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Research Paper

Evaluation of quicklime and *Beauveria bassiana* for the management of maize weevil (*Sitophilus zeamais*) under laboratory and field conditions

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Abstract. During the storage of corn (*Zea mays* L) same pests significantly damage the grain, especially maize weevil (*Sitophilus zeamais* Motschulsky), and the most common method to fight is via the use of pesticides. So thus in this work, it was evaluated the effect of fungus entomopathogenic *Beauveria bassiana* (Bals) and its combination with quicklime (calcium carbonate) as well as *Argemone mexicana* L. extract to avoid the damage caused by *S. zeamais* to stored corn in both laboratory and field settings. In laboratory assays, 9 treatments were tested under a randomized block design in which quicklime, *A. mexicana* at 2%, *B. bassiana* in concentrations of 1×10^6, 1×10^8 and 1×10^9 and the same doses were combined with lime, showing statistically significant differences in all treatments; the treatments that were more effective at the laboratory level were tested on the field in stored corn during 7 months. At 30, 60 and 90 days of corn storage no significant differences were observed between the effects of treatments for infestation of *S. zeamais*, only until 120 days, where lime + *B. bassiana* 1×10^9 and lime was the best treatment to protect the corn. The best treatment to protect stored corn was quicklime, because of its corrosive action and effect on the respiratory system. It is also an accessible and economic option for producers, and its use is strongly recommended for protecting corn from *S. zea mays*.

Keywords: *Argemone mexicana*, corn weevil, microbiological control, plant extract.

INTRODUCTION

Agriculture in Mexico, as in any part of the world, is facing a constant and intense battle against storage pests which cause yearly loss. In the Bajío agricultural area, it was reported that 63% of corn (*Zea mays* L.) stored was found infested with pests (Tigar et al., 1994a). While in humid weather regions it was reported that the presence of pests exceeded 80% of infestation, and therefore it was the first cause of loss of stored grain (Tigar et al., 1994b), while in tropical regions losses amount up to 40% (García-Lara and Bergvinson, 2007). To avoid this damage pesticides are used, and they are becoming increasingly powerful and toxic, mainly through the use of fumigants that have generated resistance in insects, and irreparable damage to the environment, besides potential

Table 1. Treatments for the control of *S. zeamais.*

Treatment and concentration
Quicklime at 2% + *B. bassiana* 1 × 10^6 conidia/ml
Quicklime at 2% + *B. bassiana* 1 × 10^8 conidia/ml
Quicklime at 2% + *B. bassiana* 1 × 10^9 conidia/ml
B. bassiana 1 × 10^6 conidia/ml
B. bassiana 1 × 10^8 conidia/ml
B. bassiana 1 × 10^9 conidia/ml
Quicklime at 2%
A. mexicana at 2%
Control

damage to human health.

Among the main pests of stored corn are the Angoumois grain moth (*Sitotroga cerealella* Olivier) and the maize weevil (*Sitophilus zeamais* Motschulsky) which can cause total damage when not controlled. One of the techniques for the control of pests is agroecological management, Altieri (2008) states that Agroecology incorporates ideas about an approach to agriculture that is more closely linked to the environment and more sensitive socially speaking; not only on the conservation but also in the ecological sustainability of the production system. An ecological and viable alternative is the management of insect pests based on the use of natural products among which we have vegetable extracts, which are inexpensive, accessible, and environment-friendly (Vázquez et al., 2016), Cuevas et al. (2006) state that for the control of maize weevil *S. zeamais*, the vegetable extracts of Chicalote (*Argemone* sp.) and *Valeriana officinalis* L. (Caprifoliaceae) caused 98.9% mortality in this pest, zero percent first-generation emergencies and zero percent grain damage.

Biological control is yet another alternative in integrating pests management and Zhang et al. (2010) state that among the main fungi for pest management are *Beauveria bassiana* (Bals.) Vuill and *Metarhizium anisopliae* (Metsch) Sorokin. The entomopathogenic fungi have been considered as an alternative or complementary treatment against insect pests, because they are natural controllers, and they do not pose a detectable danger to humans or environmental damage. Numerous studies have established the potential of entomopathogenic fungi as bioinsecticides against several insect pests of stored products (Mehmet et al., 2016). However, in highly marginalized areas where it is grown for home consumption, the most common action by producers is to put grains in the sun, and they seldom apply some vegetal extract.

Based on this, the objective of this work was to evaluate the effect of *B. bassiana* and its combination with lime, as well as *Argemone mexicana* L. aqueous extract to avoid the damage that the *S. zeamais* causes to maize stored both in laboratory field settings.

MATERIALS AND METHODS

This work was carried out in two phases, the first phase was under laboratory conditions and was carried out at the Laboratorio de Entomología of the Centro de Agroecología of the Instituto de Ciencias, Benemérita Universidad Autónoma de Puebla, and the second phase was under field conditions and was carried out at a cellar of a producer in Puebla, Mexico.

Weevil breeding

The population of *S. zeamais* was established from insects adults were collected from rustic barns in San Lucas Tulcingo, Tochimilco, Puebla in November 2019, the gender of adults was identified by following the Halstead (1963) criterion, which points out that the proboscis (*rostrum*) of the female is longer, thinner, and smoother when compared to the males. For its reproduction, 150 individuals were placed in a plastic container with a capacity of 4 L with 3 kg of "cacahuazintle" variety corn. They were covered with fine meshes for oxygen exchange to be possible, and they were placed in a climate-controlled chamber at a constants temperature of 24 ± 1°C, relative humidity of 70 ± 10% and photoperiod of 12:12 (L:D). The insects were left for 30 days, after this time, the adults were transferred to similar containers to continue breeding, the corn infested with eggs and larvae was maintained under the same conditions spraying water to add humidity to the containers once a week in this way the adults for the bioassays were obtained.

Obtaining treatments

The treatments evaluated were shown in Table 1, quicklime, or calcium carbonate ($CaCO_3$), was obtained from limestone rock which is crushed and passed through a 30-size mesh sieve to filter the dust. For the preparation of the aqueous extracts of *A. mexicana*, the methodology proposed by Aragón and Tapia (2009) was followed, where the active principles of the plant were extracted using maceration, the aerial part of the plant was collected in the field, including leaves, stems, flowers and seeds, it was left to dry in the shade for 25 days until its total dehydration was once dry, it was pulverized with an electric mill (Nixtamatic) and in this way, a powder was obtained. The vegetable powder was placed in water for 24 h, at a concentration of 30 g of the vegetable powder in 1 L of water, and at the time of applying this treatment, it was filtered to separate the solids. And obtaining the formulation of *B. bassiana* was carried out in the Centro de Investigaciones en Biotecnología Aplicada of Instituto Politécnico Nacional in Tlaxcala.

Laboratory testing

Grains of maize variety "cacahuazintle" were used, an experiment under a randomized block design with nine treatments and four replications was established, giving a total of 36 experimental units, each experimental unit consisted of a glass bottle of 250 ml capacity, in which 100 g of corn was deposited. Grains of maize were exposed to radiation in a microwave oven for 3 minutes to remove any pest and sterilize the material, once the experimental unit has cooled then each treatment was applied to experimental units sprinkling it homogeneously. Afterward, 10 female and 10 male *S. zeamais* adults of three to ten days of emergence were introduced.

The mortality percentage of S. zeamais adults was evaluated at 5, 10 and 15 days after having started the experiment; after 15 days, adult weevils were removed, to rule out natural death or any other factor beyond the treatment mortality results were corrected with Abbott's formula (Abbott, 1925). Similarly, the emergence of adults of the first generation was evaluated by performing four readings, at 60, 67, 74 and 81 days after having started the experiment, to quantify this variable, the emergence of the control treatment was considered as 100% (Silva-Aguayo et al., 2005). The loss in weight of corn was also assessed; for this parameter weight data of healthy and damaged grains were collected, and the percentage damage quotient formula by Judenko was calculated (Judenko, 1973).

Field tests

In the community of San Lucas Tulcingo, Tochimilco, Puebla, an experiment was established in a cellar measuring 3 × 3 meters, roofed with asbestos foil, cement floor, which was maintained at room temperature and humidity and intended to store corn in raffia sacks with a 50 K capacity. In this cellar, four treatments were evaluated to define the effect of lime + *B. bassiana* 1×10^9 (best treatment observed in laboratory test), quicklime (from the quarry) at 2% and *A. mexicana* at 2%, on the natural population of *S. zeamais*. The experimental design tested was randomized blocks with four treatments and five replications, considering the experimental unit as a sack of threshed native white corn with 30k corn, harvested in November 2019. The treatments were applied at the time of storing corn in December 2019, each treatment was applied to experimental units sprinkling it homogeneously. Corn was stored for 7 months, and every 30 days 100 g samples were obtained of each of the treatments of different parts of the sack, from each sample infested grains percentage was estimated, considering healthy grains as 100%, and 90 and 210 days after the storage corn loss in weight was quantified with a sample 100 g, for this parameter, weight data of healthy and damaged grains were taken, and the percentage damage quotient formula reported by Judenko was applied (Judenko, 1973).

Statistical analyses

Means were compared using an F-test of the analysis of variance (ANOVA), followed by Tukey´s multiple comparison test (α=0.05). Considering the robustness of the normality hypothesis and homoscedasticity. The tests were carried out using the STATGRAPHICS Centurion XVII software.

RESULTS AND DISCUSSION

Laboratory testing

Mortality data corrected (Table 2) revealed a significant difference between treatments, with the highest mortality for lime + *B. bassiana* 1×10^9, 100% at day 10, not significantly different with the treatments that combined lime with *B. bassiana*, as well as with lime alone at day 15, indicating that mortality increased when lime was used, rather than with the application of *B. bassiana*. When viewing adult *S. zemais* in a stereoscopic microscope, those treated with lime remained immobile, probably due to damage to the joints restraining movements, thus inhibiting feeding ability and impeding mating, resulting finally in death. These results are consistent with those in Cuevas et al. (2006), where treatments of S. zeamais with lime powder at 15 d yielded 100% mortality, and the use of *Argemone* sp. produced 98% mortality, whereas herein mortality due to *A*. *mexicana* reached only 62.5%, the difference may be due to the type of application and concentration.

Statistical analysis revealed highly significant differences between treatments on the emergence of first-generation adults in all readings (Table 3). The higher emergence at 60 d occurred with *B. bassiana* at

Table 2. Mean mortality (% ± SE) of adult *S. zeamais* with several treatments in the laboratory.

Treatments	% Mortality ± Standard Error and Significance		
	5 days	10 days	15 days
Lime+*B. bassiana* 1 × 10^6	90.0 ± 3.5[d]	97.5 ± 2.5[c]	98.7 ± 1.2[d]
Lime+*B. bassiana* 1 × 10^8	77.5 ± 3.2[c]	95.0 ± 2.8[c]	95.0 ± 2.8[d]
Lime+ *B. bassiana* 1 × 10^9	98.7 ± 1.2[d]	100.0 ± 0.0[c]	100.0 ± 0.0[d]
B. bassiana 1 × 10^6	6.2 ± 2.3[ab]	15.0 ± 4.5[ab]	21.2 ± 4.7[b]
B. bassiana 1 × 10^8	8.7 ± 1.2[ab]	25.0 ± 9.1[ab]	31.2 ± 6.2[b]
B. bassiana 1 × 10^9	12.5 ± 4.3[b]	52.5 ± 13.4[b]	60.0 ± 12.2[c]
Quicklime at 2%	87.5 ± 4.3[cd]	98.7 ± 1.2[c]	98.7 ± 1.2[d]
A. mexicana at 2%	12.5 ± 3.2[b]	52.5 ± 15.6[b]	62.5 ± 11.0[c]
Control	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]

Note: Different letters in a row indicate significant differences in a Tukey's test (P ≤ 0.05).

Table 3. Mean (% ± SE) emergence of adult *S. zeamais* under several treatments in the laboratory.

Treatments	% Emergence ± Standard Error and Significance			
	60 days	67 days	74 days	81 days
Lime + *B. bassiana* 1 × 10^6	0.3 ± 0.3[a]	0.3 ± 0.3[a]	0.3 ± 0.3[a]	0.3 ± 0.3[a]
Lime + *B. bassiana* 1 × 10^8	0.7 ± 0.7[a]	0.7 ± 0.7[a]	0.7 ± 0.7[a]	1.0 ± 1.0[ab]
Lime + *B. bassiana* 1 × 10^9	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a*]
B. bassiana 1 × 10^6	36.2 ± 4.0[c]	46.4 ± 1.8[de]	51.7 ± 3.1[de]	56.3 ± 1.9[d]
B. bassiana 1 × 10^8	28.5 ± 4.6[bc]	38.3 ± 5.8[cd]	41.5 ± 5.4[cd]	47.5 ± 1.5[d]
B. bassiana 1 × 10^9	9.8 ± 5.6[a]	13.3 ± 5.7[ab]	14.0 ± 6.5[ab]	17.9 ± 5.9[bc]
Quicklime at 2%	0.3 ± 0.3[a]	0.3 ± 0.3[a]	0.3 ± 0.3[a]	0.3 ± 0.3[a]
A. mexicana at 2%	14.4 ± 5.9[ab]	21.4 ± 5.3[bc]	23.2 ± 6.6[bc]	25.7 ± 6.1[c]
Control	44.3 ± 1.6[c]	58.4 ± 2.9[e]	65.8 ± 3.7[e]	91.8 ± 5.4[e]

Note: Different letters in a row indicate significant differences in a Tukey's test (P ≤ 0.05).

1x10^6 and the untreated control, with 36.2 and 44.3%, respectively, both statistically different from the other treatments. On day 81, the best treatments were lime+*B. bassiana* at 10^9, lime singly, and lime+*B. bassiana at* 10^6, where emergence was <0.3%. Since in these treatments the highest mortality occurred from the early days, there was no egg-laying, and therefore no new weevils to develop. This confirms that lime is the best treatment to avoid the presence of *S. zeamais*; as many insects have a pre-oviposition time, they need time to mature the ovules, from a few hours to days in some species (Faroni and García-Mari, 1992, Arnó et al., 1998, Rioja et al., 2010, Aragón-Sánchez et al., 2018). The application of *A. mexicana* contributes to protection, as it prevented emergence considerably, which was significantly less than that in the control, similarly to results in Cuevas et al. (2006), where *Argemone* sp. in dust application at 1% on stored corn infested with *S. zeamais* in the laboratory caused 98.90% mortality, 0.0% emergence of the second generation and 0.0% grain damage because the chicalote seeds contain toxic compounds to the weevil (Salinas, 2012).

When submitting the data on the percentage of weight loss to the analysis of variance, a significant difference between the effects of treatments on weight loss caused to corn by the damage of *S. zeamais* is observed (Table 4). Treatments in which there was less weight loss in comparison to the control treatment are lime, lime plus *B. bassiana* [10^9], and lime plus *B. bassiana* [10^8], treatments with a higher weight loss were *B. bassiana* [10^6] and the control. According to the observations of this work, lime works by killing the weevils by being in contact with them, which results in damage to the grains being low of 2.9% with respect to the control, where the damage was 14.8%, these data are consistent with those reported by Rodríguez (2008), who states that when corn kernels are treated with natural products, damage caused by weevils decrease up to 20%.

Field tests

In the percentage of infested grains at 30, 60 and 90 days of corn storage, with the mean comparison test, no significant differences were observed between the effects of treatments for infestation of *S. zeamais*, only until 120

Table 4. Weight loss (% ± SE) caused by *S. zeamais* to corn stored 81 d under several treatments in the laboratory.

Treatments	Weight loss ± Standard error and significance
	81 days
Lime + *B. bassiana* 1 × 10^6	3.6 ± 1.1 a
Lime + *B. bassiana* 1 × 10^8	3.9 ± 0.8 ab
Lime + *B. bassiana* 1 × 10^9	3.3 ± 0.8 a
B. bassiana 1 × 10^{66}	9.3 ± 0.3 bc
B. bassiana 1 × 10^8	7.6 ± 0.8 ab
B. bassiana 1 × 10^9	5.5 ± 0.4 ab
Quicklime at 2%	2.9 ± 1.4 a
A. mexicana at 2%	7.1 ± 0.6 ab
Control	14.8 ± 2.2 c

Note: Different letters in a row indicate significant differences in a Tukey's test (P ≤ 0.05).

Table 5. Mean infestation of corn (% ± SE) by *S. zeamais* under several treatments, in natural rustic storage

Treatments	Average % of infested corn ± Standard Error and Significance			
	120 days	150 days	180 days	210 days
Lime+*B. bassiana* 1 x 10^9	0.0 ± 0.0^a	0.0 ± 0.0^a	0.0 ± 0.0^a	0.0 ± 0.0^a
Quicklime at 2%	0.0 ± 0.0^a	0.0 ± 0.0^a	0.7 ± 0.3^a	0.9 ± 0.4^a
A. mexicana at 2%	2.4 ± 0.9^a	5.6 ± 0.5^a	9.2 ± 1.6^{ab}	18.8 ± 3.1^b
Control	8.3 ± 1.6^b	18.4 ± 2.9^b	46.8 ± 3.7^c	79.7 ± 6.4^c

Note: Different letters in a row indicate significant differences in a Tukey's test (P ≤ 0.05).

Table 6. Weight loss in corn (% ± SE) by *S. zeamais* under several treatments, in natural rustic storage.

Treatments	Corn weight loss % ± Standard error and significance	
	90 days	210 days
Lime + *B. bassiana* 1 × 10^9	0.0 ± 0.0^a	$0.0 \pm 0.0^{a*}$
Quicklime at 2%	0.0 ± 0.0^a	0.9 ± 0.4^a
A. mexicana at 2%	0.4 ± 0.2^a	9.1 ± 0.8^b
Control	2.1 ± 0.6^a	24.7 ± 4.4^c

Note: Different letters in a row indicate significant differences in a Tukey's test (P ≤ 0.05).

days a significant difference appeared, and as can be seen in Table 5, these being the best treatments to protect corn of the infestation of *S. zeamais*: lime + *B. bassiana* 1 × 10^9 and Quicklime.

The extracts from *Argemone* sp. are used to protect the stored corn, bean, and chickpeas grains at the laboratory setting against *S. zeamais*, *Zabrotes subfasciatus* (Boheman) and *Callosobruchus maculatus* (F.), causing mortalities of 98.9, 98.7 and 26.2% respectively (Cuevas et al., 2006), and also protected the grains corn infested with *S. zeamais* from damage in 100% at the laboratory level (Salinas, 2012). This work, at 210 days, did not find total protection of grain by *A. mexicana*; this is probably because, at the field level, conditions are very different from conditions that prevail when working in the laboratory, where the weevils are confined to a small space.

For weight loss in corn, it was observed that at 210 days there was a statistically significant difference between the effects of the treatments, control treatment being the one that presented the greatest weight loss. This may be because here is corn grain is not protected from damage caused to it by *S. zeamais* at the field level (Table 6).

Data in this work are consistent with those reported by Zhang et al. (2010), who states that among the most important fungi for pest management is *B. bassiana*. The effect of this fungus penetrates through the insect cuticle up to its hemocoel, the hypha of fungus contains proteins, chitin, lipids, melanin, diphenols and carbohydrates that activates the immune system of the insect, but through melanization, sclerotization, phagocytosis, nodulation, and encapsulation processes the fungus causes the weevil death (Pucheta-Diaz et al., 2006).

CONCLUSION

The best treatment to protect stored corn was quicklime, because of its corrosive action and effect on the respiratory system. It is also an accessible and economic option for producers, and its use is strongly recommended for protecting corn from *S. zeamais*.

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