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TOXICITY OF ROCK DUST AND CHARCOAL POWDER ENHANCED WITH DIATOMACEOUS EARTH AGAINST RICE WEEVIL IN MAIZE STORED BY SMALLHOLDER FARMERS

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ABSTRACT

Maize is a major staple food in Africa, its production and post-harvest generally occurs on smallholder farmers' properties, where significant food losses occur in the field during harvesting, processing and storage. Most smallholder farmers lack access to modern methods of harvesting, processing and storage. Generally, the storage structures used are ineffective against storage pests, leading to post-harvest losses due to insect pests that, in some cases, can reach about 40% of the total production. Pest control is generally scarce, and when adopted, it is based on local knowledge-based pest control alternatives or on the application of synthetic pesticides known to be harmful to the environment and human and animal health. So, alternative pest control methods commonly found include the use of inert dust as an integrated pest management approach, without residual effects and ideal for food safety. This study aimed to determine the efficacy of charcoal powder (CP) and rock dust (RD) alone or combined with diatomaceous earth (DE) in the control of rice weevil (*Sitophilus oryzae*). Maize grains were treated and infested by the addition of *Sitophilus oryzae*, on the day they were treated and, 30 and 60 days after treatment. Mortality was assessed at 10 and 20 days after each infestation. Emerged insects were counted 40 days after the second mortality assessment for each infestation period. At 90 days after treatment, the seed germination test was performed. The best treatments for *Sitophilus oryzae* control (mortality) and F1 emergence reduction were RD (0.5, 0.25 and 0.10 g/kg), RD+DE (0.5+0.05, 0.25+0.05 and 0.10+0.05 g/kg), and CP+DE (1.0+0.05, 0.50+0.05 and 0.25+0.05 g/kg). The RD and RD+DE remained efficient in maize treated and stored for 60 days. The treatments did not interfere with seed germination. Rock Dusts, RD+DE, and CP have the potential to be used as stored grain protectants, providing economically and environmentally friendly pest control alternatives, especially in small-scale agriculture. In developing countries, especially in sub-Saharan Africa, studies for optimization and introduction of inert dusts tested in this research, have the potential to reduce post-harvest losses, improving food security.

Key words: Inert dust, stored maize, biological agriculture, storage pests, diatomaceous earths

INTRODUCTION

In many countries especially on the African and South American continents, among smallholder farmers, cereals such as maize (*Zea mays*) are the staple food [1,2]. The availability of this cereal for the livelihoods of sub-Saharan African populations is seriously limited by high losses during production and storage. The weevils, *Sitophilus* spp., are important pests of stored maize, and their control has been based on synthetic insecticides, leading to resistant pest selection, environment pollution, and toxicological effects [3,4]. The use of synthetic insecticides is also associated with food contamination, leading to deleterious effects on human and animal health. This has led to increased awareness of the use of pesticides and the search for efficient and environmentally acceptable alternative stored grain pest control products.

Research and use of plant extracts and inert dust have increased in recent years. Inert dust refers to all non-reactive dry powders in nature regardless of origin [8], with important applications in industry and agriculture [9]. Among various agricultural alternatives, RD can be used for pest control, with considerable efficacy [10] depending on particle size and silicon content. It may provide alternatives for pest control in small-scale storage systems because it is low-cost, locally available and easy to prepare and use.

Using inert dust, such as diatomaceous earth (DE), against stored grain weevils had shown higher mortality due to dehydration [5-7,9]. Studies show good efficacy of DE mixed with low-toxicity substances such as insecticides, inert dust, plant extracts and fungi [9]. However, no practical solution is known based on DE mixed with inert dust used by smallholder farmers in poor regions.

This study aimed to evaluate the effectiveness of CP and RD applied alone or mixed with DE on *S. oryzae* mortality, progeny emergence and seed germination.

MATERIALS AND METHODS

Test insects

S. oryzae cultures were provided by Embrapa National Soybean Research Center and kept in wheat maintained for at least 2 years without pesticide exposure. The experiments were performed in a climate chamber (25 ± 2 °C and 12h of light).

Insects were raised using 500 ml plastic containers and assembled as follows: 200 grams of wheat grains were added to each container, and 250 adults of *S. oryzae* were released. The containers were kept in the climate chamber (25 ± 2 °C and 12h of light) for 15 days, then all insects were removed, and the grains were kept for another 45 days. Emerging adults were used in the experiments.

Products (powders and dusts)

The DE used was Keepdry®. The RD (ultrafine basalt-microgabbro) was collected as residue from the Luzia quarry, Paula Freitas, Paraná, Brazil. The CP was produced by grounding charcoal in a mill (Marconi MR 340). All materials were stored in separate containers, sealed and kept under ambient conditions in the laboratory until use. The IAPAR 51 maize grains (Paraná Agronomic Institute— IAPAR, Brazil), free from pesticides and kept in the freezer (-18 °C) for one week to eliminate possible insect infestations, were used as the substrate for treatments. Grain moisture content was measured on a moisture meter (GEHAKA AGRI G600) and dried at 12% humidity at 60 °C.

Grain treatment

Thirteen lots of 1 kg of maize grains, were separately treated in clear plastic bags with the treatments DE (0.05 g/kg), RD (0.5, 0.25 and 0.10 g/kg), CP (1.0, 0.50 and 0.25 g/kg), RD+DE (0.5+0.05, 0.25+0.05 and 0.10+0.05 g/kg), CP+DE (1.0+0.05, 0.50+0.05 and 0.25+0.05 g/kg), and untreated control. Then, the mix between maize and the dusts was manually shaken for 2 minutes. From each batch, 600g of grains were divided into 50g portions, distributed into 12 containers of 250 ml, and grouped into 3 sets of 4 containers. Grains in the first set were infested (day 0), and in the 2nd and 3rd sets at 30 and 60 days after the treatment. Due to the small insect size and to avoid damage and stress, sex determination was not done. Each container was infested with 20 adults, sealed with tulle fabric, fixed with latex elastic, and kept in the climate chamber until evaluation. The experimental design was completely randomized with 4 replications. Mortality was assessed 10 and 20 days after each infestation. In the evaluation at 20 days after infestation, the living and dead insects were removed, and the grains remained in the same conditions for another 40 days, after which the score was performed in emerging adults. This evaluation was performed for the three infestation dates: 0, 30 and 60 days after treatment.

Statistical analysis

Data were analyzed using the Variance analysis system (SISVAR) statistical package [11], corrected by the square root of $x+0.5$ and subjected to analysis of variance and comparison of means by Tukey test at 5% significance. The efficacy of tested treatments was performed by Abbott's formula [12] and for adult emergency, by Henderson and Tilton's formula [13].

The seed germination test was carried out at 90 days after treatment. Treatments consisted of seeds treated with DE (0.05%), RD (0.5%), RD (0.5%) + DE (0.05%), CP (1%), CP (1%) + DE (0.05%) and an untreated control. Twenty seeds of each treatment were arranged in boxes of polystyrene crystal (11×11×3.5 cm) (Gearbox)

with moistened paper in the germinator (De Leo) and evaluated after 8 days, at room temperature.

Diatomaceous earth was chemically analyzed and quantified at the Laboratory of Applied Nuclear Physics of the State University of Londrina, Brazil, using the X-ray Fluorescence Spectrometer, EDX-720 model from Shimadzu Co.

RESULTS AND DISCUSSION

Adults mortality

In *S. oryzae* mortality evaluation 10 days after the grain infestation, the control efficiency was greater than 70% in grains treated with DE, the highest dose of RD, RD+DE and CP+DE. The highest control efficiency was 84.2%, 80.0% and 79.2% in grain treated with RD+DE and stored for 0, 30 and 60 days, respectively. The mortality observed using the mixture of DE plus the lowest doses of RD (0.10 and 0.25) was higher than that of RD applied alone ($P < 0.05$). The treatment CP+DE showed higher efficiency than CP alone, only using the highest CP (1.0) dose in the mixture ($P < 0.05$).

In the insect mortality assessment on treated and preserved grains for 30 days, only the RD+DE (0.50 + 0.05) controlled more than 70% of the insects. For grains preserved for 60 days, a similar level of efficacy was observed in DE alone and RD+DE mixture (Table 1). In the evaluation at 20 days after infestation, the control efficiency increased, reaching 100%, using RD+DE (0.25 + 0.05). The best treatments were RD (0.50), RD+DE mixtures, and the highest dose of CP (1.0) + DE in grains infested at day 0, with control efficiency greater than 96%. After 30 days of conservation, the highest control efficiency dropped to the 76.3 to 93.2% range. The best treatments were DE (83.1%), RD+DE (85.9 to 93.2%), and CP (0.25) +DE with 76.3% efficiency. Considering grains treated and stored for 60 days, RD (0.50), RD (0.50) + DE, and RD (0.10) + DE were superior and presented control efficiency above 90%. For the remaining treatments, it was found that RD alone and mixed with DE were always among the best treatments or intermediate, with no significant difference from the best treatments ($P < 0.05$). Regarding CP alone, in the 10-day evaluation, its efficiency was intermediate, not differing from the best treatments ($P < 0.05$) (Table 1).

In the evaluation at 20 days, CP was always inferior to the best treatments (Table 1). The CP+DE, in general, were not different from the best treatments ($P < 0.05$), except CP+DE (1.0+0.05) and CP+DE (1.0 + 0.05 and 0.25+0.05) at 10 and 20-day evaluations, respectively.

There is some research regarding the use of RD and CP in the control, progeny (F1) emergence reduction, and seed germination [9,15]. However, no studies were

found about the mixes of CP or RD with DE in the stored grain pest control. However, the mixes of DE with environmentally accepted substances have been documented in previous research [9, 23-25].

In this work, the greatest *S. oryzae* control efficiency happened through the application of DE, RD, and their mixtures. The DE effectiveness has been experimentally verified against many pests of storage, namely *Rhyzopertha dominica*, *S. zeamais*, *S. oryzae*, *Tribolium* spp., *Acanthoscelides obtectus*, *Callosobruchus maculatus* among other pests [15- 17]. Concerning the RD, the effectiveness of granite dust was observed with a deterrent, insecticide, and anti-oviposition effect against *Plutella xylostella* and *Trichoplusia ni* [18].

Even though there is high control efficiency, it is reasonable to highlight that 100% of *Sitophilus oryzae* control occurred through the RD+DE mixture, which did not occur with RD or DE alone. The approach used in this work permits the assessment of fewer DE (half of those recommended by the manufacturer), which would permit his economic and efficient use. The possibility of application of the same DE amount to store twofold the quantity of grain would offer an important resource-saving. The control efficiency of CP observed in the bioassays, although lower than that of the best treatments, provides reasonable stored grain protection in the lack of more effective options. Some research describes hopeful results in testing the before-mentioned products. Applying wood ash, 87.11% of *Sitophilus oryzae* mortality, reduced progeny emergency, injury to grains, and stored maize weight loss occurred [19].

The results found in the CP+DE mixture, compared to CP and DE applied alone, show that there was an increase in the effectiveness of CP and a decrease in the effectiveness of DE. Thus, the reduction in DE effectiveness can be explained by the reduction in the amount of DE in the CP+DE mixture, which reaches the insect cuticle, reducing lipid adsorption, dehydration and insect mortality. Therefore, in the case of DE availability, the results suggest not mixing DE with CP.

Residual toxicity against adults in stored maize

The best treatments to reduce F1 emergence were DE, RD (0.50 and 0.25), RD (0.25) +DE, and CP (0.50 and 0.25) + DE, at 60 days' post-treatment evaluation. It is important to highlight the no emergence of insects in grains treated with DE and RD (0.25) + DE. At the same evaluation, the lowest dose of RD (0.10) and the highest of CP (1.0), the RD (0.50 and 0.10) + DE mixtures did not differ significantly ($P<0.05$) from the best treatments and were higher than the CP (0.50 and 0.25) (Table 2).

At the 90-day evaluation, the best treatments were DE, RD (0.25), all RD dose + DE mixtures, and CP + DE mixtures at the lowest CP doses. In the 120-days

evaluation, the best treatments were RD (0.50) and RD+DE mixtures. The remaining treatments were intermediate except CP (0.50 and 0.25) at 60 days, CP (0.25) at 90 days, and CP (1.0 and 0.25) at 120 days. All treatments have a lower insect emergence than control (Table 2).

The high effectiveness of RD and DE in decreasing insect emergencies is consistent with the results of published by Siteo and collaborators [15], though the results are available for DE and very scarce for RD. The effectiveness of CP in decreasing the progeny emergence was notable, even seeing as a study limitation, the fact that small insect size and the need to avoid injury and stress, sex determination was not done. Research show that some powders, such as clay, sand and ashes, used in high doses (10% w/w), offer a physical obstacle against insects [9], which might partially explain the reduced progeny emergence observed in grains treated with CP.

Seed germination

Except for CP+DE, with lower seed germination (77.5%), there was no significant difference in the germination of the treated seeds compared to the control, which shows that the treatments tested do not interfere with the seed germination ($P < 0.05$) (Table 3).

The fact that the tested treatments did not inhibit seed germination was observed in similar studies. Some research show that, in general, inert dust, namely DE, rice husk ash, wood ash, fly ash, and cow dung, do not inhibit seed germination [15, 20, 21].

Inert powders' chemical composition

The results for DE chemical composition analysis were SiO_2 (88.89%), Al_2O_3 (9.05%), Fe_2O_3 (0.81%), and CaO (0.29%). For RD, the results were SiO_2 (51.57%), Al_2O_3 (13.99%), Fe_2O_3 (12.58%), and CaO (7.68%). The average particle size of RD was determined on LAMIR-UFPR to be 20 μm , and for DE, it was 5 μm [14]. For CP, whose chemical composition is described in previous documents [22] and, given the lack of silica in its composition, no chemical composition was performed.

The specific weights for RD, CP and DE were 860, 260 and 160 g L⁻¹, respectively. Thus, RD is 3.30 and 5.38 times heavier than CP and DE, respectively.

The high efficacy of DE and RD perceived in the bioassays can be related to the levels of SiO_2 content in DE (88.89%) and RD (51.57%), with 5 and 7 μm granulometry, respectively. Research shows that high levels of SiO_2 have been associated with lipid adsorption in the insect cuticle, resulting in death from

dehydration. Powders with a particle size $<45\ \mu\text{m}$ are considered abrasive, particularly if they are angular, which can injure the insect cuticle, resulting in death [9]. These facts can explain the high mortality of insects treated with DE and RD observed in this study.

Overall, the results of this study strengthen the notion that inert dust constitutes an essential tool in the integrated management of stored grain pests. This research carries RD as an efficient and cheap option to control stored grain pests, with a noticeably high efficacy comparable to that of DE. However, it is critical to constantly consider the source of the RD under study and the particle size [9]. Similarly, essential are considerations regarding the increased efficacy of mixing CP and DE over CP applied alone.

CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

This study has shown that RD locally available, less expensive than synthetic inorganic pesticides and easy to use by farmers, can be used as grain protectants both, applied alone and in combination with DE. On the other hand, CP has the potential to be used as grain protectant, especially in small-scale family farming systems. Using these inert powders alone, or in combination with DE, provides economically and environmentally acceptable alternatives, an essential consideration in the development of insecticidal treatment for stored grain. Therefore, it is essential that more quarries are analyzed as potential sources of RD, especially if their silica contents are high. So, small farming communities in developing countries would have more local and ecologically acceptable alternatives for controlling stored product pests.

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DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version.



Table 1: Percentage mortality (mean±SE, N=20) of *Sitophilus oryzae* at 10 days evaluation, after different storage duration, treated with different inert dust

-----10 days evaluation-----													
Ttrat.	Dose %)					Storage	Time	(days)*					
		0					30	60					
DE	0.05	76.3	±	2.6	a	60.0	±	3.4	ab	74.0	±	3.1	ab
RD	0.50	71.1	±	3.8	a	59.0	±	3.9	ab	28.6	±	2.0	abc
	0.25	64.7	±	2.8	ab	32.5	±	0.7	abc	40.1	±	2.0	abc
	0.10	35.8	±	3.9	abc	37.5	±	0.7	ab	33.9	±	1.5	abc
RD+DE	0.50+0.05	71.1	±	1.3	a	80.0	±	1.8	a	79.2	±	1.2	a
	0.25+0.05	70.0	±	1.7	a	51.5	±	3.1	ab	54.7	±	2.8	ab
	0.10+0.05	84.2	±	1.8	a	56.5	±	1.6	ab	55.7	±	1.6	ab
CP	1.0	41.1	±	3.9	abc	17.5	±	0.9	bc	18.2	±	1.7	bc
	0.50	28.9	±	0.6	abc	26.3	±	2.7	abc	39.1	±	1.0	abc
	0.25	9.5	±	1.1	bc	22.5	±	1.1	abc	37.5	±	1.9	abc
CP+DE	1.0+0.05	71.1	±	1.0	a	47.5	±	1.9	ab	21.9	±	0.7	abc
	0.50+0.05	54.2	±	2.6	ab	27.5	±	0.7	abc	26.0	±	1.2	abc
	0.25+0.05	52.6	±	3.3	abc	41.5	±	1.9	ab	39.1	±	2.2	abc
Control	0	0.0	±	0.0	c	0.0	±	0.0	c	0.0	±	0.0	c
		CV	=	26.5		CV	=	29.7		CV	=	26.8	
-----20 days evaluation-----													
Ttrat.	Dose %)					Storage	time	(days)*					
		0					30	60					
DE	0.05	86.8	±	0.3	abc	83.1	±	1.6	a	89.0	±	0.8	ab
RD	0.50	97.5	±	0.5	a	81.7	±	1.5	ab	90.7	±	0.6	a
	0.25	86.8	±	1.9	abc	63.4	±	1.6	ab	76.9	±	0.8	abc
	0.10	85.8	±	1.1	abc	63.4	±	2.9	ab	72.5	±	0.5	abc
RD+DE	0.50+0.05	98.7	±	0.3	a	91.5	±	1.0	a	90.7	±	0.3	a
	0.25+0.05	100	±	0.0	a	85.9	±	1.0	a	83.5	±	0.6	abc
	0.10+0.05	98.7	±	0.3	a	93.2	±	0.8	a	91.8	±	0.7	a
CP	1.0	42.1	±	3.8	bcd	31.0	±	1.0	b	23.1	±	2.0	de
	0.50	42.1	±	3.9	cd	33.8	±	1.6	b	47.8	±	1.7	cd
	0.25	22.6	±	1.0	de	38.0	±	1.1	b	49.5	±	1.3	bcd
CP+DE	1.0+0.05	96.3	±	0.5	a	59.3	±	2.1	ab	72.5	±	0.9	abc
	0.50+0.05	75.3	±	3.1	abc	57.6	±	0.7	ab	65.9	±	0.4	abc
	0.25+0.05	92.1	±	0.7	ab	76.3	±	0.3	a	67.0	±	1.6	abc
Control	0	0.0	±	0.0	e	0.0	±	0.0	c	0.0	±	0.0	e
		CV	=	15.8		CV	=	10.6		CV	=	10.7	

*Means (± SE) represent 4 replicates of 20 insects each. Means in the same column followed by the same letter are not significantly different (P<0.05). DE – diatomaceous earth; RD – rock dust; CP – charcoal powder

Table 2: Percentage of adult *Sitophilus oryzae* (mean±SE) emerged from grain treated, infested (N=20) for 20 days, and left without insects for 40 days

Treat.	Dose (%)	Emergence time (days)											
		60				90				120			
DE	0.05	0.00	±	0.0	a	4.68	±	0.63	a	9.52	±	0.65	ab
RD	0.50	5.00	±	0.25	a	5.21	±	0.65	ab	1.59	±	0.48	a
	0.25	6.67	±	0.71	a	4.69	±	1.13	a	6.35	±	0.91	ab
	0.10	25.00	±	1.25	abc	8.33	±	1.22	abc	11.64	±	1.85	ab
RD+DE	0.50+0.05	5.00	±	0.75	abc	4.17	±	0.82	a	3.17	±	0.87	a
	0.25+0.05	0.00	±	0.00	a	3.65	±	0.88	a	3.70	±	1.18	a
	0.10+0.05	5.00	±	0.75	abc	3.65	±	0.85	a	3.70	±	1.03	a
CP	1.0	35.00	±	2.32	abc	23.44	±	2.39	bc	30.16	±	2.89	bc
	0.50	51.67	±	1.38	bc	21.35	±	2.78	abc	16.93	±	1.41	abc
	0.25	60.00	±	1.83	c	25.52	±	1.65	c	40.74	±	2.43	c
CP+DE	1.0+0.05	16.67	±	1.32	ab	6.77	±	1.63	ab	19.58	±	1.03	abc
	0.50+0.05	1.00	±	0.71	a	4.69	±	0.48	a	6.88	±	0.63	ab
	0.25+0.05	0.25	±	0.25	a	3.13	±	0.65	a	5.29	±	1.04	ab
Control	0	100.0	±	0.00	d	100.0	±	0.00	d	100.0	±	0.00	d
		CV	=	35.47		CV	=	29.61		CV	=	23.14	

*Means (± SE) represent 4 replicates. Means in the same column followed by the same letter are not significantly different (P<0.05). DE – diatomaceous earth; RD – rock dust; CP – charcoal powder.

Table 3: Percentage of seed germination 98 days after treatment

Treatment	Dose (%)	Germination (%)			
Control	-	90.0	±	0.4	a
DE	0.05	92.5	±	0.3	a
RD	0.5	87.5	±	0.9	ab
RD+DE	0.5+0.05	88.8	±	0.6	ab
CP	1	86.3	±	0.5	ab
CP+DE	1+0.05	77.5	±	0.3	b
		CV=		6.2	

*Means (± SE) represent 4 replicates. Means in the same column followed by the same letter are not significantly different (P<0.05). DE – diatomaceous earth; RD – rock dust; CP – charcoal powder

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