was applied to observe them with a stereoscopic microscope (Olympus SZX7 and Nikon Eclipse 80i) and it was noticed that the joints of the weevils were covered with lime, which prevented them from move, this prevented copulation, oviposition and reproduction. Other inert powders such as volcanic ash and diatomaceous earth are viable for managing *S. zeamais*, since they act in a similar way to quicklime Jairoce *et al.* (2016), obtained a mortality rate of 100 % five days after establishing the experiment in the laboratory.

The Secretaria de Agricultura, Ganadería, Desarrollo rural, Pesca y Ganadería (SAGARPA, 2016), indicates that when seeds are stored in a modified atmosphere, gaseous and moisture exchange is avoided, grain respiration decreases and the low concentration of Oxygen kills insects.

Regarding the botanical insecticide evaluated, the dust from the fruits of *R. communis* had no effect on the adults of *S. zeamais*, allowing the population of weevils to increase significantly throughout the experiment. According to Arboleda (2012), it produces secondary metabolites such as albumin, ricin and alkaloids (ricinin), which are toxic compounds and are present in all parts of the plant, although they have a higher concentration in the fruit. Millán (2008), presenting toxicity on mites, nematodes, insects and rats. It is very likely that this effect is due to the fact that substances of natural origin are unstable, because they are highly biodegradable in the face of solar radiation and microclimatic humidity, reducing their action and making frequent applications necessary (Morales and García, 2000; Silva *et al.*, 2005).

In relation to the treatment based on *B. bassiana*, this did not have the expected effect at the concentration used (1.10^9 spores.mL^{-1}), since it was where the largest number of individuals appeared, these results agree with those obtained by Molina and Espinal (2000), who determined that the populations of *S. zeamais* where *B. bassiana* was incorporated at a concentration of 1.10^{10} conidia were similar to that of the control, the authors explaining that this was due to the fact that the pest was already within the cobs before the application of the product, hindering the contact of the pest with the conidia of the entomopathogenic fungus.

Percentage of grain damage

The lowest percentage values of grain damage were obtained in the treatments with quicklime, storage in a modified atmosphere and storage in an airtight environment, as a consequence of the low populations of weevils found in them (table 3). These results are similar to those reported by Rodríguez *et al.* (2017), who evaluated in the laboratory the effect of white marble powder on *S. zeamais* (1.74 g per 105 g of corn), recording that there were no percentages of losses in the corn seed by the weevil, they also determined that the higher the dose of the powder, the lower the affectation of the grain by this insect, obtaining a repellency index of 0.9 %. This grain conservation method, due to its ancestral knowledge and its easy acquisition, has been used in the North of Puebla, for which a very thin layer of quicklime or calcium oxide (CaO) is applied, alternated with a layer of 30 cm of corn shelled or on the cob, avoiding the damage of the corn weevil for a period of six months (Rodríguez, 2008). Quicklime is an economical treatment unlike other treatments and it is very easy to handle, it is also easily removed from the corn grain, at the time of sale it is passed through a sieve and in the case of nixtamalization the cooking of corn is done with water and quicklime.

Table 3. Percentage of grain damage for each treatment at 127 and 247 days of evaluation.

Treatment	Grain damage percentage 127 d	Treatment	Grain damage percentage 247
Control	2.12 + 0.7c	Control	50.30 + 4.1c
R. communis	1.72 + 0.2bc	*B. bassiana*	34.90 + 2.8b
B. bassiana	0.44 + 0.1ab	*R. communis*	26.20 + 2.5b
Storage in modified atmosphere	0.30 + 0.1ab	Storage in hermetic environment	0.90 + 0.2a
Storage in hermetic environment	0.04 + 0.0a	Storage in modified atmosphere	0.16 + 0.1a
Quicklime	0.02 + 0.0a	Quicklime	0.02 + 0.0a

[a, b, c]Mean value ± standard error followed by different lowercase letters between columns indicate significant differences ($p < 0.05$), by Tukey's test.

According to Garcia *et al.* (2007), the use of warehouses with modified atmosphere and airtight environment reduces the percentage of damage caused by insects, also does not leave toxic residues in food, decreases environmental contamination, reduces selective pressure on resistant insects and lengthens shelf life. of the grains. According to Ortiz *et al.* (2015), a good storage system allows to reduce losses caused by pests and diseases, which allows, in turn, to face periods of food scarcity, it is for this reason that the storage of corn grains in Modified atmosphere conditions in this research is considered an effective and simple method to apply by producers since weevils are not introduced, and the decrease in oxygen inside the container does not allow fungi and insects to proliferate.

Regarding the Rc and Bb treatments, on the two evaluation dates there were no significant differences with the control and at 247 days a grain damage percentage of 26.2 and 34.9 %, respectively, was found. The effectiveness of *R. communis* contrasts with that reported by Gómez *et al.* (2018), who cited that this treatment causes a 4 % repellency to the insect under laboratory conditions, Aragón *et al.* (2021), mentions that the Bb bioinsecticide for the

control *S. zeamais* at the same concentration, the percentage of damage was not as effective as when a combination of *B. bassiana* 1.10^9 + Quicklime is made, reducing damage by 3.3 % at 81 days in the laboratory.

To ensure that grain damage was caused by weevils and not by the temperature and humidity concentrations in the store, it was determined that, for quicklime treatments, *R. communis*, *B. bassiana* and the control, stored in sacks of plastic, the temperature was kept below 35 °C and humidity at 14 %, values that are within the parameters of the Mexican Standard NMX-FF-034/1-SCFI-PARTE-1 (2002). These two climatic factors act as catalysts for metabolic processes, increasing the respiration rate of the grains and the loss of dry matter; increases in temperature and humidity affect the quality of stored grains (Chulze, 2010).

Conclusions

The quicklime, the modified atmosphere and the hermetic environment allow an agroecological management of *Sitophilus zeamais* in corn grains in prolonged storage.

These management practices are feasible to carry out, especially by small farmers who store corn for self-consumption.

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